Adv Exp Med Biol - Advances in Microbiology, Infectious Diseases and Public Health (2016) 4: 65–92
DOI 10.1007/5584_2016_27

Published online: 28 June 2016

Clostridium difficile in Food and Animals: A Comprehensive Review

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Abstract

Zoonoses are infections or diseases that can be transmitted between animals and humans through direct contact, close proximity or the environment. *Clostridium difficile* is ubiquitous in the environment, and the bacterium is able to colonise the intestinal tract of both animals and humans. Since domestic and food animals frequently test positive for toxigenic *C. difficile*, even without showing any signs of disease, it seems plausible that *C. difficile* could be zoonotic. Therefore, animals could play an essential role as carriers of the bacterium. In addition, the presence of the spores in different meats, fish, fruits and vegetables suggests a risk of foodborne transmission. This review summarises the current available data on *C. difficile* in animals and foods, from when the bacterium was first described up to the present.

Keywords

Clostridium difficile • Epidemiology • Animals • Food • Transmission

1 Introduction

Clostridium difficile is a spore-forming anaerobic bacterium recognised as the leading cause of antibiotic-associated diarrhoea in hospitalised patients. However, in recent years *C. difficile* infection (CDI) is increasingly common in the community, in younger patients without a previous history of hospitalisation or antibiotic treatment (Gupta and Khanna 2014). Studies worldwide have reported the presence of the bacterium in animals and foods (Songer and Anderson 2006; Hoover and Rodriguez-Palacios 2013; Rodriguez-Palacios et al. 2013) with a prevalence that varies according to the methodology used, the geographical area, the age and the animal species studied. While *C. difficile* is

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well known as enteric pathogen in some food producing, wild and companion animal species (Donaldson and Palmer 1999; Songer and Uzal 2005), there are several reports describing the presence of the bacterium in the intestinal contents of apparently healthy animals (Rodriguez et al. 2012; Hawken et al. 2013). Moreover, data recently published suggests that besides the nosocomial transmission, animals are an important source of human CDI, whether through environmental contamination, direct or indirect contact, or food contamination, including carcass and meat contamination at slaughter – or in the case of vegetables and other fruits, by the use of organic fertilizer or contaminated water (Rupnik and Songer 2010; Hoover and Rodriguez-Palacios 2013; Rodriguez-Palacios et al. 2013).

The European Food Safety Authority (EFSA) defines zoonoses as infections or diseases that can be transmitted directly or indirectly between animals and humans (through direct contact or close proximity with infected animals, or through the environment). As noted before (Rodriguez-Palacios et al. 2013), the relevance of the presence of *C. difficile* in some environments, animals and foods is little understood. This review describes the current knowledge regarding *C. difficile* in animals, foods, and the environment, as well as the prevalence among animals with and without signs of disease. The available data about animals and foods as vectors of CDI in humans has also been reviewed.

2 The Evolutionary History of *C. difficile* Detection in Animals and the Natural Environment

C. difficile was first reported in animals in 1960 (McBee 1960). The bacterium was isolated from a sample of a Weddell seal's large intestine contents, obtained during the course of a brief biological survey in the Ross Sea area of Antarctica. In 1974, a doctoral thesis described for the first time the presence of *C. difficile* in hay, soil, sand, and mud from the bank of the river, and in stools from diverse animals such as donkeys, horses, cows and camels, in Pakistan (Hafiz 1974). In an experimental study conducted in 1979 to reproduce neonatal diarrhoea in young gnotobiotic hares, the authors concluded that *C. difficile* was the causal agent of neonatal diarrhoea and that other strains of *Clostridium* enhanced its pathogenic effect (Dabard et al. 1979). CDI in pigs was first confirmed in 1980 when gnotobiotic pigs were accidentally exposed to *C. difficile* and accordingly suffered dehydration and excreted mucoid faeces containing specks of blood (Nagy and Bilkei 2003). In 1981 *C. difficile* was isolated from a goat (Hunter et al. 1981) and in 1982 the bacterium was obtained from rectal samples of healthy cattle in Nigeria of different breeds aged 6 months and above (Princewell and Agba 1982). Borriello et al. (1983) were the first to report the carriage of *C. difficile* in household pets and their immediate environment, including dogs, cats, ducks, geese, chicken, ring-necked parakeets, rabbits, goats, hedgehogs and guinea pigs. However, most of the recovered isolates were identified as non-cytotoxigenic. In the same year, *C. difficile* was recovered from pigs (Jones and Hunter 1983) and identified as the causative agent of antibiotic-associated colitis in a Kodiak bear (Orchard et al. 1983). Interest in the study of *C. difficile* in animals continued to increase during this period. From 1984 to 1987 three new studies described the bacterium as causal agent of enteric disease and diarrhoea in hares, European and cottontail rabbits (Carman and Evans 1984), horses (Ehrich et al. 1984) and foals (Jones et al. 1987). These findings raised the first concerns that domestic animals might be vectors of *C. difficile* among humans (Weber et al. 1988). From 1978 onwards, several studies focused on the isolation procedures and characterisation of *C. difficile* from healthy and diarrhoeic animals, including not only domestic animals such as foals (Jones 1989), cats, dogs (Weber et al. 1989; Riley et al. 1991; Martirossian et al. 1992) and captive ostriches (Frazier et al. 1993), but also wild animals such as cotton-top tamarinds (Snook et al. 1989). In 1995, *C. difficile* toxins were detected in the

small intestine and cecum of three juveniles and one adult rabbit with clinical signs of anorexia, decreased faecal output, nasal exudate and laboured breathing before death (Perkins et al. 1995). A later study in 1996 also reported the presence of *C. difficile* in animals (dogs, cats, horses, sheep and poultry) and in the environment: in soils, in river, sea and lake waters, and in swimming pool and tap waters (al Saif and Brazier 1996). Waters et al. (1998) described an outbreak of *C. difficile* in suckling piglets, and in 1999, Rieu-Lesme and Fonty isolated the bacterium from the ruminal reservoir of newborn lambs (Rieu-Lesme and Fonty 1999).

Besides clinical reports of CDI in exotic animals, such as Asian elephants (Bojesen et al. 2006) and ocelots (Silva et al. 2013a), *C. difficile* has been also isolated from faecal samples of captive white-tailed deer (*Odocoileus virginianus*) in confinement facilities in Ohio, USA, with a prevalence of 36.7 % (French et al. 2010). Furthermore, different studies have investigated the presence of the bacterium in wild animals, including wild passerine birds (Bandelj et al. 2011) and barn swallows (Bandelj et al. 2014); zoo animals (chimpanzees, dwarf goats, Iberian ibexes and plains zebras) (Álvarez-Pérez et al. 2014); sea otters (Miller et al. 2010); free-living South America coatis (Silva et al. 2014); small and medium-size wild mammals (raccoons, shrews, deer and house mice, rats, voles, opossum and groundhogs) (Jardine et al. 2013); black and Norway rats (Firth et al. 2014; Himsworth et al. 2014); feral pigs (Thakur et al. 2011) and Iberian free-range pigs (Álvarez-Pérez et al. 2013).

In the natural environment, *C. difficile* has recently been described in soils of studfarms and farms with mature horses in Sweden (Båverud et al. 2003), in homestead soils and household-stored water in Zimbabwe (Simango 2006), in tropical soils in Costa Rica (del Mar Gamboa et al. 2005) and in Slovenian rivers (Zidaric et al. 2010). In a study conducted in marine environments in the South of Italy, toxigenic *C. difficile* was also detected in seawater and zooplankton (Pasquale et al. 2011).

3 *Clostridium difficile* in Household Pets: Dogs and Cats

Rodriguez-Palacios et al. (2013) refer to the importance of household pets as common transmission routes for human infections of *C. difficile*: in modern lifestyles dogs and cats are considered family members and have access to all parts of the house, including beds, sofas, kitchens and dining rooms. Children under 16 years old often have close contact with their pets, as dogs often licked their faces and both cats and dogs usually sleep in the child's bed. In a study conducted in Canada, it was reported that very few of these children (2.9–4.4 %) recognised the need for washing their hands after contact with pets (Stull et al. 2013). A further study evaluating *C. difficile* in dogs and in the household environment indicated that 10 % of dogs were colonised by the bacterium and 31 % of households were contaminated with its spores, suggesting that exposure to this pathogen may be common (Weese et al. 2010a). In this environment, children, elderly and immune-compromised people could be more at risk of being colonised and developing CDI. In the same study, molecular characterisation of the isolates revealed that household and dog strains were different, concluding that there are sources of household *C. difficile* contamination other than dogs (Weese et al. 2010a). In any case, all dog isolates were indistinguishable from those circulating in human hospitals in the same geographical area (Rodriguez-Palacios et al. 2013). Therefore, the potential transmission of *C. difficile* between pets and humans is currently unclear.

Conversely, it has been reported that pets owned by an immune-compromised person or dogs living with a human receiving antimicrobial treatment were at greater risk of being colonised, presumably because the owner is at greater risk of developing the disease and in turn becoming a source of infection for the pet (Rodriguez-Palacios et al. 2013; Weese 2011). *C. difficile* has been detected in very high rates in healthy

dogs that visit human hospitals (58 %) (Lefebvre et al. 2006a). The risk seems to be particularly high when they accepted treats during the visit or licked patients (Lefebvre et al. 2009). However, it is not yet clear whether the contamination comes from patients or the hospital environment (Weese and Fulford 2011). Lefebvre et al. (2006b) reported the first human epidemic strain PCR-ribotype 027 in a healthy 4-year-old toy poodle that visited patients in healthcare settings in Ontario on a weekly basis. In 2009, Lefebvre and Weese (2009) reported the acquisition of toxigenic *C. difficile* by a therapy dog on its paws during a visit to an acute care facility. In this visit, the dog had been encouraged to 'shake paws' with patients. With these findings authors demonstrated that transient contamination of pet therapy animals (without colonisation) could be a source of pathogen transmission.

Regarding *C. difficile* as a cause of disease in pets, it seems that infection is more commonly community-associated rather than acquired at veterinary hospitals or after antimicrobial therapy (Weese 2011). However, the prevalence and causes of infections acquired in veterinary practices is largely unknown. A previous study identified administration of antimicrobials prior to admission, or administration of immunosuppressive drugs during hospitalisation, as risk factors for veterinary hospital-associated colonisation (Clooten et al. 2008). Murphy et al. (2010) described an important proportion of veterinary hospitals (58 %) with positive environmental swabs for *C. difficile*. While signs of disease could range from mild self-limiting diarrhoea to chronic or fatal diarrhoea (Berry and Levett 1986), the relevance of the bacterium in small veterinary clinics is still uncertain (Weese 2011; Busch et al. 2014). Different other studies have associated the presence of *C. difficile* in faeces with diarrhoea in dogs and cats (Weese et al. 2001a; 2001b; Weese and Armstrong 2003; Koene et al. 2012; Wetterwik et al. 2013). However, dogs can also be healthy carriers of *C. difficile* strains belonging to human epidemic PCR-ribotypes (Schneeberg et al. 2012; Silva et al. 2013b; Spigaglia et al. 2015), with a high colonisation in the first period of live (Perrin et al. 1993; Álvarez-Pérez et al. 2015).

Regarding CDI in cats, little information is available. It seems that colonisation rates are relatively low in the general population (0–21 %), but slightly higher among cats in veterinary hospitals (9.4–31 %) (Marks et al. 2011). The same *C. difficile* strains were recovered from cats and floor drains in the same veterinary hospital, suggesting the clinical environment was a possible source of contamination (Madewell et al. 1999).

Pet nutrition has been identified as a possible source of *C. difficile*, via pet treats (as bully sticks for dogs) and other raw or processed foods (Freeman et al. 2013; Rodriguez-Palacios et al. 2013). In a study conducted in France, *C. difficile* was not detected in any feline raw foods ($n = 20$) purchased from 20 Paris stores (Bouttier et al. 2010). However, a further study conducted in Ontario reported the presence of toxigenic *C. difficile* in turkey-based pet food. In the same study the authors recommended disinfecting food and water bowls daily with a 10 % bleach solution to reduce the potential burden of bacteria. Furthermore, it was proposed owners should not feed pets with raw diets in households with young children or immunosuppressed or elderly individuals (Weese et al. 2005).

4 Clostridium difficile in Horses

C. difficile toxins were associated with equine diarrhoea for the first time in 1984, in a study of horses in Potomac River area. In this study, Ehrich et al. (1984) concluded that toxins appeared not to be primary determinants of diarrhoea but they may have contributed to the disease. Currently, *C. difficile* is considered one of the most important causes of diarrhoea and enterocolitis in foals and horses (Arroyo et al. 2006; Weese et al. 2006; Uzal et al. 2012; Diab et al. 2013b). The prevalence of *C. difficile* in foals and adult horses with gastrointestinal disease varies considerably among studies, ranging between 5 % and 63 % (Diab et al. 2013b).

In newborn foals, *C. difficile* has been associated with spontaneous watery or bloody diarrhoea immediately after birth, depression, dehydration, toxaemia and finally death (Diab et al. 2013a). While in some cases the disease can occur without a history of antibiotic therapy or hospitalisation (Diab et al. 2013b), the major risk factors for the development of CDI in horses are antimicrobial treatment, hospitalisation, pre- or post-surgical feed withdrawal or changes in diet. The antimicrobials that have been most frequently associated with *C. difficile* diarrhoea in horses are erythromycin, clindamycin, rifampicin and gentamicin (Diab et al. 2013b).

Like other species, horses can carry *C. difficile* without showing signs of disease. In healthy foals the reported prevalence can vary between 0 and 29 % depending on different factors such the type of the study, the diagnostic test used and the method of sample collection (Diab et al. 2013b). A colonisation rate of up to 44 % has been reported in non-diarrhoeic foals under antibiotic treatment (Båverud et al. 2003). Mare-foal pairs can harbour *C. difficile* subclinically and potentially serve as reservoirs for cross-colonisation (Magdesian and Leutenegger 2011). In hospitalised horses without clinical signs of *C. difficile* disease, the observed prevalence ranged from 4.8 to 11 % (Medina-Torres et al. 2011; Rodriguez et al. 2014a), possibly under the influence of stresses that alter the intestinal flora (such as change of diet, transportation to the hospital, hospitalisation, and surgical or medical treatments) (Båverud 2004). Some studies have suggested a transient shedding of *C. difficile* in adult horses (Schoster et al. 2012) but also in other animal species including cattle (Rodriguez-Palacios et al. 2011b) and humans (Ozaki et al. 2004).

A recent study has evaluated the effect of probiotics on foals developing diarrhoea within 6 months of birth. The authors concluded that there was no benefit observable of administering a 3-week course of probiotics. Furthermore, a significantly higher incidence of diarrhoea in foals receiving probiotics than in control groups suggested a negative impact of probiotics (Schoster et al. 2015), although in vitro inhibition of *C. difficile* and *C. perfringens* by commercial probiotic strains has also been reported (Schoster et al. 2013).

5 *C. difficile* in Food-Producing Animals

In the twenty-first century the possibility of human exposure to *C. difficile* spores via environments and foods contaminated with feces of colonised animals has aroused considerable interest. Furthermore, besides the concern for zoonotic transmission, *C. difficile* is also a costly disease on companion animals and livestock production. There are no financial loss estimates for the treatment of household pets, but veterinary services and medical treatment for a case of acute diarrhoea without further complications costs between 100 and 200 euros in Europe. In production animals, *C. difficile* losses and treatment costs have also not been estimated, but *C. difficile* can produce mortality in breeding, weight loss, and delayed weight gain in animals (Rodriguez-Palacios et al. 2013; Squire and Riley 2013).

5.1 Food-Producing Animals: Swine

C. difficile has been widely described in both healthy pigs and pigs with diarrhoea (Table 1). In neonatal piglets (<15 days old), *C. difficile* has been proposed as the most common cause of diarrhoea (Songer and Anderson 2006) with a mortality rate of up to 50 % in suckling piglets (Songer 2000). Previous studies reported spore or toxin detection ranging between 23 and 93 % in faeces of diarrhoeic piglets and between 1.4 and 96 % in piglets with normal faeces (Table 1). The presence of *C. difficile* toxins in the colon of neonatal swine has been associated with: profuse non-haemorrhagic yellow pasty-to-watery diarrhoea, colitis, typhocoloitis, severe mesocolonic edema, other microscopic lesions such as erosive or ulcerative colonic lesions, infiltration of neutrophils in the lamina propia, and exudation of fibrin into the lumen, resulting

Table 1 Presence of *C. difficile* in piglets and adult pigs at farms, slaughterhouses and clinics

Area	Country/ State	Year[a] From 2000	Age or situation	With (D) Without (ND) diarrhoea (%)	Prevalence (%)	T (% of toxigenic strains)	Main PCR-ribotypes[b]	Study
Europe	Slovenia	08	1–10 days	D and ND	133/257 (51.8)	T (100)	–	Pirs et al. (2008)
		09	<10 days	D (77.7 of litters)	247/485 (50.9)	T (99.6)	066 (68.3)	Avbersek et al. (2009)
	Spain	09	1–7 days	D (49.7)	58/254 (22.8)	T (100)	–	Alvarez-Perez et al. (2009)
				ND (50.3)	76/257 (29.6)	T (90.8)		
			1–2 months	D (6)	0/12 (0)	–		
				ND (94)	0/187 (0)			
	Belgium	11	<15 days	ND (100)	18/23 (78.3)	T (100)	078 (66.7)	Rodriguez et al. (2012)
							002 (16.7)	
			At slaughter (5–6 months)	ND (100)	0/194 (0)	–	–	
		11–12	At slaughter (5–6 months)	ND (100)	1/100 (1)	T (100)	078 (100)	Rodriguez et al. (2013)
	Sweden	12	Neonatal	D and ND	45/67 (67)	T (100)	046 (100)	Norén et al. (2014)
	Germany	12	0–1 days	D (70.5)	19/31 (61)	T (100)	078 (55)	Schneeberg et al. (2013a)
				ND (29.5)	11/13 (85)		126 (20)	
			2–14 days	D (77.8)	78/84 (93)			
				ND (22.2)	23/24 (96)			
			15–77 days	D (71.4)	11/35 (31)			
				ND (28.6)	5/14 (36)			
	Austria	08	At slaughter	ND (100)	2/61 (3.3)	T (100)	–	Indra et al. (2009)
	The Netherlands	09	At slaughter	ND (100)	14/50 (28)	T (100)	015 (35.7)	Hopman et al. (2011b)
		09–10	At slaughter	ND (100)	0/100 (0)	–	–	Koene et al. (2012)
			In clinics	D (100)	9/25 (36)	T (100)	078 (77.8)	
							023 (11.1)	
							005 (11.1)	
		09–10	At slaughter	ND (100)	58/677 (8.6)	–	078 (31)	Keessen et al. (2011b)
							014 (15.5)	
							013 (12.1)	
	Switzerland	10	At slaughter	ND (100)	0/165 (0)	–	–	Hoffer et al. (2010)

USA	Texas	04–07	At farrowing	–	175/702 (24.9)	T (97.2)	078 (26.2)	Norman et al. (2011)
			Nursery		14/274 (5.1)			
			Breeding		26/604 (4.3)			
			Growth or finishing (at farm)		37/1370 (2.7)			
		06–07	Suckling	ND (100)	61/122 (50)	T93	–	Norman et al. (2009)
			Nursery		10/119 (8.4)			
			Growth or finishing (at farm)		15/382 (3.9)			
	Midwest	06	Neonatal	D (1000)	241/513 (47)	T [578 isolates]	–	Baker et al. (2010)
	Michigan	08	At farm	–	1/56	T (100)	–	McNamara et al. (2011)
	Ohio	08	16–20 weeks (at farm)	–	1/150 (0.67)	T (100)	–	Rodriguez-Palacios et al. (2014)
	Ohio	10	At farrowing	ND (100)	183/251 (73)	T (83.6)	–	Thakur et al. (2010)
	North Carolina		Sows after farrowing		32/68 (47)	T (90.6)		
	North Carolina	08–10	At farrowing (conventional farms)	ND (100)	120/350 (34.3)	T (97.8)	–	Susick et al. (2012)
			At farrowing (antimicrobial-free farms)		56/241 (23)			
			Nursing (conventional farms)		34/651 (5.2)			
			Nursing (antimicrobial-free farms)		7/491 (1.4)			
			Conventional sows in the farrowing barns		24/70 (34.3)			
			Antimicrobial-free sows in the farrowing barns		2/39 (5.1 %)			
			Finishing at conventional farms		2/579 (0.3)			

(continued)

Table 1 (continued)

Area	Country/ State	Year[a] From 2000	Age or situation	With (D) Without (ND) diarrhoea (%)	Prevalence (%)	T (% of toxigenic strains)	Main PCR-ribotypes[b]	Study
Canada	Ontario	10	On day 2	–	90/121 (74)	T (100)	078 (94)	Weese et al. (2010c)
			On day 7		66/117 (56)			
			On day 30		45/113 (40)			
			On day 44		23/101 (23)			
			On day 62		2/54 (3.7)			
			Sows prior to farrowing		4/10 (40)	T (100)	078 (12)	
	Ontario	13	1 day	–	28/30 (93)	T (100)	078	Hawken et al. (2013)
			Market age (188 days)		1/26 (3.8)	T (100)	078 (100)	
	Various provinces	11	At slaughter	–	30/436 (6.9)	T (93.3)	078 (67)	Weese et al. (2011)
Australia	Western Australia	09	Neonatal	D (94.1)	103/174 (59.19)	T (100)	273 (100)	Squire et al. (2013)
				ND (5.9)	11/11 (100)			
	Five states	12–13	<7 days	D (12 farms with idiopathic diarrhoea) ND (9 farms without idiopathic diarrhoea)	154/229 (67.2)	T (87)	014 (23)	Knight et al. (2015b)
	Georgia	11	Before slaughter	–	55/345 (15.9)	–	–	Thitaram et al. (2011)
Japan	Kanto-Tokai	12	Finishing at farm (13–27 weeks)	–	2/250 (0.8)	T (50)	–	Asai et al. (2013)

[a]Year when the study was conducted or year when the study was published
[b]Main PCR-ribotypes found with standard Cardiff nomenclature
(–) Data not available or not applicable

in 'volcano lesions' (Lizer 2010). Scrotal edema, dyspnoea, mild abdominal distension, hydrothorax, ascites, anorexia and dehydration are other extra-intestinal symptoms probably caused by systemic sepsis (Squire and Riley 2013). However, an absence of diarrhoea does not discount possible *C. difficile* colonisation (Yaeger et al. 2007). Why some colonised piglets with toxigenic strains of *C. difficile* do not develop any signs of disease remains unclear and may be explained by the variability in colostrum intake and colostrum antibody concentration (Squire and Riley 2013). Similarly, the presence of *C. difficile*-negative piglets has been described in litters where most of the members carried the bacterium. The reason why these piglets were negative despite being constantly exposed to the bacterium is also unknown (Weese et al. 2010c). The prevalence of the bacterium decreases with age, varying from 0 to 23 % at finishing in the farm or at slaughter (Table 1). Furthermore, outbreaks in adult pigs have only been reported in periparturient sows (Kiss and Bilkei 2005). It appears that sows are more likely to be colonised by *C. difficile* before or after farrowing (Thakur et al. 2010; Weese et al. 2010c; Susick et al. 2012), which may be due to environmental stress or the administration of antibiotics (Kiss and Bilkei 2005). While it seems sows would pose an obvious contamination source for piglets during farrowing, one study describes the predominance of different PCR-ribotypes in each group, suggesting that external sources other than sows could be responsible for CDI in piglets (Weese et al. 2010c; Hopman et al. 2011a). Widespread aerial dissemination of *C. difficile* on a pig farm was demonstrated and associated with personnel activity. Furthermore, possible aerial dispersal of the bacterium between farrowing pens was revealed by the detection of spores in the hallway following relocation of piglets (Keessen et al. 2011a). On pig farms, vermin such as house mice, drain flies, lesser houseflies and yellow mealworms were found positive for *C. difficile* and proposed as vectors for bacteria transmission (Burt et al. 2012). Despite the progress made in these studies, the sources of *C. difficile* in pig farms and aspects of the infection cycle still remain unclear. Several procedures, like surface disinfection and the use of gloves, have been proposed to reduce disease-associated mortality in piggeries (Squire and Riley 2013).

5.2 Food-Producing Animals: Cattle

As in the case of swine, the reported prevalence of *C. difficile* in cattle can vary wildly from one study to another depending on the geographical location studied, with percentages as diverse as 0 % in farms in North America and 60 % in Iran (Doosti and Mokhtari-Farsani 2014; McNamara et al. 2011) (Table 2). Furthermore, the pathogenicity of *C. difficile* in cattle is not fully understood. The bacterium and its toxins have been associated with diarrhoea in calves and dairy cows (Table 2). Using post-mortem analysis of calves infected with *C. difficile*, it has been showed that the bacterium was more frequently encountered in the cecum, where histologic lesions were also more severe (Rodriguez-Palacios et al. 2007b).

A higher prevalence (up to 56 %) has been reported in apparently healthy calves aged less than three months old (Table 2). One experimental study investigated the infection of neonatal calves by oral inoculation (in the colostrum) of toxigenic *C. difficile* spores. Results showed faecal shedding but did not detect toxins or the induction of enteric disease, and suggested that simple exposure to *C. difficile* could not cause disease in calves (Rodriguez-Palacios et al. 2007b). Colostrum can also play a protective role, providing passive immunity in neonatal calves. A natural protective effect of this first milk when ingested by calves immediately after birth is plausible (Rodriguez-Palacios et al. 2007b) and merits further investigation. In the literature, many studies have investigated hyperimmune bovine colostrum (obtained by repeated immunisation of pregnant cows) as an effective treatment for CDI in human patients (Steele et al. 2013). However, with or without signs of enteric disease, a decrease in the prevalence rate of *C. difficile* is observed in adult

Table 2 Presence of *C. difficile* in calves, dairy cattle and beef cattle at farms and slaughterhouses

Area	Country/ State	Year[a] From 2000	Age	With (D) Without (ND) diarrhoea (%)	Prevalence (%)	T (% of toxigenic strains)	Main PCR-ribotypes[b]	Study
Europe	Slovenia	08	>21 days	D (100)	1/56 (1.8)	T (100)	033 (100)	Pirs et al. (2008)
		09	<12 weeks	D (76.1)	4/42 (9.5)	T (100)	077 (50)	Avbersek et al. (2009)
							038 (25)	
							002 (25)	
	Belgium	10	14 days (at arrival)	D (60)	5/50 (10)	T (95)	126 (36.8)	Zidaric et al. (2012)
			18 days		8/50 (16)		078 (31.6)	
			25 days		6/50 (12)		045 (10.5)	
			32 days		1/50 (2)		033 (7.9)	
			46 days		1/50 (2)		012 (7.9)	
			194 days (just before slaughter)		6/50 (0)			
		11	<3 months	ND (100)	4/18 (22.2)	T (100)	078 (75)	Rodriguez et al. (2012)
							015 (25)	
			At slaughter (11–52 months)	ND (100)	14/202 (6.9)	T (71.4)	002 (7.1)	
							014 (7.1)	
							081 (7.1)	
		11–12	At slaughter (15–56 months)	ND (100)	10/101 (9.9)	T (80)	078 (54.5)	Rodriguez et al. (2013)
							029 (18.2)	
	The Netherlands	09–10	Veal calves	ND (100)	6/100 (6)	T (100)	012 (83.3)	Koene et al. (2012)
							033 (16.6)	
			Dairy cows	D (100)	0/5 (0)	–	–	
				ND (100)	1/100 (1)	T (100)	012 (100)	
	Austria	08	At slaughter	ND (100)	3/67 (4.5)	T (3)	–	Indra et al. (2009)
	Switzerland	10	At slaughter	ND (100)	1/204 (4.2)	T (100)	078 (100)	Hoffer et al. (2010)
		10	Cows	–	1/63 (1.6)	T (100)	137 (100)	Romano et al. (2012)
			Calves		6/47 (12.7)	T (83.3)	033 (16.7)	
							003 (16.7)	
							066 (16.7)	
							070 (16.7)	

	Germany	10–12	Calves	D (100)	176/999 (17.6)	–	033 (57)	Schneeberg et al. (2013b)
							078 (17)	
							045 (9)	
		11–12	Dairy cattle	–	25/29 (86.2)	T (17)	–	Schmid et al. (2013)
			Beef cattle		4/29 (13.8)			
Asia	Iran	13	3–25 days	–	90/150 (60)	T (41)	–	Doosti and Mokhtari-Farsani (2014)
USA	Georgia	11	Dairy cattle	ND (100)	32/1325 (2.4)	–	–	Thitaram et al. (2011)
			Beef cattle		188/2965 (6.3)			
	Ohio	08	Dairy cow	–	2/330 (0.61)	T (100)	027 (50)	Rodriguez-Palacios et al. (2014)
			Beef cow					
		07	On arrival	ND (100)	24/186 (12.9)	T (92.8)	078	Rodriguez-Palacios et al. (2011b)
			Week 1		0/176 (0)			
			Week 4		3/176 (1.7)			
			Week 12		0/168 (0)			
			Week 20		5/168 (3.6)			
			Prior to slaughter		2/167 (1.2)			
			At slaughter (intestinal contents)		2/168 (1/2)			
		11	At harvest (meat processing plants)	ND (100)	17/944 (1.8)	T (0.4)	078 (5.9)	Rodriguez-Palacios et al. (2011a)
	Michigan	08	At farm	–	0/50 (0)	–	–	McNamara et al. (2011)
	South-western	08	1–6 weeks	D (82.7)	64/253 (25.3)	T (23)	–	Hammitt et al. (2008)
				ND (17.3)	7/53 (13.2)	T (30.2)		
	Pennsylvania	12	<2 weeks	ND (100)	8/200 (4)	T (7.1)	–	Houser et al. (2012)
			4–6 weeks		18/200 (9)			
			8–10 weeks		6/200 (3)			
			12–18 weeks		10/200 (5)			
			20–22 weeks		18/200 (9)			

(continued)

Table 2 (continued)

Area	Country/ State	Year[a] From 2000	Age	With (D) Without (ND) diarrhoea (%)	Prevalence (%)	T (% of toxigenic strains)	Main PCR-ribotypes[b]	Study
Canada	Ontario	04	<1 month	D (51.8)	11/144 (7.6)	T (100)	078 (25.8)	Arroyo et al. (2005)
				ND (48.2)	20/134 (14.8)		017 (29)	
				014 (12.9)				
				027 (12.9)				
				033 (9.7)				
	Ontario	08–09	2–10 days (48 h after arrival)	–	56/174 (32)	T (98.7)	078 (67)	Costa et al. (2011)
			1 week		88/172 (51.1)			
			17 weeks		4/183 (2)			
			21 weeks		4/156 (2)			
	Alberta	09	At feedlot on arrival	–	18/539 (3.3)	T (100)	078 (100)	Costa et al. (2012)
			Mid-feeding period		18/335 (5.4)			
Australia	Western Australia	07–08	Adult cattle at slaughterhouse (intestinal contents)	ND (100)	0/158 (0)	T (98.6)	127 (50.2)	Knight et al. (2013)
							033 (19.6)	
							16 (7.7)	
	New South Wales	08–09	Adult cattle at slaughterhouse (faeces)		5/280 (1.8)		126 (5.7)	
	Queensland						3 (1.4)	
	South Australia						103 (1.4)	
							002 (1)	
	Victoria						137 (0.5)	
	Western Australia						7 (3.3)	
	Victoria	12	Calves aged < 7 days at slaughterhouse		203/360 (56)			
	Queensland							
			Calves 2–6 months at slaughterhouse		1/26 (3.8)			

[a]Year when the study was conducted or year when the study was published

[b]Main PCR-ribotypes found with standard Cardiff nomenclature (−) Data not available or not applicable

animals (Table 2). While the reason for this age effect is still unknown, a probable explanation is that the bacterium is better able to colonise and proliferate in the intestinal tract of younger animals, where the gut microbiota is less developed (Rodriguez-Palacios et al. 2006).

5.3 Food-Producing Animals: Poultry

A wide variety of zoonotic diseases can be transmitted by poultry. However, few studies have focused on the study of *C. difficile* in these animals. The limited data available shows that the situation is similar to other species, with prevalence decreasing with increasing age (ranging from 100 % in faecal samples of 14-day-old birds to 0.29 % in mature farm animals), and with bacterial colonisation observable with or without development of disease (Table 3).

Only one outbreak of *C. difficile* has been described in newly hatched ostriches (Cooper et al. 2013). In this outbreak, more than 90 % of birds died within three days of the onset of diarrhoea. At necropsy, the colon and rectum were dilated and diffusely haemorrhagic. Microscopic examination also revealed necrotizing typhilitis and colitis in all the birds. After this report, 300 additional birds from a subsequent hatching were also affected by an epidemic of necrotic enteritis. Identical symptoms were observed which may suggest that CDI is a common and important problem in captive ostrich chicks (Frazier et al. 1993).

In rural communities in Zimbabwe, chickens were identified as major reservoirs of *C. difficile*. Water probably acted as a source of the bacterium for these chickens, as spores were detected in well water and household-stored water. Sources of water contamination may be faeces of domestic animals or humans, although this was not investigated in the study. In addition, soils were also heavily contaminated with *C. difficile* by chicken faeces. The free movement of chickens between neighbouring homesteads highlights the importance of these colonised animals as vectors for widespread distribution of *C. difficile* in rural communities (Simango 2006).

5.4 Food-Producing Animals: Sheep and Goats

Other production animals such as lambs, sheep and goats have been also described as carriers of the bacterium, with a prevalence varying between 0.6 and 10.1 % (Table 3). As in other animal species, the rate of *C. difficile* detection seems to decrease with age.

On average, a lower prevalence has been reported in sheep and lambs than in swine. This may be associated with the greater use of antimicrobials in production of pigs than in sheep (Knight and Riley 2013). However, as stated before, the few studies available in the literature studying the effect of antibiotics did not find a direct relation between the use of antimicrobials and *C. difficile* colonisation or infection (Romano et al. 2012; Susick et al. 2012). While the presence of *C. difficile* in apparently healthy sheep and goats in farms and at slaughter could play a role in animal-to-animal, environmental or zoonotic transmission, there are no reports identifying the bacterium as responsible for outbreaks of enteropathogen in these animal species.

6 Clostridium difficile in Foods

Recent studies have described the presence of *C. difficile* spores in a variety of food products of both animal and plant origin. These findings highlight the potential risk of infection associated with consuming foods, particularly if they are not cooked prior to eating (Lund and Peck 2015).

6.1 Prevalence and Food Products Concerned

The contamination by *C. difficile* spores has been detected in different types of food products,

Table 3 Presence of *C. difficile* in other food-producing animals

Animal species (Origin)	Area	Country	Year[a] From 2000	Age	With (D) Without (ND) diarrhoea (%)	Prevalence (%)	T (% of toxigenic strains)	Main PCR-ribotypes[b]	Study
Poultry	Europe	Slovenia	07–08	14 weeks (flock 1)	–	5 /7 (71.4)	T (100)	023 (6.8)	Zidaric et al. (2008)
				1 day (flock 2)		0/8 (0)	–		
				15 days (flock 2)		24/24 (100)	T (96.3)		
				18 weeks (flock 2)		9/22 (40.9)	T (90)		
		The Netherlands	09–10	Clinics	D (100)	2/21 (9.5)	T (57.1)	014 (28.6)	Koene et al. (2012)
				At slaughter	ND (100)	5/100 (5)		010 (28.6)	
		Austria	08	Broiler chicken at slaughter	ND (100)	3/59 (5)	T (66.7)	001 (33.3)	Indra et al. (2009)
								446 (33.3)	
	Africa	Zimbabwe	06	Chicken faeces (home leads rural community)	–	20/115 (17.4)	T (55)	–	Simango (2006)
			08	Broiler chickens at market places	–	29/100 (29)	T (89.7)	–	Simango and Mwakurudza (2008)
	USA	Ohio	08	At farm	–	1/340 (0.29)	T (0)	–	Rodriguez-Palacios et al. (2014)
		Texas	09	Broiler chicken 42 days-old at barns	–	6/300 (2.3)	T (100)	078	Harvey et al. (2011a)
Sheep	Europe	The Netherlands	09–10	Clinics	D (100)	2/11 (18.2)	T (100)	015 (50)	Koene et al. (2012)
								097 (50)	
		Slovenia	09–11	Adult sheep >1 year at farms	D (12.4)	0/27 (0)	T (100)	056 (16.7)	Avberšek et al. (2014)
				Lambs between 1 day and 4 months	D (7.6)	6/78 (7.7)		061 (16.7)	
	Australia	South Australia	11–12	Sheep at slaughterhouse	ND (100)	1/156 (0.6)	T (93.3)	056 (40)	Knight and Riley (2013)
		New South Wales						101 (40)	
		Victoria		Lambs at slaughterhouse or farm	–	14/215 (6.5)		m	
		Western Australia							
	USA	Michigan	08	At farm	–	0/57 (0)	–	–	McNamara et al. (2011)

Goats	Europe	Slovenia	09–11	Adult goats >1 year at farms	ND (100)	0/10 (0)	T (90)	045 (40)	Avberšek et al. (2014)
								010 (10)	
				Goats between 1 day and 4 months		10/99 (10.1)		014 (10)	
								020 (10)	
		Switzerland	10	At farm	–	3/40 (7.5)	T (100)	001 (66.7)	Romano et al. (2012)
								066 (33.3)	
	USA	Michigan	08	At farm	–	0/14 (0)	–	–	McNamara et al. (2011)

[a]Year when the study was conducted or year when the study was published
[b]Main PCR-ribotypes found with standard Cardiff nomenclature
(–) Data not available or not applicable (–) Data not available or not applicable

including seafood, vegetables and meats, with a prevalence ranging between 2.9 and 66.7 % (Tables 4 and 5). Considering that *C. difficile* is present in healthy food-producing animals at slaughter, it is not surprising that its spores have also been found in meats (Table 4). The mean prevalence of *C. difficile* spores in these products ranges between 0 and 15 %. While early studies conducted in North America reported a much higher contamination rate than elsewhere (Rupnik and Songer 2010), recent studies show the situation to be similar to other countries (Table 4). Rodriguez-Palacios et al. (2009), noting an increased recovery of the bacterium from ground beef and chops in winter in Canada, suggested a seasonal component in *C. difficile* contamination in meats, and also hypothesised a possible epidemiological connection between the prevalence of *C. difficile* in food animals, some foods and humans (Rodriguez-Palacios et al. 2013).

If the initial contamination of food products with *C. difficile* is low, the preservation method used may play a fundamental role in the spores' survival. One of the key features of *C. difficile* in foods is if the pathogen grows or resides in the dormant state, especially if there are anaerobic conditions and the cool chain is not respected. *C. difficile* has been reported in vacuum-packaged meat in France (Bouttier et al. 2010) and in New Zealand, where the bacterium was isolated from chilled vacuum-packed meats in which 'blown pack' spoilage had been observed (Broda et al. 1996). The impact of *C. difficile* survival in these storage conditions clearly demands further study.

There has also been interest with respect to thermal inactivation of *C. difficile* spores by thermal treatment. Rodriguez-Palacios and Lejeune (2011) reported that cooking food at a minimum of 96 °C for 15 min produced an inhibitory effect on *C. difficile* spores. However, minimally-processed fruits and vegetables are treated below these temperatures and therefore could be potential vectors of human infection (Rodriguez-Palacios et al. 2013). The contamination source of these fruits and vegetables could be the use of organic fertilizer containing *C. difficile* spores, or irrigation or washing with contaminated water.

6.2 Routes of Food Contamination

As stated before, *C. difficile* is present in the intestinal contents of apparently healthy food-producing animals, suggesting carcasses and meats could be contaminated during the slaughter process. A few studies have addressed the contamination of carcasses at slaughter. In pigs, *C. difficile* was detected in a total of 3 out of 20 carcasses (15 %) sampled at post-bleed and a further 3 out of 20 (15 %) at post-evisceration in a processing facility in Canada (Hawken et al. 2013). A further study reported a prevalence of 2.2 % and 2.5 % in antimicrobial-free pigs at post-evisceration and post-chill respectively (Susick et al. 2012). Harvey et al. (2011b) detected 3 positive samples from a total of 10 sponge swabs collected from carcass hide, post-excision hides and ears from pigs in a processing plant in Texas. In Belgium, the prevalence reported in carcasses from slaughter pigs was 7 % (7/100) (Rodriguez et al. 2013).

C. difficile has also been described in cattle carcasses. In Belgium, the observed prevalence in cattle carcasses reached up to 7.9 % (8/101) (Rodriguez et al. 2013). In a study conducted in Pennsylvania, Houser et al. (2012) detected the *tpi* housekeeping gene in 4 out of 100 cattle carcass swabs by PCR, but *C. difficile* was not isolated using culture techniques. The same data has been reported in an Australian study of cattle carcasses sampled in the processing area of the slaughter line where none of the samples taken ($n = 151$) were positive for *C. difficile* (Knight et al. 2013). Rodriguez-Palacios et al. (2011b) reported 0 positive carcasses from a total of 168 samples analysed.. In a further study conducted in the USA, samples were collected from pig hides, pre-evisceration carcasses, post-intervention carcasses and ground beef. The bacterium was detected in hides with a prevalence of 3.2 %. However, none of the carcass or meat samples tested positive, evidencing a low

Table 4 Presence of *C. difficile* in meats (in processing plants or the retail trade) and other foods (at farms or markets)

Area	Country/State	Year[a]	Sample Type	Prevalence (%)	T (% of toxigenic strains)	Main PCR-ribotypes[b] (%)	Study
Asia	Iran (Isfahan)	2014	Chopped beef	1/35 (2.8)	T (100)	–	Esfandiari et al. (2014a)
			Ground beef	1/46 (2.1)			
			Chopped mutton	2/55 (3.6)			
			Ground mutton	4/64 (6.2)			
		2012	Beef meat samples	3/54 (5.6)	T (100)	–	Esfandiari et al. (2014b)
			Beef hamburger	4/56 (7.1)			
	Iran (Isfahan/ Khuzestan)	2012	Buffalo meat	6/67 (9)	T (92.3)	078 (53.8)	Rahimi et al. (2014)
			Goat meat	3/92 (3.3)			
			Beef meat	2/121 (1.7)			
			Cow meat	1/106 (0.9)			
			Sheep meat	1/150 (0.7)			
			Camel meat	0/124 (0)			
USA	Connecticut	2015	Ground beef	0/100 (0)	T (0)	–	Mooyottu et al. (2015)
			Ground pork	2/100 (2)			
			Chicken wings	0/100 (0)			
	Pennsylvania	2011–2012	Beef meat samples	5/72 (6.9)	T (100)	027 (40)/078 (40)	Varshney et al. (2014)
			Pork meat samples	9/78 (11.5)	T (66.7)	078 (44)	
			Turkey meat samples	11/76 (14.5)	T (81.8)	027 (9.1)/078 (18.2)	
			Chicken meat samples	6/77 (7.8)	T (33.3)	–	
		2012	Pork sausages	2/102 (2)	T (100)	078 (100)	Curry et al. (2012)
		2012	Ground veal products	4/50 (8)	T (100)	–	Houser et al. (2012)
	Nine different states	2009–2011	Retail meat products	0/1755 (0)	–	–	Limbago et al. (2012)
	Nebraska	2013	Ground beef	0/956 (0)	–	–	Kalchayanand et al. (2013)
	Texas	2004–2009	Ground pork and turkey	23/243 (9.5)	T (100)	078 and variants (95.7)	Harvey et al. (2011b)
			Pork chorizo and trim				
	Arizona	2007	Ground beef uncooked	13/26 (50)	T (100)	078 (2.2)/027 (4.4)	Songer et al. (2009)
			Summer sausage (cooked)	1/7 (14.3)			
			Ground pork (uncooked)	3/7 (42.9)			

(continued)

Table 4 (continued)

Area	Country/State	Year[a]	Sample Type	Prevalence (%)	T (% of toxigenic strains)	Main PCR-ribotypes[b] (%)	Study
			Braunschweiger (cooked)	10/16 (62.5)			
			Chorizo (uncooked)	3/10 (30)			
			Pork sausage (uncooked)	3/13 (23.1)			
			Ground turkey (uncooked)	4/9 (44.4)			
		2010	Poultry meats	4/32 (12.5)	T (100)	078 and variants (100)	Harvey et al. (2011a)
Africa	Abidjan	2009–2010	Cooked kidney beef	19/172 (11)	–	–	Kouassi et al. (2014)
			Cooked flesh beef	30/223 (13.4)			
Europe	Belgium	2012	Ground and burger beef	3/133 (2.3)	T (100)	078 (33.3)/014 (66.7)	Rodriguez et al. (2014b)
			Ground and sausage pork	5/107 (4.7)	T (80)	078 (20)/014 (40)	
	The Netherlands	2008–2009	Beef meat	0/145 (0)	–	–	de Boer et al. (2011)
			Pork meat	0/63 (0)	–	–	
			Calf meat	0/19 (0)	–	–	
			Lamb meat	1/16 (6.3)	T (100)	045 (100)	
			Chicken meat	7/257 (2.7)	T (2.7)	001/003/087/071	
	Switzerland	2010	Minced meat products	0/46 (0)	–	–	Hoffer et al. (2010)
	France	2007–2008	Ground beef	2/105	T (100)	012 (100)	Bouttier et al. (2010)
			Pork sausage	0/59			
	Austria	2007–2008	Ground meat	3/100	T (66.7)	053 (33.3)	Jöbstl et al. (2010)
		2008	Beef meat	0/51 (0)	–	–	Indra et al. (2009)
			Pork meat	0/27 (0)			
			Chicken meat	0/6 (0)			
	Sweden	2008	Ground meat	2/82 (2.4)	T (100)	–	Von Abercron et al. (2009)

Canada	Manitoba	2007	Ground beef	2/24 (8.3)	T (100)	–	Visser et al. (2012)
			Ground pork	1/24 (4.2)			
			Ground pork				Metcalf et al. (2010a)
			Chopped pork				
	Ontario	2008–2009	Chicken thighs	10/11 (9)	T (100)	078 (100)[2]	Weese et al. (2010b)
			Chicken wings	13/72 (18)			
			Chicken legs	3/20 (15)			
	Four provinces	2008	Ground beef	14/115 (12)	T (100)	078 (71.4)/027 (7.1)	Lefebvre and Weese (2009)
			Ground pork	14/115 (12)	T (100)	078 (85.7)/027 (7.1)	
	Various provinces	2006	Ground beef	22/149 (14.8)	T (89.3)	027 (30.8)/077 (23.1)/014 (15.4)	Rodriguez-Palacios et al. (2009)
			Veal chops	6/65 (9.2)			
	Ontario and Quebec	2005	Beef and veal ground meat	12/60 (20)	T (91.7)		Rodriguez-Palacios et al. (2007a)
Central America	Costa Rica	2013	Beef meat	1/67 (1.5)	T (100)	–	Quesada-Gómez et al. (2013)
			Pork meat	2/66 (3)			
			Poultry meat	1/67 (1.5)			

[a]Year when the study was conducted or year when the study was published
[b]Main PCR-ribotypes found with standard Cardiff nomenclature
(–) Data not available or not applicable

Table 5 Presence of *C. difficile* in other foods sampling from farms, wholesalers or markets

Food Type	Area	Country/ State	Year[a]	Sample Type	Prevalence (%)	T (% of toxigenic strains)	Main PCR-ribotypes [b] (%)	Study
Seasoned ingredients	Asia	Iran	2015	Defrosted onions	0/14 (0)	–	–	Esfandiari et al. (2014b)
				Textured soy proteins	0/14 (0)			
				17 seasoning	0/17 (0)			
Seafood	USA	Texas	2012	Fresh mussel	3/67 (4.5)	T (100)	078 (66.7)	Norman et al. (2014)
				Frozen salmon/ shrimp				
	Europe	Italy	2010–2011	*Mytilus galloprovincialis*	16/33 (48.5)	T (43.7)	014/020/078/045/ 012/	Pasquale et al. (2012)
				Tapes philippinarum	10/19 (52.6)	T (80)	002/001/003/106	
				Venus verrucosum	0/1 (0)	–	–	
			2009	*Mytilus galloprovincialis*	2/3 (66.7)	T (50)	066 (50)/010 (50)	Pasquale et al. (2011)
				Tapes philippinarum	1/1 (100)	T (0)	010 (100)	
				Callista chione	1/2 (50)	T (100)	005 (100)	
	Canada	Ontario	2010	Frozen scallop and shrimp	5/119 (4.8)	T (80)	078 (80)	Metcalf et al. (2011)
				Fresh perch and salmon				
				Cooked shrimp				
Ready-to-eat/raw vegetables	Europe	France	2013	Heard of lettuce	3/104 (2.9)	T (100)	001 (33.3)	Eckert et al. (2013)
				Lambs lettuce salad			014/020/077 (33.3)	
				Peat sprouts			015 (33.3)	
	Canada	Ontario	2008	Ginger	5/111 (4.5)	T (100)	078 (60)	Metcalf et al. (2010b)
				Carrot				
				Eddoes				
Milk	Europe	Austria	2008	Bactofugates	0/50 (0)	–	–	Jöbstl et al. (2010)

[a]Year when the study was conducted or year when the study was published
[b]Main PCR-ribotypes found with standard Cardiff nomenclature
(–) Data not available or not applicable

contamination of the production chain (Kalchayanand et al. 2013).

Regarding the environmental shedding of *C. difficile* in processing facilities, little data is available. In seven hamburger processing plants in Iran, *C. difficile* was detected in 3.5 % (2/56) of swabs taken from the environment. The authors suggested that this environmental contamination might be due to biofilm formation which could facilitate the attachment of spores (Esfandiari et al. 2014b). In contrast, in a further study conducted in three sausage-manufacturing plants, sponge swabs collected from equipment and facilities yielded no *C. difficile* isolates (Harvey et al. 2011b), while meat samples tested positive for the bacterium, indicating meat contamination with *C. difficile* from the intestinal contents of food animals.

The hands of food handers, especially of those who produce ready-to-eat food, are well-known vectors of foodborne pathogens, in most cases due to poor hygiene. However the impact of contamination of *C. difficile* by humans who handle foods without washing their hands has not yet been evaluated. In a previous study investigating the *C. difficile* contamination of foods prepared in-house at a Belgian nursing hom, only 1 out of 188 food samples tested positive for *C. difficile*. This positive sample was recovered from a meal composed of carrot salad, mustard sauce and pork sausage. However, as they were analysed together, contamination could have originated from any of the ingredients or as a result of manipulation (Rodriguez et al. 2015).

7 The Threat of Zoonotic and Foodborne Transmission

The literature of the last decade has presented several hypotheses about *C. difficile* transmission (Bauer and Kuijper 2015). Weese et al. (2002) reported a risk of zoonotic transmission of some animal diseases, including *C. difficile*, especially in small veterinary hospitals. Goorhuis et al. (2008) described PCR-ribotype 078 as frequently encountered in human CDI and in pigs with diarrhoea in The Netherlands. A further study reported that this ribotype was the most prevalent type in pig, cattle and horse species worldwide, and also reported an increase in its prevalence in humans in different countries (Rupnik et al. 2008). Other studies conducted in 2008 (Jhung et al. 2008) and in 2009 (Debast et al. 2009) showed a high degree of similarity between pig and animal *C. difficile* PCR-ribotype 078 toxinotype V strains, suggesting a common origin. Recently, Janezic et al. (2014) showed that the most prevalent *C. difficile* types in humans are also prevalent in different animals from different geographic areas, evidencing the potential for global dissemination of some strains.

In the twenty-first century, the development of different typing methods has allowed genome analysis and the comparison of animal, food and human strains (Griffiths et al. 2010). The first study investigating the phylogeny of *C. difficile* by multilocus sequence typing (MLST) analysis reported that differences between phylogenetic lineages do not correlate with the type of host (human or animal) (Pons 2004). Lemée et al. (2004) studied the genetic relationships and population structures of 72 *C. difficile* isolates from various hosts and geographic sources, including human, dog, horse, cow and rabbit stools. Results obtained in the study showed that animal isolates did not constitute a distinct lineage from human isolates. In subsequent works, the same study group (Lemée et al. 2005; Lemée and Pons 2010) observed that animal isolates were intermixed with human isolates. In the recent years, clade 5 has been largely studied as it contains *C. difficile* PCR-ribotype 078 (Knight et al. 2015a). This type was classically associated with animals, especially pigs (Álvarez-Pérez et al. 2013). However, lately it has been also reported in hospitals (Indra et al. 2015). At present, clade 5 seems to be highly heterogeneous and divergent from the rest of population (Janezic and Rupnik 2015).

Marsh et al. (2010) used multiple-locus variable number tandem repeat analysis (MLVA) to show that toxinotype V (REA group BK) human

and animal isolates were highly related but differentiated. In another study conducted in the Netherlands (Koene et al. 2012), faecal samples from healthy and diarrhoeic animals were compared with human strains isolated from patients with diarrhoea and hospitalised patients. MLVA analysis showed a genotypic correlation between animal and human PCR-ribotype 078, but a distinction between human and animal PCR-ribotypes 012 and 014.

Whole genome sequencing (WGS) has recently been used to study the epidemiology of CDI and the genetics of *C. difficile* (Knight et al. 2015a). One such study investigated the evolutionary relatedness of *C. difficile* PCR-ribotype 078 isolated from humans and pigs (in farms) (Knetsch et al. 2014). Results revealed that farmers and pigs were colonised with identical or nearly identical *C. difficile* clones (with zero or less than two single nucleotide polymorphism differences). These results supported the hypothesis of interspecies transmission between animals and humans; however, the existence of a common contamination source (in the environment) was also possible.

It seems that *C. difficile* occurs as a low-level contaminant in meats and other food products. Therefore foodborne transmission may be responsible for only a small proportion of human CDI cases (Curry et al. 2012). However, other authors have reported no molecular relationship between clinical human and meat isolates and, therefore, that sources other than meat are responsible for CDI (Esfandiari et al. 2014a). At present, the human infectious dose for *C. difficile* is not known (Hoover and Rodriguez-Palacios 2013) and the risk posed by the presence of its spores in meat and other foods is still not clarified. Among healthy people with normal intestinal flora, the ingestion of low quantities of spores may not have major repercussions. However, the consumption of these contaminated foods by vulnerable populations with gastrointestinal perturbations could lead to *C. difficile* colonisation and infection, or can contribute to the asymptomatic *C. difficile* carriage and transmission in the community.

8 Conclusions and Perspectives

Eighty years after its discovery, *C. difficile* continues to be the focus of attention in hospitals and an important topic for many research groups worldwide. Comparisons of strains have revealed that in some regions animals and humans are colonised with identical *C. difficile* clones or these strains cluster in the same lineage. Therefore, it is suggested that *C. difficile* should be considered as a zoonotic pathogen and that animals play an important role as reservoirs of the bacterium.

While many questions remain unanswered, next generation typing techniques must be applied in the future to study the relatedness of strains of human and animal origins. In this context, it will be interesting to assess the presence of *C. difficile* in close related human and animal populations, like pets and their owners or farmers in close contact with their animals. The analysis of the isolates by WGS analysis will definitively confirm the absence of host tropism of certain strains and the zoonotic transmission of the bacterium.

Acknowledgements Our most sincere thanks go to Cate Chapman and Josh Jones for their support in editing the manuscript.

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