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Research Article

The Occurrence of *Clostridium difficile* in Different Animal Species in Egypt

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ABSTRACT

Clostridium difficile is an anaerobic Gram-positive endospore forming bacterium that is the most substantial cause of nosocomial acquired infection in humans. In addition to the nosocomial dissemination, farm and companion animals are a possible source of human *C. difficile* infection either by direct or indirect contact, environmental contamination, or food infection. The present study was conducted to evaluate the prevalence of *C. difficile* and its toxins in different animal species within the veterinary clinics in Egypt. A total of 249 fecal samples including apparently healthy and diseased animals were collected from 60 cows; 26 buffaloes; 24 sheep; 30 goats; 40 horses; 21 dogs and 30 cats. The samples were evaluated for the occurrence of *C. difficile* using culture method. All positive *C. difficile* isolates were screened for the presence of toxin A and B toxin genes. *C. difficile* was detected in both apparently and diseases animals even though in goats *C. difficile* could be detected neither in apparently healthy nor animals showing clinical signs. In addition, no *Clostridium difficile* has been recovered from apparently healthy Buffaloes and cat. Toxigenic *C. difficile* was detected from ten isolates in apparently healthy animals and eleven isolates from diseased animals. All isolates contained tcdB and tcdA. The occurrence of other toxicgenic *C. difficile* strains indicates the public health significance.

Key words: Toxicgenic *C. difficile*, Farm animals, Dogs and cats, Public health, Veterinary clinics, Reservoirs

INTRODUCTION

Clostridium difficile is a spore forming Gram positive strictly anaerobic bacterium that causes hospital and antimicrobial-associated intestinal disease in humans and some animal species ranging from asymptomatic colonization to diarrhea and colitis (Warny *et al*., 2005; McFarland, 2008). *C. difficile* produce two exotoxins A and B and / or a binary toxin, leading to *C. difficile* infection (CDI) symptoms (Clark and Wiselka, 2008; Heinlen and Ballard, 2010). *C. difficile* infection (CDI) predominantly affect elderly persons and may be life threatening (Warny *et al*., 2005). A remarkable change in the epidemiology of CDI has been encountered over the past recent years, with more than double increase in the incidence, severity, and recurrence rates in humans compared to the early 2000s (McFarland, 2008; CDCP 2005; Juneau *et al*., 2013; Indra *et al*., 2009; Zilberberg *et al*., 2008). Additionally, there are increasing reports of community-associated CDI, including disease in young individuals and people with few or no conventional risk factors. Interestingly, community-associated CDI cases were linked with the emergent hypervirulent antimicrobial-resistant strains, called epidemic PCR ribotypes 027 and 078, which present also in livestock (Warny *et al*., 2005; Rupnik, 2007; Jhung *et al*., 2008; CDCP, 2008; Songer *et al*., 2009; Kuntz *et al*., 2011;).

The source of infection for community-associated cases of CDI remains uncertain (Weese *et al*., 2010a; Jhung *et al*., 2008). As *C. difficile* is a ubiquitous bacterium inhabiting the environment and can colonize the intestinal tract of both humans and animals, recent prevalence studies suggested that besides the nosocomial dissemination, farm and companion animals are a potential source of human CDI, either by direct or indirect contact, environmental contamination, or food infection (Avbersek *et al*., 2009; Koene *et al*., 2012; Hoover and Rodriguez-Palacios 2013; Rodriguez-Palacios *et al*., 2013; Wetterwik *et al*., 2013; Álvarez-Pérez *et al*., 2015; Rabold *et al*., 2018).

Since domestic livestock and companion animals frequently tested positive for toxigenic *C. difficile*, even without displaying any clinical signs, it looks probable that *C. difficile* could be zoonotic. Therefore, animals could play an essential role as carriers of the bacterium (Rodriguez *et al*., 2016).

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Therefore, the aim of this study was to determine the prevalence of *C. difficile* and its toxins in different animal species within the veterinary clinics and different localities in Egypt.

MATERIALS AND METHODS

Samples

A total of 249 fecal samples were collected from apparently healthy and diseased animals (60 cows; 26 buffaloes; 24 sheep; 30 goats; 40 horses; 21 dogs; 30 cats) (Table 1) raised in Cairo, Giza, Ismailia and El-fayoum governorates in Egypt, during the period between January 2015 to June 2016.

Table 1: The number of fecal samples collected from apparently healthy and diseased domestic and companion animals

Animal species	Apparently healthy	Diseased animals	Total
Cows	24	36	60
Buffaloes	15	11	26
Sheep	14	28	42
Goat	3	27	30
Horse	40	-	40
Dogs	21	-	21
Cats	13	17	30

All samples were transferred in icebox to the laboratory of the department of Zoonoses, Faculty of Veterinary Medicine, Cairo University, Egypt, with minimum delay for bacteriological examination.

Culturing for *C. difficile*

Fecal samples were cultured for isolation of *C. difficile*, as described in Avbersek *et al*., 2009.

Molecular characterization of isolates

Suspected colonies of *C. difficile* were sub-cultured onto blood agar plates and incubated anaerobically at 37°C/24 h. DNA extraction was extracted by transferring 3-5 colonies into 100 µl of sterile distilled water, heating at 95°C for 3 min and then the suspension was centrifuged at 10000 × g for 5 min. The supernatant was used as a DNA-template.

PCR detection of genes encoding triose phosphate isomerase (tpi), toxin A and B (tcd A and tcd B, respectively) as described by Lemee *et al*. (18); Stubbs *et al*. (19).

Statistical analysis

Infection rates were compared by the $\chi 2$ and Fisher's Exact test. Data analysis was performed using PASW Statistics, Version 18.0 software (SPSS Inc., Chicago, IL, USA). A P-value <0.05 was considered statistically significant.

RESULTS

Infection rates in animals

In cows (n = 60) *C. difficile* was detected in 4.17% (1/24) of apparently healthy cows, and in 5.56% (2/36) of cows showing clinical signs. While in buffaloes (n = 26), *C. difficile* could only be detected in 9.09% (1/11) of animals showing clinical signs (Table 2). In sheep (n = 42) *C. difficile* was detected in 14.29% (2/14) of apparently healthy sheep, and in 14.29% (4/28) of sheep showing clinical signs. While in goats (n = 30), *C. difficile* could be detected neither in apparently healthy nor animals showing clinical signs (Table 2). In apparently healthy horses (n = 40), *C. difficile* could be detected in 10% (4/40) of examined animals (Table 2).

In apparently healthy dogs (n = 21), *C. difficile* was detected in 14.29% (3/21) of examined animals. While in cats (n = 30), *C. difficile* could only be detected in 23.53% (4/17) of animals showing clinical signs (Table 2). The results of molecular characterizing of the ten *C. difficile* isolates from apparently healthy animals and eleven isolates from the diseased one are presented in Table 2. Interestingly, all isolates contained tcdB and tcdA.

The infection rates of *C. difficile* in different animal species didn't differ significantly (P>0.05), suggesting that *C. difficile* recovery was not associated with the animal species.

Table 2: Rate of *C. difficile* infection in the examined apparently healthy and clinically diseased domestic and companion animals (N= 249)

	Apparently healthy animals			Diseased animals		
	Examined samples	Positive cases		Examined samples	Positive cases	
		No.	%		No.	%
Cows	24	1	4.17	36	2	5.56
Buffaloes	15	0	0.00	11	1	9.09
Sheep	14	2	14.29	28	4	14.29
Goat	3	0	0.00	27	0	0.00
Horses	40	4	10.00	-	-	-
Dogs	21	3	14.29	-	-	-
Cats	13	0	0.00	17	4	23.53

Table 3: Molecular characterization *C. difficile* strains isolated from different animals.

	Apparently healthy animals		Diseased animals	
	Examined samples	Toxin typing	Examined samples	Toxin typing
Cows	1	tcdA+ tcdB+	2	tcdA+ tcdB+
Buffaloes	0	-	1	tcdA+ tcdB+
Sheep	2	tcdA+ tcdB+	4	tcdA+ tcdB+
Goat	0	-	0	-
Horses	4	tcdA+ tcdB+	-	-
Dogs	3	tcdA+ tcdB+	-	-
Cats	0	-	4	tcdA+ tcdB+
Total	10	tcdA+ tcdB+	11	tcdA+ tcdB+

DISCUSSION

This study provides insights into the epidemiology of *C. difficile* in both farm and companion animals.

Cows and buffaloes: Low rates of infection with *C. difficile* in apparently healthy and diseased cows were observed (4.17% and 5.56%; respectively), while a 9.09% infection rate in diseased buffaloes was recorded. Dahms *et al*., 2014 stated that the pathogenicity of *C. difficile* for cattle and calves, is still questionable and that diarrhea was found to be associated mostly with toxin producing strains. Rodriguez *et al.*, 2017 isolated toxigenic *C. difficile* from 5.5% to 11.3% of calves and cattle and considered them as persistent reservoirs that probably indirectly disseminate the pathogen to humans, *via* the environment. Rahimi *et al.*, 2014 evaluated meet of different animals for the presence of *C. difficile* and found the highest prevalence was in buffalo meat (9%).

Sheep and goats: Rates of infection with *C. difficile* in apparently healthy and diseased sheep were observed (14.29%), while in goats, no infection with *C. difficile* could be detected. Previous studies showed infection rates ranging from 0 to 18.2% (al Saif and Brazier, 1996; Rieu-Lesme and Fonty, 1999). Recent study reported *C. difficile* colonization in 4.2% to 11.2% of lambs and 0.6% of sheep, of which all *C. difficile* isolates from lambs were positive for tcdA and tcdB (A+, B+) but negative for binary toxin genes (CDT-), while the isolate from sheep was A- B+ CDT+ (Knight and Riley, 2013). Another recent study had detected *C. difficile* in 9.2% of neonatal goats, while none of the adult goats were positive (Avberšek *et al*., 2014). Romano *et al*., 2012 isolated *C. difficile* from 7.5% of goats irrespective to age.

Horses: The rate of infection with *C. difficile* in apparently healthy horses was 10%. Recent studies had considered *C. difficile* as one of the most significant causes of diarrhoea and enterocolitis in horses (Arroyo *et al*. 2006; Weese *et al*. 2006; Uzal *et al*. 2012; Diab *et al*. 2013b). Prevalence studies of *C. difficile* in horses with gastrointestinal disease recorded varied infection rates, ranging between 5% and 63%, while in apparently healthy horses, the reported prevalence varied between 0 and 29% (Diab *et al.* 2013b). Båverud *et al*. 2003 reported up to 44% colonization rate in apparently healthy foals under antibiotic treatment, which is considered the main risk factor leading to CDI in horses (Diab *et al*., 2013b). Similar infection rates (from 4.8 to 11%) were observed in hospitalized horses without clinical signs of *C. difficile* disease (Rodriguez *et al*., 2013).

Dogs: In this study, the rate of infection with *C. difficile* in apparently healthy dogs was 14.29%. Struble *et al*., 1994 reported similar prevalence, in canines visited veterinary hospital in California, as they found that 13.8% of canines with normal stools shed *C. difficile*. Another study done by Weese *et al*., 2010b found that 10 % of dogs in households were colonized by *C. difficile*. Weese *et al.*, 2001 reported no infection rates (0%) with *C. difficile*, but *C. difficile* toxins A, B, or both were present in feces of 7% normal dogs. While, Lefebvre *et al.*, 2006 reported higher infection rates, as they isolated *C. difficile* from feces of 58% apparently healthy dog. Rodriguez-Palacios *et al*., 2013 noted that, within the same geographical area, all dog isolates resembled those found in human hospitals. Other studies (Schneeberg *et al*., 2012; Silva *et al*., 2013; Spigaglia *et al.,* 2015) confirmed that dogs can be healthy carriers of human epidemic PCR-ribotypes *C. difficile* strains.

Cats: There were no infection with *C. difficile* reported in apparently healthy cats (0%). While, the rate of infection with *C. difficile* in diseased cats was 23.53%. These results agree with Madewell *et al.*, 1999 who could not detect *C. difficile* in healthy adult cats, but isolated *C. difficile* from 9.4% of diseased ones, and identified A and B toxins sequences in 34.8% of that infected group. Wei *et al*., 2019 isolated *C. difficile* from 7% of apparently healthy cats.

Our results showed that the toxigenic *C. difficile* is a common member of the fecal flora of companion and farm animals, which indicate that those animals are reservoirs and sources of pathogenic *C. difficile*, and that household, nosocomial and contact transmission of *C. difficile* from animals to humans cannot be denied. Moreover, as *C. difficile* is a spore former, it can survive for unspecified period in the environment and could be ingested by man via various routes.

The presence of *C. difficile* in both apparently healthy and diseased animals of different species suggests that contamination may affect the external environment and may play an important role in the expansion of pathogenic *C. difficile* and also in transmission to humans *via* food.

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