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 diseased 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: Toxigenic *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; Rodríguez-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).
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.

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 χ2 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 tcdA+ tcdB+.

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

<table>
<thead>
<tr>
<th>Animal species</th>
<th>Apparently healthy</th>
<th>Diseased animals</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cows</td>
<td>24</td>
<td>36</td>
<td>60</td>
</tr>
<tr>
<td>Buffaloes</td>
<td>15</td>
<td>11</td>
<td>26</td>
</tr>
<tr>
<td>Sheep</td>
<td>14</td>
<td>28</td>
<td>42</td>
</tr>
<tr>
<td>Goat</td>
<td>3</td>
<td>27</td>
<td>30</td>
</tr>
<tr>
<td>Horse</td>
<td>40</td>
<td>-</td>
<td>40</td>
</tr>
<tr>
<td>Dogs</td>
<td>21</td>
<td>-</td>
<td>21</td>
</tr>
<tr>
<td>Cats</td>
<td>13</td>
<td>17</td>
<td>30</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Examined samples</th>
<th>Positive cases</th>
<th>Examined samples</th>
<th>Positive cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Cows</td>
<td>24</td>
<td>1</td>
<td>4.17</td>
</tr>
<tr>
<td>Buffaloes</td>
<td>15</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Sheep</td>
<td>14</td>
<td>2</td>
<td>14.29</td>
</tr>
<tr>
<td>Goat</td>
<td>3</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Horses</td>
<td>40</td>
<td>4</td>
<td>10.00</td>
</tr>
<tr>
<td>Dogs</td>
<td>21</td>
<td>3</td>
<td>14.29</td>
</tr>
<tr>
<td>Cats</td>
<td>13</td>
<td>0</td>
<td>0.00</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Examined samples</th>
<th>Toxin typing</th>
<th>Examined samples</th>
<th>Toxin typing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cows</td>
<td>tcdA+ tcdB+</td>
<td>2</td>
<td>tcdA+ tcdB+</td>
</tr>
<tr>
<td>Buffaloes</td>
<td>-</td>
<td>1</td>
<td>tcdA+ tcdB+</td>
</tr>
<tr>
<td>Sheep</td>
<td>tcdA+ tcdB+</td>
<td>4</td>
<td>tcdA+ tcdB+</td>
</tr>
<tr>
<td>Goat</td>
<td>-</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Horses</td>
<td>tcdA+ tcdB+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dogs</td>
<td>tcdA+ tcdB+</td>
<td>4</td>
<td>tcdA+ tcdB+</td>
</tr>
<tr>
<td>Cats</td>
<td>-</td>
<td>11</td>
<td>tcdA+ tcdB+</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 tdA and tdB (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). Bäverud 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; Spagaglia 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.

REFERENCES


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