Clostridium difficile is an emerging enteropathogen responsible for pseudomembranous colitis in humans and diarrhoea in several domestic and wild animal species. Despite its known importance, there are few studies about C. difficile polymerase chain reaction (PCR) ribotypes in Brazil and the actual knowledge is restricted to studies on human isolates.

The aim of the study was therefore to compare C. difficile ribotypes in Brazil and the actual knowledge is restricted to studies on human isolates. The aim of the study was therefore to compare C. difficile ribotypes isolated from humans and animals in Brazil. Seventy-six C. difficile strains isolated from humans (n = 25), dogs (n = 23), piglets (n = 12), foals (n = 7), calves (n = 7), one cat, and one manned wolf were distributed into 24 different PCR ribotypes. Among toxigenic strains, PCR ribotypes 014/020 and 106 were the most common, accounting for 14 (18.4%) and eight (10.5%) samples, respectively. Fourteen different PCR ribotypes were detected among human isolates; nine of them have also been identified in at least one animal species. PCR ribotype 027 was not detected, whereas 078 were found only in foals. This data suggests a high diversity of PCR ribotypes in humans and animals in Brazil and support the discussion of C. difficile as a zoonotic pathogen.

Key words: C. difficile - pseudomembranous colitis - zoonosis
PCR ribotype 014/020 was previously reported in three humans with confirmed CDI (Balassiano et al. 2009, Secco et al. 2014) and in free-living coatis (*Nasua nasua*) in an urban park in Brazil (Silva et al. 2014b). At this time, it was again detected in four hospitalised humans with confirmed CDI. Moreover, the PCR ribotype 014/020 was also found in six dogs, three piglets, and in one foal, four of them with CDI, which suggest a high frequency of this PCR ribotype in humans and animals in Brazil. PCR ribotype 014/020 is currently the main cause of CDI in the European community and has been reported in animals in several countries including Germany, Netherlands, United States of America, and Slovenia (Bauer et al. 2011, Janezic et al. 2012, 2014). Thus, the present study corroborates previous reports that showed PCR ribotype 014/020 can colonise a broader range of species and also it is present in different geographic regions, although this type is not recognised as being hypervirulent (Janezic et al. 2014).

Among toxigenic strains, PCR ribotype 106 was the second most frequent in this study. Until 2009, PCR ribotype 106 was reported only in the United Kingdom when then it was identified in one human with CDI (Balassiano et al. 2009) in Brazil and recently detected also in coatis (Silva et al. 2014b). In the present study, 106 has been identified in humans (4 isolates) and dogs (4 isolates), suggesting that this PCR ribotype might be common in humans and also in animals in the country.

The PCR ribotype 009 (also known as 53-like) was the second most common in the present study. This type was found in 13 (17.1%) isolates, one from human and four from different animal species, which corroborates previous studies (Janezic et al. 2012) and suggest that this PCR ribotype, similar to 014/020, is common and might have a high capacity to colonise different species. Among eight isolates from calves, five were classified as PCR ribotype 009. Considering that the strains were isolated from calves between 10-60 days of age from five different farms, the present result suggest a low diversity in this group compared with the other species or with previous studies with strains from calves (Janezic et al. 2012, Koene et al. 2012, Knight et al. 2013). Finally, it is also interesting to note that recent studies have shown that nontoxigenic strains has a potential to prevent CDI in humans and piglets (Songer et al. 2007, Oliveira Junior et al. 2016, Zhang et al. 2015). Considering that the nontoxigenic ribotype 009 has a high capacity to colonise different species, this may be a good candidate for future studies focusing on CDI prevention by colonisation with nontoxigenic strains.

Some PCR ribotypes previously reported in humans in Brazil (Balassiano et al. 2009, 2010, 2011) were not identified in the present study, including PCR ribotypes 038, 133, 135, and 233. Together with the present report, these results suggest a high diversity of PCR ribotypes in humans and animals in Brazil, similar to previously reported in other countries. Moreover, the prevalence of some PCR ribotypes appears to vary in different geographical regions. Also, the present study is the first to report the isolation of *cdtB* in humans in Brazil.

It is remarkable that some common PCR ribotypes in Europe and North America, such as 002, 015, 045, and 150, seem to be rare in Brazil. Recently, some reports suggest the rapid emergence of the epidemic 027 strain in Latin America, with confirmed cases in Costa Rica, Panama, and Chile (Hernández-Rocha et al. 2012, Quesada-Gómez et al. 2012, López-Ureña et al. 2014). Anyway, this *C. difficile* ribotype is still not reported in animals and humans in Brazil. PCR ribotypes 078 has also drawn the attention of researchers for its increased frequency in cases in humans and/or animals (Hensgens et al. 2012). Again in contrast with these findings, 078 were found

**TABLE I**

| Host          | Clinical history                          | Isolates n (%) | Total n (%) |
|---------------|-------------------------------------------|----------------|-------------|
| Humans        | CDI                                       | 20 (26.3)      | 25 (32.9)   |
|               | Diarrhoeic but negative for A/B toxin     | 5 (6.6)        |             |
|               | Not diarrhoeic                            | 16 (21)        |             |
|               |                                          | 5 (6.6)        |             |
| Dogs          | CDI                                       | 2 (2.6)        | 23 (30.3)   |
|               | Diarrhoeic but negative for A/B toxin     | 3 (3.9)        |             |
|               | Not diarrhoeic                            | 1 (1.3)        |             |
| Piglets       | CDI                                       | 8 (10.5)       | 12 (15.8)   |
|               | Diarrhoeic but negative for A/B toxin     | 3 (3.9)        |             |
|               | Not diarrhoeic                            | 1 (1.3)        |             |
| Foal          | CDI                                       | 4 (5.3)        | 7 (9.2)     |
|               | Diarrhoea                                 | 3 (3.9)        |             |
| Calves        | Diarrhoea                                 | 8 (10.5)       | 8 (10.5)    |
| Cat           | Not diarrhoeic                            | 1 (1.3)        | 1 (1.3)     |
| Maned wolf    | Diarrhoeic but negative for A/B toxin     | 1 (1.3)        | 1 (1.3)     |

CDI: *C. difficile* infection.
### TABLE II

| Ribotype       | Total isolates n (%) | Host       | Clinical history (number of isolates) |
|----------------|----------------------|------------|----------------------------------------|
| 001/072        | A′B′CDT              | 3 (3.9)    | Human CDI (2)                          |
|                |                      |            | Piglet Not diarrhoeic (1)              |
| 009            | A B CDT              | 13 (17.1)  | Human Diarrhoea (1)                    |
|                |                      |            | Dog Diarrhoea (3)                      |
|                |                      |            | Cat Not diarrhoeic (2)                 |
|                |                      |            | Calf Diarrhoea (5)                     |
|                |                      |            | Foal Diarrhoea (1)                     |
| 010            | A′B′CDT              | 3 (3.9)    | Human Diarrhoea (1)                    |
|                |                      |            | Dog Diarrhoea (2)                      |
| 011/049        | A′B′CDT              | 1 (1.3)    | Piglet Diarrhoea (1)                   |
| 012            | A′B′CDT              | 3 (3.9)    | Piglet CDI (3)                         |
| 014/020        | A′B′CDT              | 14 (18.4)  | Human CDI (4)                          |
|                |                      |            | Dog CDI (2)                            |
|                |                      |            | Dog Diarrhoea (3)                      |
|                |                      |            | Piglet Not diarrhoeic (1)              |
| 050(CE)        | A′B′CDT              | 1 (1.3)    | Human Diarrhoea (1)                    |
| 053            | A B CDT              | 4 (5.3)    | Dog Diarrhoea (1)                      |
| 078            | A′B′CDT              | 3 (3.9)    | Foal CDI (3)                           |
| 084(CE)        | A′B′CDT              | 1 (1.3)    | Piglet CDI (1)                         |
| 106            | A′B′CDT              | 8 (10.5)   | Human CDI (3)                          |
|                |                      |            | Dog Diarrhoea (3)                      |
|                |                      |            | Piglet Not diarrhoeic (1)              |
| 126            | A′B′CDT              | 2 (2.6)    | Piglet CDI (2)                         |
| 131            | A′B′CDT              | 1 (1.3)    | Human CDI (1)                          |
| 602(CE)        | A′B′CDT              | 1 (1.3)    | Dog Diarrhoea (1)                      |
| SLO002         | A B CDT              | 6 (7.9)    | Human CDI (1)                          |
|                |                      |            | Dog Diarrhoea (4)                      |
| SLO046         | A′B′CDT              | 1 (1.3)    | Piglet CDI (1)                         |
| SLO147         | A′B′CDT              | 2 (2.6)    | Human CDI (1)                          |
|                |                      |            | Foal Diarrhoea (1)                     |
| SLO179         | A B CDT              | 1 (1.3)    | Calf Diarrhoea (1)                     |
| SLO197         | A′B′CDT              | 1 (1.3)    | Human CDI (1)                          |
| SLO198         | A′B′CDT              | 2 (2.6)    | Human CDI (1)                          |
|                |                      |            | Calf Diarrhoea (1)                     |
| SLO199         | A′B′CDT              | 2 (2.6)    | Human CDI (1)                          |
|                |                      |            | Dog Diarrhoea (1)                      |
| SLO224         | A′B′CDT              | 1 (1.3)    | Human CDI (1)                          |
| SLO225         | A′B′CDT              | 1 (1.3)    | Human Diarrhoea (1)                    |
| SLO231         | A′B′CDT              | 1 (1.3)    | Dog Not diarrhoeic (1)                 |

CDI: *C. difficile* infection.
only in three foals (4%) with confirmed CDI. The present study suggests that Brazil has some marked differences in the pattern of \textit{C. difficile} ribotypes in humans and animals compared to those reported in the rest of the world. Anyway, considering the continental dimensions of the country and the limited number of isolates evaluated, these results should be analysed cautiously.

This is the first study to evaluate \textit{C. difficile} strains from domestic animals in Brazil and shows a high diversity of PCR ribotypes, also with a considerable number of ribotypes present in both humans and various domestic animals.

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