TITLE: CHARACTERIZATION OF PATHOGENIC RIBOTYPES OF *Clostridioides difficile* ISOLATED FROM PET DOGS IN THE RIO DE JANEIRO STATE

AUTHORS: Rainha, K.; Fernandes, R. F.; Carneiro, L.G.; Penna, B.; Endres, B.; Alam, J.; Garey, K.W.; Domingues, R. M. C.P.; Ferreira, E. O.

INSTITUTION: Universidade Federal Do Rio De Janeiro, Instituto De Microbiologia Paulo De Góes, Rio De Janeiro, RJ (Av. Carlos Chagas Filho, 373, Bloco I, 2° Andar – Sala 006, Cep 21941-902, Cidade Universitária – RJ, Brazil)

Clostridioides difficile is the major etiologic agent of nosocomial bacterial diarrhoea and pseudomembranous colitis associated with the use of antibiotics. The main virulence factors responsible for the cause enteric disease of *C. difficile*, commonly called CDI (*Clostridioides difficile* Infection), are the cytotoxins (TcdA,TcdB and CDT) released by toxigenic strains. Since *C. difficile* has started to be isolated from animals, a potential zoonotic disease has been suggested after the isolation of ribotypes in common between animals and humans. So, the aim of this study is to characterize phenotypic and genotypic *C. difficile* ribotypes isolated from pet dogs in the Rio de Janeiro state. One hundred and fifty one dog stools samples (from 2 months to 18 years old), solid and/or diarrheic, were select regardless of gender or race distinction. For the identification, approximately 0.5 g of stool was inoculated in a differential medium, and incubated under anaerobiosis at 37°C for at least 7 days. All colonies characteristic of *C. difficile* (resemble broken glass) and gram-positive rods was confirmed by MALDI-TOF MS (BRUKER®) and by the polymerase chain reaction (PCR) using oligonucleotides for the species-specific gene (*tpi*-triose phosphate isomerase). After confirmation, a phenotypic characterization (biofilm formation, motility and antimicrobial susceptibility profile; and a genotypic characterization (PCR, aiming the toxins TcdA, TcdB and CDT, analysis of the PCR-ribotypes, MSLT and WGS) will be performed. In parallel, will be evaluated the gene expression of the toxins, adhesins, flagellar proteins and protein associated with the sporulation process by qPCR. N**ine strains were identified as *C. difficile*. The PCR-ribotype revealed that most of the strains (56%) are the ribotype 106 and are toxigenic (A^+B^+) and belong the ST 42 (MLST), the same ST of the epidemic ribotype, 014/020. Concerning the antibiotic resistance profile, 28.6% strains were resistant to clindamycin (≥ 256µg/mL) and 28.6% strains displayed full resistance to metronidazole (≥ 32 µg/mL). All strains were sensitive to vancomycin, strong biofilm producers and showed great motility. We truly believe that** our results will allow us to evaluate the genetic relationship between ribotypes isolated in dogs and humans, reinforcing the discussion of a possible zoonotic pathway for the CDI.

Keywords: *Clostrioides difficile*, ribotype, dogs, zoonotic

Development agency: CAPES, CNPq, FAPERJ