ISOLATION OF *Clostridium difficile* FROM PUPPIES : A LONGITUDINAL STUDY

E-mail: megarcia@vet.ucm.es

J.L. Blanco[1], S. Alvarez-Perez[2], T. Pelaez[2], M.P. Lanzarot[1], B. Gama[2], C. Harmanus[3], E. Kuijper[3], E. Bouza[2], M.E. Garcia[1]

[1] Veterinary Faculty, Universidad Complutense, 28040 Madrid, Spain.
[2] Hospital General Universitario "Gregorio Marañón", 28007 Madrid, Spain.
[3] Center of Infectious Diseases, Leiden University Medical Center, Leiden, The Netherlands.

INTRODUCTION

Although a relationship between *Clostridium difficile* (CD) and development of canine diarrhea has been suggested in several case reports, most authors agree that, on the basis of current data, a causative link between CD and symptomatic diarrhea has not been established. This could be due, at least in part, to disparate results obtained in studies on the rate of the asymptomatic carriership status of dogs for CD, which, depending on the studied population, have reported prevalence rates even ≥50%. Additionally, longitudinal studies of CD presence in dogs are still lacking. The aim of this study was to assess the incidence of CD in puppies from the same litter at different ages and the length of CD shedding in feces.

MATERIAL AND METHODS

Nine Black Labrador Retriever puppies (5 males and 4 females) from the same litter were sampled weekly from parturition to day 50 postpartum, resulting in a total of 63 fecal samples. All samples were plated on solid selective medium (CLO agar, bioMérieux) after ethanol shock (70%, v/v) and growth in selective broth for enrichment. Recovered isolates were ribotyped and further characterised by standard procedures.

RESULTS

Of 63 samples, 24 (38.1%) yielded CD growth. In general, CD was recovered from samples up to day 29 postpartum, but differences on bacterial recovery were detected between age groups. CD incidence was 33.3% (3/9) in 7-day-animals, but 100% (9/9) at day 14. Fecal samples from from ≥30-day-old puppies never yielded CD colonies. 15 CD isolates were ribotyped, and belonged to PCR ribotype 056 and harboured the genes encoding for toxins A and B, but no binary toxin loci were detected. In addition, most isolates (87.5 %) showed in vitro resistance to daptomycin (MIC_{50} = 2 µg/ml, MIC_{90} = 3 µg/ml), but were sensitive to linezolid (MIC_{90} = 2 µg/ml) , metronidazole (MIC_{90} = 0.38 µg/ml), moxifloxacin (MIC_{90} = 1.5 µg/ml) , rifampicin (MIC_{90} ≤ 0.002 µg/ml), teicoplanin (MIC_{90} = 0.250 µg/ml), tigecycline (MIC_{90} = 0.064 µg/ml) and vancomycin (MIC_{90} = 1 µg/ml).

Table 1: Isolation of *Clostridium difficile* from puppies.

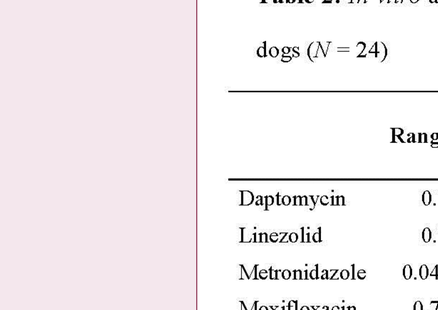
	Puppies								
Age	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
7 days		**+** **056**			+	+			
14 days	**+** **056**	**+** **056**	+	**+** **056**	**+** **056**	**+** **056**	**+** **056**	**+** **056**	**+** **056**
22 days	+	+			**+** **056**		**+** **056**	**+** **056**	**+** **056**
29 days		+	+	+	+	**+** **056**			**+** **056**
36 days									
43 days									
50 days									

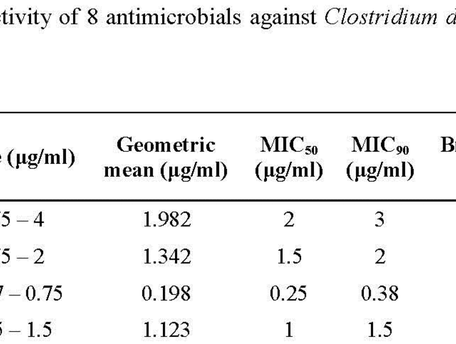
+ : Isolated by direct plating and enrichment in liquid medium.
+ : Isolated only by enrichment in liquid medium.
056: Ribotype

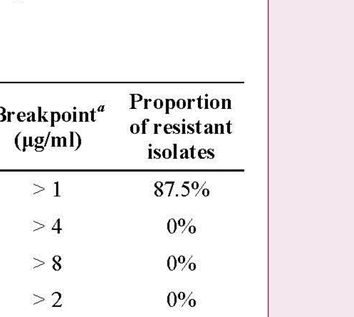
Table 2: *In vitro* activity of 8 antimicrobials against *Clostridium difficile* isolates from dogs (N = 24)

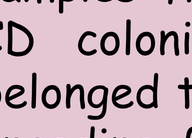
	Range (µg/ml)	Geometric mean (µg/ml)	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)	Breakpoint[a] (µg/ml)	Proportion of resistant isolates
Daptomycin	0.75 – 4	1.982	2	3	> 1	87.5%
Linezolid	0.75 – 2	1.342	1.5	2	> 4	0%
Metronidazole	0.047 – 0.75	0.198	0.25	0.38	> 8	0%
Moxifloxacin	0.75 – 1.5	1.123	1	1.5	> 2	0%
Rifampicin	≤ 0.002 – 0.002	0.002	0.002	0.002	> 0.5	0%
Teicoplanin	0.125 – 0.38	0.159	0.125	0.250	> 2	0%
Tigecycline	0.032 – 0.094	0.049	0.047	0.064	> 0.5	0%
Vancomycin	0.25 – 1.5	0.759	0.75	1	> 2	0%

[a] The only breakpoints for resistance established by the CLSI for anaerobic bacteria are those of metronidazole, clindamycin, moxifloxacin, meropenem, and ertapenem. The remaining breakpoints were based on CLSI recommendations for aerobic bacteria and other sources.

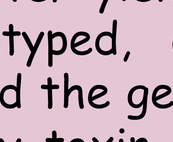








CONCLUSIONS



We conclude that puppies carry asymptomatically toxinogenic *C. difficile* PCR ribotype 056 up to 30 days postpartum. Interestingly, CD ribotype 056, which has also been isolated from other animal species and environmental sources, has been implicated in severe cases of human CD-associated disease [1].

REFERENCES

[1] Bauer et al. 2011. Lancet, 377: 63-73.

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