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FIRST DETECTION OF CANINE PARVOVIRUS TYPE 2C IN BRAZIL

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ABSTRACT

The presence of canine parvovirus type 2 (CPV-2), 2a and 2b has been described in Brazil, however, the type 2c had not been reported until now. In the current study, seven out of nine samples from dogs with diarrhea were characterized as CPV-2c, indicating that this virus is already circulating in the Brazilian canine population.

Key words: canine parvovirus type 2c, sequence analysis, Brazil.

The canine parvovirus type 2 (CPV-2) emerged as a novel pathogen in the late 70`s (1) and rapidly spread worldwide. Within few years, the virus underwent a rapid evolution and, new antigenic types, termed CPV-2a and CPV-2b (16, 17), completely replaced the original type 2 (21, 24). In 2001, an antigenic variant was reported in Italy (3, 4). That mutant virus has an amino acid substitution (Asp-426 to Glu-426) in a residue of the capsid protein that is considered antigenically important. This mutant has also been detected in several countries (9, 15, 20, 26) and, recently, it was detected in Uruguay (20). In the last years, serological studies performed in Brazil, Rio Grande do Sul State, indicated that CPV frequently infects the canine population (10, 27). However, until now, the CPV-2c has not been reported in Brazil yet.

The emergency and spread of CPV-2c is considered a sanitary threat worldwide. The monitoring of CPV field isolates has been fundamental to understand the virus epidemiology and to develop preventive measures (20). The present communication describes the first record of the CPV-2c detection in Brazil.

Twenty faecal samples from vaccinated and unvaccinated dogs (one to six months of age) with diarrhea were collected from January to July 2008 in private and public (Hospital de Clínicas Veterinárias) animal Hospitals located in Porto Alegre, Brazil (Table 1). Samples were

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Table 1. Nucleotide and amino acid differences in the VP2 sequence and characteristics of their hosts of the CPV-2 strains analyzed in the present study with other CPV-2 isolates from Brazil (DQ340409 and DQ340434) and Uruguay (EF375479).

Accession number	Breed	Sex	Age	Vaccination program[a]	CPV Type	Codon position and amino acid					
						426	430 (Leu)	452 (Gly)	491 (Gln)	546 (Asn)	555 (Val)
EF375479	Short terrier	NA	NA	Complete	2c	GAA (Glu)	TTG	GGT	CAA	AAT	GTA
DQ340409	NA	NA	NA	NA	2b	GAT (Asp)					
DQ340434	NA	NA	NA	NA	2a	AAT (Asn)					
EU797726[b]	Mongrel	♂	5 m	None	2c					C	
EU797727[b]	German shepherd	♀	4 m	Complete	2c					C	
EU797728[b]	Mongrel	♀	NA	None	2c					C	
FJ236063[b]	German shepherd	♀	3 m	Incomplete	2c					C	
FJ236064[b]	Pinscher	♀	2 m	Incomplete	2c					C	
FJ236065[b]	NA[c]	NA	2 m	NA	2c					C	
FJ236066[b]	NA	NA	2 m	NA	2c					C	
FJ236067[b]	NA	NA	6 m	NA	2b	GAT (Asp)					
FJ236068[b]	NA	♀	NA	NA	2b	GAT (Asp)					

[a] Complete vaccination program was considerered at least three doses of CPV vaccine.

[b] This study.

[c] Not available.

homogenized (20%, w/v) in PBS (pH 7.4) frozen and defrozen three times and subsequently clarified by centrifuging at 1000 x *g* for 10 min. DNA was extracted from the supernatants using a protocol based on silica (2). PCR was carried out according previous study (3), using primer pair 555for/555rev that amplifies a 583 base pairs (bp) fragment of the VP2 gene (position 4003 to 4585). The positive PCR products were purified with GFX DNA and Gel Band Purification (Amersham Bioscience, USA) and sequenced by using an ABI-PRISM 3100 Genetic Analyzer (Applied Biosystems, USA).

Nucleotide sequences were submitted to GenBank database (http://www.ncbi.nlm.nih.gov) and their accession numbers are displayed in Table 1. Alignments and sequence analysis were performed using Bioedit software package, version7.0.1(http://www.mbio.ncsu.edu/BioEdit/bioedit.html) For sequence comparison, the nucleotide sequence of CPV-2a (DQ340434 and EF375482), CPV-2b (DQ340409) and CPV-2c (EF375479, EF375480, EF375481, AY742942 and AY380577) were retrieved from GenBank. Nucleotide and amino acid positions in this study are referred to strain CPV-2b (accession number M38245).

A single band of the expected size (583 pb) was observed in nine out of the 20 field samples. Sequencing of the amplification products revealed that all had the same nucleotide identity, with exception of two nucleotide sites (Table 1). Seven samples had the presence of a GAA codon at position 426 of the VP protein. This GAA codes for Glutamate, what characterizes the type 2c. The sequence alignment of the Brazilian 2c strains displayed high homology with the 2a strains (99.2% to 99.4%), 2b (99.4%) and 2c (99.6% to 99.8%). One distinct nucleotide in the position 4424 (codon 546) was displayed in the seven 2c strains from Brazil but not in the strains from other countries. This mutation (T to C) corresponds to a transition in the third position of the codon that did not change the amino acid sequence. The other two strains were identified as type 2b and exhibited 100% nucleotide identity with one 2b strain previously described in Brazil (18).

In the present study, seven out of the nine samples were type 2c, indicating that this type was already introduced in Brazil. In the last years, authors from many countries analyzed CPV samples to determine the circulating types, finding a high frequency of the type 2c (13, 14, 28). One study performed in Uruguay found that 24 out of the 25 CPV strains were type 2c (20). Probably, this new viral type could have some adaptative advantage that leads it to replace the types 2a and 2b (11, 24).

Previously, the canine parvovirus types 2a and 2b had completely replaced the type 2 worldwide. CPV appeared in Brazil around the 80's and this type change was also observed in previous studies (6, 18, 19). The 2c samples analyzed in the present study presented the nucleotide C instead of T in the codon 526. This single nucleotide polymorphism was not displayed in previously reported strains and indicates that, probably, the Brazilian CPV-2c had an independent origin. However, more samples from other geographic regions of the country are necessary to have a more representative figure of the situation.

All the puppies sampled had clinical signs of gastro-enteritis. Differences in clinical signs induced by distinct viral types have been reported (5, 20). The efficiency of the current CPV-2 vaccines against this mutant is another question that must be addressed. There is only one report showing a complete protection by an attenuated vaccine based in other CPV type against challenge by the 2c type (25). On the other hand, there are several works reporting vaccine failures when the challenge virus is from the type 2c

(7, 8, 20). Also, in the present study we cannot discuss vaccination failure because in nine positive cases only one puppy had completed the vaccination protocol.

This is the first report of the presence of CPV-2c in Brazil. The present study aims to warm the veterinarians about these new threat and possible changes among parvovirus pathology and protection afforded by the current vaccines.

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RESUMO

Detecção de parvovírus canino tipo 2c no Brasil

No Brasil, a presença do parvovírus canino do tipo 2 (CPV-2), 2a e 2b já havia sido
descrita, contudo, ainda não havia sido verificada a presença do tipo 2c. No presente trabalho, sete de nove amostras de cães com diarréia foram caracterizadas como CPV-2c, indicando que este vírus já está circulando na população canina no Brasil.

Palavras-chave: parvovírus canino tipo 2c, análise se seqüências, Brasil.

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