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Molecular characterization of canine parvovirus strains in Argentina: Detection of the pathogenic variant CPV2c in vaccinated dogs

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PCR amplification with sequence-specific primers was used to detect canine parvovirus (CPV) DNA in 38 rectal swabs from Argentine domestic dogs with symptoms compatible with parvovirus disease. Twenty-seven out of 38 samples analyzed were CPV positive. The classical CPV2 strain was not detected in any of the samples, but nine samples were identified as CPV2a variant and 18 samples as CPV2b variant. Further sequence analysis revealed a mutation at amino acid 426 of the VP2 gene (Asp426Glu), characteristic of the CPV2c variant, in 14 out of 18 of the samples identified initially by PCR as CPV2b. The appearance of CPV2c variant in Argentina might be dated at least to the year 2003. Three different pathogenic CPV variants circulating currently in the Argentine domestic dog population were identified, with CPV2c being the only variant affecting vaccinated and unvaccinated dogs during the year 2008.

1. Introduction

Canine parvovirus (CPV) emerged in 1978 as the etiological agent of an epizootic severe gastroenteritis of dogs characterized by depression, loss of appetite, vomiting, diarrhea (mucoid or hemorrhagic) and leucopenia (Kelly, 1978; Appel et al., 1979; Burtonboy et al., 1979; Decaro et al., 2005a). The virus was referred as CPV2 to distinguish it from the antigenically unrelated minute virus of canines (MVC or CPV1), which is responsible for neonatal death in pups (Binn et al., 1970; Carmichael et al., 1994; Parrish, 1999).

CPV2 belongs to the feline parvovirus subgroup of the genus *Parvovirus* (Decaro et al., 2005a). The origin of CPV2 is still unknown, although its derivation from feline panleukopenia virus (FPV) or from FPV-like viruses of wild carnivores has been hypothesized (Truyen et al., 1998; Truyen, 1999).

CPV is a small (26 nm-diameter), non-enveloped virus carrying a single stranded DNA genome of approximately 5200 nucleotides (Nakamura et al., 2004). The viral genome is enclosed into an icosahedral capsid made up of a combination of two proteins, VP1 and VP2, which are translated from alternatively spliced mRNAs (Martella et al., 2004).

A few years after its emergence, two new antigenic variants, named CPV2a and CPV2b, were characterized (Parrish et al., 1985, 1991; Decaro et al., 2005b). At present, the original CPV2 is not circulating in dog populations, although it is still present in vaccine formulations (Parrish et al., 1991; Martella et al., 2005a; Decaro et al., 2006b), whereas the variants CPV2a and CPV2b are distributed worldwide (Mochizuki et al., 1993; De Ybanez et al., 1995; Truyen et al., 1996; Greenwood et al., 1996; Sagazio et al., 1998; Steinel et al., 1998; Buonavoglia et al., 2000; Pereira et al., 2000; Truyen et al., 2000; Buonavoglia et al., 2001; Martella et al., 2004, 2005b; Decaro et al., 2006b).

The antigenic types CPV2a and 2b differ from the original CPV2 in at least five or six amino acids (aa) of the VP2 capsid protein. Mutations affecting important residues of the capsid protein VP2 of CPV, such as residues 297, 300, 426 and 555 have been recognized recently. This is the case of a new CPV2a variant carrying a Val at the 555 position, which can be differentiated from the CPV2b variants only by the presence of a substitution (Asn426Asp) in the major antigenic site of the VP2 protein (Martella et al., 2006).

A new antigenic variant, carrying the aa substitution Asp426Glu, located at the major antigenic region over the 3-fold spike of the CPV capsid, was reported in Italy in the year 2001 (Buonavoglia et al., 2001; Martella et al., 2004, 2006; Decaro et al., 2005b; Desario et al., 2005). This new mutant, designated CPV2c, has been detected later in Vietnam (Nakamura et al., 2004), Spain (Decaro et al., 2006b), USA (Hong et al., 2007), Portugal, Germany, the United

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Kingdom (Decaro et al., 2007) and recently in Uruguay (Perez et al., 2007).

Antigenic and genetic analysis of the CPV2 variants isolated since the year 2002 have revealed that the CPV2c is currently replacing the CPV2b variant in the Italian dog population (Decaro et al., 2005a,c). Clinical symptoms produced by infection with the viral variant CPV2c are somewhat different from those caused by the CPV2a/b variants (i.e., mucoid instead of hemorrhagic diarrhea) (Decaro et al., 2005a). This may complicate the diagnosis of the disease, increasing the need to use serological and/or molecular techniques in order to assess accurately the real incidence of CPV2c in dog populations.

In this study, a rapid, sensitive and specific PCR method was used for the detection of CPV DNA in clinical specimens from Argentine dogs showing symptoms compatible with CPV disease.

2. Materials and methods

2.1. Clinical specimens

A total of 38 rectal swabs samples were obtained from domestic dogs from Buenos Aires city and from Tandil, Río Negro, Bahía Blanca and Mar del Plata cities (located at 350, 1500, 690 and 400 km from Buenos Aires, respectively). Clinical specimens were submitted to the laboratory for diagnostic purposes between the years 2002 and 2008. Information about the animals with positive test samples for CPV, such as clinical symptoms, age, gender, breed and vaccination status, are shown in Table 1. Two commercial CPV vaccines (Vanguard® Plus CPV, Pfizer, and Duramune® Max 5, Fort Dodge, USA) were also tested as positive controls.

2.2. Preparation of samples for PCR

CPV genomic DNA was extracted directly from rectal swabs and from commercial vaccines in a lysis buffer containing 50 mM Tris–HCl pH 8, 100 mM NaCl, 25 mM sucrose, 10 mM EDTA and 1% SDS. After lysis, the extracts were digested with proteinase K (Invitrogen®, USA) at 56 °C for 30 min and the DNA was extracted with phenol–chloroform.

2.3. Primers and PCR amplification

Three different sets of primers, whose sequences had been selected from variable regions of the gene coding for VP2 capsid protein, were used. The primer pairs P2 (which detects CPV2) and Pb (which detects the CPV2b variant) were designed by Pereira et al. (Pereira et al., 2000). The primers sequences were as follows: P2 sense, 5′-GAAGAGTGGTTGTAAATAATA-3′; P2 anti-sense, 5′-CCTATATCACCAAAGTTAGTAG-3′; Pb sense, 5′-CTTTAACC-TTCCTGTAACAG-3′, and Pb anti-sense, 5′-CATAGTTAAATTGGTT-ATCTAC-3′. P2 primers were used to amplify the genomic region between nucleotides 3025 and 3706, while Pb primers amplified the region between nucleotides 4043 and 4470, giving rise to products of 681 and 427 bp, respectively. It is worth mentioning, that the new CPV2a strains, with a Val-555, may be amplified eventually with the Pb primers. The third primer pair, Pab (Pab sense, 5′-GAAGAGTGGTTGTAAATAATT-3′ and Pab anti-sense 5′-CCTATATAACCAAAGTTAGTAC-3′), which detects CPV2a and CPV2b variants, has been reported previously by Senda et al. (Senda et al., 1995).

The difference in the nucleotide sequence between P2 and Pab primer pairs is restricted to one base in the 3′ end of each primer, which is essential for polymerase initiation in PCR.

PCR amplification was performed using Taq recombinant polymerase (Invitrogen, USA) in an MJ Research cycler (PTC-100, Sierra Point, CA), using an initial denaturation step at 95 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min, and extension at 72 °C for 1 min. A final extension step was performed at 72 °C for 5 min.

Table 1
Age, gender, vaccination records and clinical signs of dogs infected with CPV.

Strain	Year	Age (month)	Sex	Breed	Vaccination status	Clinical signs	CPV strain	Procedence
Arg 1	2002	NA	NA	R	NA	NA	CPV2a	NA
Arg 2	2003	4	F	D	V (C)	+	CPV2a	Buenos Aires
Arg 3	2003	6	M	R	NV	+	CPV2b	Buenos Aires
Arg 4	2003	4	M	LR	V (I)	+	CPV2a	Buenos Aires
Arg 5	2003	135	M	NA	V (C)	+	CPV2b	Buenos Aires
Arg 6	2003	5	M	MB	V (C)	+	CPV2a	Buenos Aires
Arg 7	2003	NA	F	MB	V (NA)	+	CPV2a	Buenos Aires
Arg 8	2003	6	F	C	V (C)	+	CPV2c	Buenos Aires
Arg 9	2003	5	M	MB	NV	+	CPV2a	Buenos Aires
Arg 10	2003	4	F	R	V (C)	+	CPV2b	Buenos Aires
Arg 11	2003	4	F	MB	V (C)	+	CPV2a	Buenos Aires
Arg 12	2005	NA	M	AD	NA	NA	CPV2c	Buenos Aires
Arg 13	2005	NA	NA	NA	NV	NA	CPV2c	Buenos Aires
Arg 14	2007	1	M	ST	V (C)	+	CPV2c	Bahía Blanca
Arg 15	2007	NA	M	SFT	V (NA)	+	CPV2c	Bahía Blanca
Arg 16	2007	3	M	YT	V (C)	NA	CPV2b	Mar del Plata
Arg 17	2007	3	F	P	V (C)	NA	CPV2a	Mar del Plata
Arg 18	2007	2	M	MP	V (C)	+	CPV2a	Mar del Plata
Arg 19	2008	2	M	R	V (C)	+	CPV2c	Buenos Aires
Arg 20	2008	2	NA	AD	V (C)	+	CPV2c	Bahía Blanca
Arg 21	2008	2	NA	AD	V (C)	+	CPV2c	Bahía Blanca
Arg 22	2008	2	NA	AD	V (C)	+	CPV2c	Bahía Blanca
Arg 23	2008	2	NA	AD	V (C)	+	CPV2c	Bahía Blanca
Arg 24	2008	5	M	MB	V (C)	+	CPV2c	Buenos Aires
Arg 25	2008	2	F	MS	V (C)	+	CPV2c	Tandil
Arg 26	2008	2	M	GS	V (C)	+	CPV2c	Río Negro
Arg 27	2008	2	M	GS	V (C)	+	CPV2c	Río Negro

F: female; M: male; MB: mixed breed; LR: Labrador Retriever; GS: German Shepherd; R: Rottweiler; D: Doberman; AD: Argentine Dogo; MS: Miniature Schnauzer; ST: Skye Terrier; YT: Yorkshire Terrier; SFT: Smooth Fox Terrier; C: Cocker; P: Poodle; MP: Miniature Poodle; V: vaccinated; NV: non-vaccinated; C: complete vaccination according to its age; I: incomplete vaccination; NA: no information available; Y: yes.