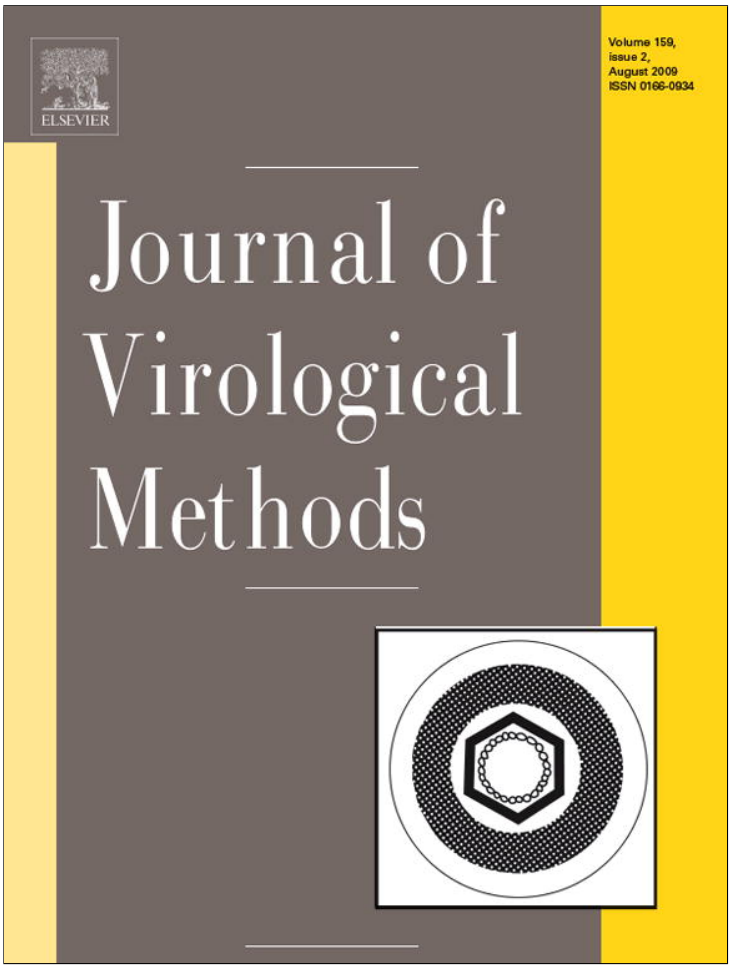

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Molecular characterization of canine parvovirus strains in Argentina: Detection of the pathogenic variant CPV2c in vaccinated dogs

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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 antisense, 5′-CCTATATCACCAAAGTTAGTAG-3′; Pb sense, 5′-CTTTAACCTTCCTGTAACAG-3′, and Pb anti-sense, 5′-CATAGTTAAATTGGTTATCTAC-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.

The final concentration of $MgCl_2$ was 1.5 mM in all cases. Each clinical sample was typed in three independent PCR reactions using the primer pairs P2, Pab and Pb.

2.4. Cloning and sequencing of the amplified DNA

The amplified DNA fragments obtained with the Pab primer pair from two clinical samples (Arg 3 and Arg 4) and with the Pb primer pair from 18 clinical samples (Arg 3, 5, 8, 10, 12 to 16, and 19–27) were cloned into the pGEM-T Easy vector (Promega, Madison, WI). The same procedure was followed with the DNA fragments obtained by amplification with the P2 primer pair from the commercial vaccines Vanguard® Plus CPV, Pfizer (Vac1), and Duramune® Max 5, Fort Dodge (Vac2).

For each DNA fragment, two recombinant plasmids were sequenced in both directions by the dideoxy-mediated chain-termination method (Macrogen Inc, Korea).

Sequence analysis was performed using ClustalW. The GenBank accession numbers of the aa and nucleotide sequences of reference CPV strains are as follows: CPV-b variant (CPV2, M38245); CPV-15 variant (CPV2a, M24003); CPV-39 variant (CPV2b, M74849). The GenBank accession numbers of the nucleotide sequences of the DNA fragments amplified from local variants with the Pab primer pair are: FJ349323 (Arg3) and FJ349324 (Arg4). The GenBank accession numbers of the nucleotide sequences of the DNA fragments amplified from local variants with the Pb primer pair are: FJ349305 (Arg 3), FJ349306 (Arg 5), FJ349307 (Arg 8), FJ349308 (Arg 10), FJ349309 (Arg12), FJ349310 (Arg13), FJ349311 (Arg14), FJ349312 (Arg15), FJ349313 (Arg 16), FJ349314 (Arg 19), FJ349315 (Arg 20), FJ349316 (Arg 21), FJ349317 (Arg 22), FJ349318 (Arg 23), FJ349319 (Arg 24), FJ349320 (Arg 25), FJ349321 (Arg 26), FJ349322 (Arg 27).

3. Results

3.1. CPV detection by PCR

During this study, 38 rectal swabs samples from animals showing symptoms compatible with CPV disease were tested. CPV DNA was detected in 27 of 38 (71%) rectal swabs, and in both commercial vaccine samples. Negative samples were assayed twice to confirm the results.

As shown in Table 1, 22 of the 27 positive clinical specimens (81.5%) were obtained from dogs which had been vaccinated against CPV at least once, with different brands of vaccines. Three samples were obtained from unvaccinated dogs, and the vaccination status of the remaining two animals was unknown. Nineteen of the 22 vaccinated dogs had completed the vaccination schedule with classical type 2-based vaccines.

Twenty-two of the 27 CPV positive animals (81.5%) presented gastroenteritis, while no clinical data were available for the other five dogs.

3.2. Identification of CPV variants by PCR

In order to identify and differentiate CPV variants in local clinical samples, PCR was undertaken using differential primers P2, Pab and Pb. None of the 27 CPV positive samples was amplified with the primer pair P2. This result is coincident with the observation that CPV2 strain is not circulating at present in the dog population (Decaro et al., 2006b). The DNA obtained from the commercial vaccines was amplified with P2, Pab and Pb primer pairs (data not shown). However, sequencing of the amplified DNA fragments showed the presence of only the CPV2 strain (Fig. 1, see discussion).

```
                                  101
Vac1                RVVVNNMDKTAVNGNMALDDIHAQIVTPWSLVDANAWGVWFNPGDWQLIVNTMSELHLVS 60
Vac2                RVVVNNMDKTAVNGNMALDDIHAQIVTPWSLVDANAWGVWFNPGDWQLIVNTMSELHLVS 60
CPV-bCPV2           RVVVNNMDKTAVNGNMALDDIHAQIVTPWSLVDANAWGVWFNPGDWQLIVNTMSELHLVS 60
CPV-15CPV2a         RVVVNNLDKTAVNGNMALDDTHAQIVTPWSLVDANAWGVWFNPGDWQLIVNTMSELHLVS 60
CPV-39CPV2b         RVVVNNLDKTAVNGNMALDDTHAQIVTPWSLVDANAWGVWFNPGDWQLIVNTMSELHLVS 60
Arg 3-CPV2b         RVVVNNLDKTAVNGNMALDDTHAQIVTPWSLVDANAWGVWFNPGDWQLIVNTMSELHLVS 60
Arg 4-CPV2a         RVVVNNLDKTAVNGNMALDDTHAQIVTPWSLVDANAWGVWFNPGDWQLIVNTMSELHLVS 60
                    ******:************ ************************************

Vac1                FEQEIFNVVLKTVSESATQPPTKVYNNDLTASLMVALDSNNTMPFTPAAMRSETLGFYPW 120
Vac2                FEQEIFNVVLKTVSESATQPPTKVYNNDLTASLMVALDSNNTMPFTPAAMRSETLGFYPW 120
CPV-bCPV2           FEQEIFNVVLKTVSESATQPPTKVYNNDLTASLMVALDSNNTMPFTPAAMRSETLGFYPW 120
CPV-15CPV2a         FEQEIFNVVLKTVSESATQPPTKVYNNDLTASLMVALDSNNTMPFTPAAMRSETLGFYPW 120
CPV-39CPV2b         FEQEIFNVVLKTVSESATQPPTKVYNNDLTASLMVALDSNNTMPFTPAAMRSETLGFYPW 120
Arg 3-CPV2b         FEQEIFNVVLKTVSESATQPPTKVYNNDLTASLMVALDSNNTMPFTPAAMRSETLGFYPW 120
Arg 4-CPV2a         FEQEIFNVVLKTVSESATQPPTKVYNNDLTASLMVALDSNNTMPFTPAAMRSETLGFYPW 120
                    ************************************************************

Vac1                KPTIPTPWRYYFQWDRTLIPSHTGTSGTPTNIYHGTDPDDVQFYTIENSVPVHLLRTGDE 180
Vac2                KPTIPTPWRYYFQWDRTLIPSHTGTSGTPTNIYHGTDPDDVQFYTIENSVPVHLLRTGDE 180
CPV-bCPV2           KPTIPTPWRYYFQWDRTLIPSHTGTSGTPTNIYHGTDPDDVQFYTIENSVPVHLLRTGDE 180
CPV-15CPV2a         KPTIPTPWRYYFQWDRTLIPSHTGTSGTPTNIYHGTDPDDVQFYTIENSVPVHLLRTGDE 180
CPV-39CPV2b         KPTIPTPWRYYFQWDRTLIPSHTGTSGTPTNIYHGTDPDDVQFYTIENSVPVHLLRTGDE 180
Arg 3-CPV2b         KPTIPTPWRYYFQWDRTLIPSHTGTSGTPTNIYHGTDPDDVQFYTIENSVPVHLLRTGDE 180
Arg 4-CPV2a         KPTIPTPWRYYFQWDRTLIPSHTGTSGTPTNIYHGTDPDDVQFYTIENSVPVHLLRTGDE 180
                    ************************************************************
                                                    297 300 305
Vac1                FATGTFFFDCKPCRLTHTWQTNRALGLPPFLNSLPQSEGATNFGDI 226
Vac2                FATGTFFFDCKPCRLTHTWQTNRALGLPPFLNSLPQSEGATNFGDI 226
CPV-bCPV2           FATGTFFFDCKPCRLTHTWQTNRALGLPPFLNSLPQSEGATNFGDI 226
CPV-15CPV2a         FATGTFFFDCKPCRLTHTWQTNRALGLPPFLNSLPQSEGGTNFGYI 226
CPV-39CPV2b         FATGTFFFDCKPCRLTHTWQTNRALGLPPFLNSLPQSEGGTNFGYI 226
Arg 3-CPV2b         FATGTFFFDCKPCRLTHTWQTNRALGLPPFLNSLPQNEGGTNFGYI 226
Arg 4-CPV2a         FATGTFFFDCKPCRLTHTWQTNRALGLPPFLNSLPQNEGGTNFGYI 226
                    ***********************************.**.**** *
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Fig. 1. Alignment of the deduced amino acid sequences of the DNA fragments amplified by PCR from commercial vaccines and clinical samples, using P2 and Pab primer pairs, respectively. The sequences of two local field variants, one identified as CPV2b (Arg 3) and the other as CPV2a (Arg 4), and two commercial vaccines strains (Vac1 and Vac2) are shown aligned with sequences of reference strains CPV-b (CPV2); CPV-15 (CPV2a) and CPV-39 (CPV2b) obtained from the GenBank. Sequence accession numbers are described in Section 2. Amino acid positions 101, 300 and 305 are grey shaded, as well as the unique mutation S297N of Argentine CPV variants.

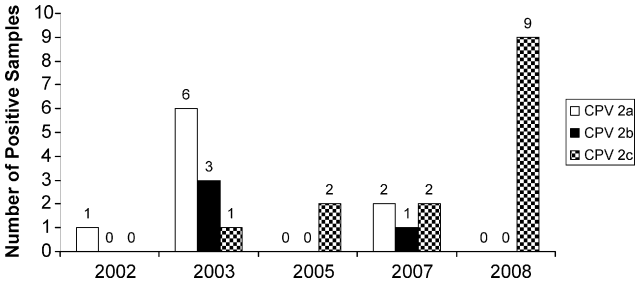

Fig. 2. Evolution of CPV variants in Argentina. The number of cases detected in the years 2002–2008 belonging to each CPV type are shown on top of the bars.

According to the results of the PCR amplification, nine of the 27 CPV positive samples were identified as CPV2a, whereas 18 were identified initially as CPV2b.

3.3. Sequencing of the fragments amplified with primer pairs P2 and Pab

The DNA fragments amplified with P2 and Pab primer pairs from the commercial vaccines and field variants, respectively, were sequenced subsequently. The deduced aa sequences (226 aa) were aligned with those of the reference CPV2 strain and CPV2a and CPV2b variants, using the Clustal W program. The sequence alignments showed a complete identity among the vaccine strains (Vac1, Vac2 and CPV-b), indicating that they were classical, type 2-based vaccines (Fig. 1). On the other hand, field variants Arg 3 and Arg 4 showed a replacement Ser297Asn, which was unique for the local types, and the expected residues for CPV2a/b variants at positions 101, 300 and 305.

3.4. Identification of CPV2c variants

The sequencing results showed that 14 out of 18 (77.7%) DNA fragments amplified from local field variants with the Pb primer pair, presented the Asp426Glu mutation, characteristic of the CPV2c variants. The remaining four samples were confirmed to be genuine CPV2b variants (Table 1). These results demonstrated the presence in the Argentine domestic dog population of the variant CPV2c, reported previously in other regions of the world. The 14 Argentine CPV2c variants had a 100% aa identity within the sequenced fragment.

Briefly, nine of the 27 CPV positive samples (33.4%) were identified as CPV2a, 4 as CPV2b (14.8%) and 14 as CPV2c (51.8%). CPV2c biotype became prevalent in the year 2008 (Fig. 2).

4. Discussion

During the years 2007 and 2008, an increasing number of cases of sick dogs showing symptoms compatible with CPV, including vaccinated animals, were reported in Argentina. This situation raised concerns among breeders, owners and veterinary practitioners about the ability of the current vaccines to protect theirs pups, as noted previously in other countries (Decaro et al., 2008; Lamm and Rezabek, 2008).

Therefore, the main objective of this study was to identify CPV circulating variants using PCR and sequencing of canine rectal swabs samples. The analysis of the general and clinical data provided by veterinarians did not show any particular association between the disease and animal sex or breed, but interestingly, 70.4% of the animals infected by CPV had been subjected to the full vaccination schedule (Table 1). The presence of CPV gastroenteritis in vaccinated animals has been reported previously (Decaro et al., 2008; Lamm and Rezabek, 2008). It should also be emphasized that nine out of 14 CPV2c samples belonged to vaccinated pups less than two months old that had received only one dose of vaccine.

Using PCR, the presence of CPV2a and CPV2b variants in field samples was demonstrated. The classical strain CPV2 was only found in commercial vaccine extracts. From 18 samples originally identified by PCR as CPV2b, 14 of them (77.7%) were found to be CPV2c after sequence analysis. These findings reinforce the suggestion made by other researchers that PCR alone is not a suitable tool for CPV variant strain identification (Buonavoglia et al., 2001; Decaro et al., 2005c, 2006a). Moreover, the unexpected detection of the presence of CPV2b variants in classical type 2-based commercial vaccines reported in this study may be explained by the failure of conventional PCR assays, based on primers differing in a single nucleotide, to discriminate correctly the CPV types. Recently, new molecular methods based on real-time PCR assays using minor groove binding probes have proved to be highly specific for CPV characterization and for discrimination between field and vaccine strains (Decaro et al., 2006c,d).

Although it has been reported that the Pb primer pair might amplified not only CPV2b variants but also the new CPV2a variants, mischaracterizing them as 2b, all the local samples amplified with this primer pair presented either an Asp426 or a Glu426, excluding the presence of an Asn426, typical of the 2a variant.

It has been reported previously that, compared with other DNA viruses, CPV shows a higher rate of nucleotide substitution during replication, being similar to that reported for RNA viruses (Shackelton et al., 2005). This high mutation rate, together with eventual selective pressure that might be generated by suboptimal levels of neutralizing antibodies due to incomplete or improper vaccination of dogs, might contribute to the emergence of new CPV variants.

In addition, it has been reported that the antigenic variants might be poorly neutralized by antibodies elicited by vaccination with CPV2 strain (Cavalli et al., 2008; Decaro et al., 2008). This might be the case of a vaccinated adult dog (135 months of age) infected by a CPV2b variant (Arg 5).

Mutations affecting important residues of the capsid protein of CPV, such as residues 297, 300 and 426, have been recognized recently, suggesting that CPV is still evolving (Martella et al., 2006; Truyen, 2006). In this regard, field variants Arg 3 and Arg 4 showed a replacement Ser297Asn, which is unique for the local types.

Regarding the evolution of CPV strains in Argentina, it is clear that although CPV2c has become the predominant variant in the year 2008, CPV2a and CPV2b variants are still circulating in the dog population, whereas CPV2 was not detected in field samples. The appearance of CPV2c variant in Argentina might be dated at least to the year 2003.

The results of the present study and those in other parts of the world suggest the necessity of updating CPV vaccines, using formulations containing CPV strains that are circulating currently in the field.

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References

Appel, M.J., Scott, F.W., Carmichael, L.E., 1979. Isolation and immunisation studies of a canine parvo-like virus from dogs with haemorrhagic enteritis. Vet Rec 105, 156–159.

Binn, L.N., Lazar, E.C., Eddy, G.A., Kajima, M., 1970. Recovery and characterization of a minute virus of canines. Infect Immun 1, 503–508.
Buonavoglia, D., Cavalli, A., Pratelli, A., Martella, V., Greco, G., Tempesta, M., Buonavoglia, C., 2000. Antigenic analysis of canine parvovirus strains isolated in Italy. New Microbiol 23, 93–96.
Buonavoglia, C., Martella, V., Pratelli, A., Tempesta, M., Cavalli, A., Buonavoglia, D., Bozzo, G., Elia, G., Decaro, N., Carmichael, L., 2001. Evidence for evolution of canine parvovirus type 2 in Italy. J Gen Virol 82, 3021–3025.
Burtonboy, G., Coignoul, F., Delferriere, N., Pastoret, P.P., 1979. Canine hemorrhagic enteritis: detection of viral particles by electron microscopy. Arch Virol 61, 1–11.
Carmichael, L.E., Schlafer, D.H., Hashimoto, A., 1994. Minute virus of canines (MVC, canine parvovirus type-1): pathogenicity for pups and seroprevalence estimate. J Vet Diagn Invest 6, 165–174.
Cavalli, A., Martella, V., Desario, C., Camero, M., Bellacicco, A.L., De Palo, P., Decaro, N., Elia, G., Buonavoglia, C., 2008. Evaluation of the antigenic relationships among canine parvovirus type 2 variants. Clin Vaccine Immunol 15, 534–539.
De Ybanez, R.R., Vela, C., Cortes, E., Simarro, I., Casal, J.I., 1995. Identification of types of canine parvovirus circulating in Spain. Vet Rec 136, 174–175.
Decaro, N., Desario, C., Campolo, M., Elia, G., Martella, V., Ricci, D., Lorusso, E., Buonavoglia, C., 2005a. Clinical and virological findings in pups naturally infected by canine parvovirus type 2 Glu-426 mutant. J Vet Diagn Invest 17, 133–138.
Decaro, N., Elia, G., Campolo, M., Desario, C., Lucente, M.S., Bellacicco, A.L., Buonavoglia, C., 2005b. New approaches for the molecular characterization of canine parvovirus type 2 strains. J Vet Med B Infect Dis Vet Public Health 52, 316–319.
Decaro, N., Elia, G., Martella, V., Desario, C., Campolo, M., Trani, L.D., Tarsitano, E., Tempesta, M., Buonavoglia, C., 2005c. A real-time PCR assay for rapid detection and quantitation of canine parvovirus type 2 in the feces of dogs. Vet Microbiol 105, 19–28.
Decaro, N., Elia, G., Desario, C., Roperto, S., Martella, V., Campolo, M., Lorusso, A., Cavalli, A., Buonavoglia, C., 2006a. A minor groove binder probe real-time PCR assay for discrimination between type 2-based vaccines and field strains of canine parvovirus. J Virol Methods 136, 65–70.
Decaro, N., Martella, V., Desario, C., Bellacicco, A.L., Camero, M., Manna, L., d'Aloja, D., Buonavoglia, C., 2006b. First detection of canine parvovirus type 2c in pups with haemorrhagic enteritis in Spain. J Vet Med B Infect Dis Vet Public Health 53, 468–472.
Decaro, N., Elia, G., Martella, V., Campolo, M., Desario, C., Camero, M., Cirone, F., Lorusso, E., Lucente, M.S., Narcisi, D., Scalia, P., Buonavoglia, C., 2006c. Characterisation of the canine parvovirus type 2 variants using minor groove binder probe technology. J Virol Methods 133, 92–99.
Decaro, N., Martella, V., Elia, G., Desario, C., Campolo, M., Buonavoglia, D., Bellacicco, A.L., Tempesta, M., Buonavoglia, C., 2006d. Diagnostic tools based on minor groove binder probe technology for rapid identification of vaccinal and field strains of canine parvovirus type 2b. J Virol Methods 138, 10–16.
Decaro, N., Desario, C., Addie, D.D., Martella, V., Vieira, M.J., Elia, G., Zicola, A., Davis, C., Thompson, G., Thiry, E., Truyen, U., Buonavoglia, C., 2007. The study molecular epidemiology of canine parvovirus, Europe. Emerg Infect Dis 13, 1222–1224.
Decaro, N., Desario, C., Elia, G., Martella, V., Mari, V., Lavazza, A., Nardi, M., Buonavoglia, C., 2008. Evidence for immunisation failure in vaccinated adult dogs infected with canine parvovirus type 2c. New Microbiol 31, 125–130.
Desario, C., Decaro, N., Campolo, M., Cavalli, A., Cirone, F., Elia, G., Martella, V., Lorusso, E., Camero, M., Buonavoglia, C., 2005. Canine parvovirus infection: which diagnostic test for virus? J Virol Methods 126, 179–185.
Greenwood, N.M., Chalmers, W.S., Baxendale, W., Thompson, H., 1996. Comparison of isolates of canine parvovirus by monoclonal antibody and restriction enzyme analysis. Vet Rec 138, 495–496.
Hong, C., Decaro, N., Desario, C., Tanner, P., Pardo, M.C., Sanchez, S., Buonavoglia, C., Saliki, J.T., 2007. Occurrence of canine parvovirus type 2c in the United States. J Vet Diagn Invest 19, 535–539.
Kelly, W.R., 1978. An enteric disease of dogs reselmbing feline panleucopaenia. Aust Vet J 54, 593.
Lamm, C.G., Rezabek, G.B., 2008. Parvovirus infection in domestic companion animals. Vet Clin North Am Small Anim Pract 38, 837–850 (viii–ix).
Martella, V., Cavalli, A., Pratelli, A., Bozzo, G., Camero, M., Buonavoglia, D., Narcisi, D., Tempesta, M., Buonavoglia, C., 2004. A canine parvovirus mutant is spreading in Italy. J Clin Microbiol 42, 1333–1336.
Martella, V., Cavalli, A., Decaro, N., Elia, G., Desario, C., Campolo, M., Bozzo, G., Tarsitano, E., Buonavoglia, C., 2005a. Immunogenicity of an intranasally administered modified live canine parvovirus type 2b vaccine in pups with maternally derived antibodies. Clin Diagn Lab Immunol 12, 1243–1245.
Martella, V., Decaro, N., Elia, G., Buonavoglia, C., 2005b. Surveillance activity for canine parvovirus in Italy. J Vet Med B Infect Dis Vet Public Health 52, 312–315.
Martella, V., Decaro, N., Buonavoglia, C., 2006. Evolution of CPV-2 and implication for antigenic/genetic characterization. Virus Genes 33, 11–13.
Mochizuki, M., Harasawa, R., Nakatani, H., 1993. Antigenic and genomic variabilities among recently prevalent parvoviruses of canine and feline origin in Japan. Vet Microbiol 38, 1–10.
Nakamura, M., Tohya, Y., Miyazawa, T., Mochizuki, M., Phung, H.T., Nguyen, N.H., Huynh, L.M., Nguyen, L.T., Nguyen, P.N., Nguyen, P.V., Nguyen, N.P., Akashi, H., 2004. A novel antigenic variant of canine parvovirus from a Vietnamese dog. Arch Virol 149, 2261–2269.
Parrish, C.R., O'Connell, P.H., Evermann, J.F., Carmichael, L.E., 1985. Natural variation of canine parvovirus. Science 230, 1046–1048.
Parrish, C.R., 1999. Host range relationships and the evolution of canine parvovirus. Vet Microbiol 69, 29–40.
Parrish, C.R., Aquadro, C.F., Strassheim, M.L., Evermann, J.F., Sgro, J.Y., Mohammed, H.O., 1991. Rapid antigenic-type replacement and DNA sequence evolution of canine parvovirus. J Virol 65, 6544–6552.
Pereira, C.A., Monezi, T.A., Mehnert, D.U., D'Angelo, M., Durigon, E.L., 2000. Molecular characterization of canine parvovirus in Brazil by polymerase chain reaction assay. Vet Microbiol 75, 127–133.
Perez, R., Francia, L., Romero, V., Maya, L., Lopez, I., Hernandez, M., 2007. First detection of canine parvovirus type 2c in South America. Vet Microbiol 124, 147–152.
Sagazio, P., Tempesta, M., Buonavoglia, D., Cirone, F., Buonavoglia, C., 1998. Antigenic characterization of canine parvovirus strains isolated in Italy. J Virol Methods 73, 197–200.
Senda, M., Parrish, C.R., Harasawa, R., Gamoh, K., Muramatsu, M., Hirayama, N., Itoh, O., 1995. Detection by PCR of wild-type canine parvovirus which contaminates dog vaccines. J Clin Microbiol 33, 110–113.
Shackelton, L.A., Parrish, C.R., Truyen, U., Holmes, E.C., 2005. High rate of viral evolution associated with the emergence of carnivore parvovirus. Proc Natl Acad Sci U S A 102, 379–384.
Steinel, A., Venter, E.H., Van Vuuren, M., Parrish, C.R., Truyen, U., 1998. Antigenic and genetic analysis of canine parvoviruses in southern Africa. Onderstepoort J Vet Res 65, 239–242.
Truyen, U., Platzer, G., Parrish, C.R., 1996. Antigenic type distribution among canine parvoviruses in dogs and cats in Germany. Vet Rec 138, 365–366.
Truyen, U., Geissler, K., Parrish, C.R., Hermanns, W., Siegl, G., 1998. No evidence for a role of modified live virus vaccines in the emergence of canine parvovirus. J Gen Virol 79 (Pt 5), 1153–1158.
Truyen, U., 1999. Emergence and recent evolution of canine parvovirus. Vet Microbiol 69, 47–50.
Truyen, U., Steinel, A., Bruckner, L., Lutz, H., Mostl, K., 2000. Distribution of antigen types of canine parvovirus in Switzerland, Austria and Germany. Schweiz Arch Tierheilkd 142, 115–119.
Truyen, U., 2006. Evolution of canine parvovirus—a need for new vaccines? Vet Microbiol 117, 9–13.