

Available online at [www.sciencedirect.com](http://www.sciencedirect.com)

Veterinary Microbiology xxx (2007) xxx–xxx

**veterinary  
microbiology**[www.elsevier.com/locate/vetmic](http://www.elsevier.com/locate/vetmic)

Short communication

## First detection of canine parvovirus type 2c in South America

Ruben Pérez<sup>\*</sup>, Lourdes Francia, Valeria Romero, Leticia Maya,  
Ignacio López, Martín Hernández

*Sección Genética Evolutiva, Instituto de Biología, Facultad de Ciencias, Universidad de la República,  
Iguá 4225, 11400 Montevideo, Uruguay*

Received 6 March 2007; received in revised form 9 April 2007; accepted 17 April 2007

### Abstract

Since its sudden emergence in the early 1970s, canine parvovirus type-2 (CPV-2) has been evolving through the generation of novel genetic and antigenic variants (CPV-2a/b/c and a number of additional mutations) that are unevenly distributed throughout the world. In order to develop strategies to control the spread of these variants and to understand virus evolution is fundamental to genotype field isolates from different geographic locations. In the present paper we have examined 25 isolates of CPV from clinical samples of Uruguayan dogs collected during year 2006. A fragment of the VP2 gene of the virus was analyzed using polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP) and DNA sequence analysis. Out of the 25 isolates analyzed, only one was characterized as CPV-2a and 24 were characterized as CPV-2c, indicating that this type is currently the prevalent field CPV circulating in Uruguay. This is the first report of CPV-2c in the American continent and it also represents the highest frequency of this type observed in a dog population so far. Its presence in South America supports the assumption that CPV-2c is reaching a worldwide distribution as occurred with 2a/2b antigenic types.

© 2007 Elsevier B.V. All rights reserved.

**Keywords:** Canine parvovirus; CPV-2c; Genetic variability

### 1. Introduction

The canine parvovirus type 2 (CPV-2) is a member of the autonomous replicating viruses of the *Parvoviridae* family, with a single-stranded DNA genome of negative polarity. Its 5.2 kb DNA has two open reading frames that encode nonstructural (NS) and structural (VP) viral proteins. The VP proteins (VP1 and VP2)

are translated from alternatively spliced mRNA, and the VP2 sequence is completely contained within VP1 (Reed et al., 1988). VP2 mainly comprises the nonenveloped icosahedral capsid of CPV, and only a few amino acid (aa) substitutions in its sequence can alter relevant biological characteristics of the virus (Parrish and Carmichael, 1986; Strassheim et al., 1994).

CPV-2 emerged as a novel pathogen in the late 1970s and rapidly spread worldwide, causing a new enteric and myocardial disease in dogs (Appel et al., 1979). The new virus presumably originated as a host

<sup>\*</sup> Corresponding author. Tel.: +598 2 525 86 19x144;  
fax: +598 2 525 86 17/31.

E-mail address: [rperez@fcien.edu.uy](mailto:rperez@fcien.edu.uy) (R. Pérez).

range variant from feline panleukopenia virus (FLV), that adapted to the new canine host via wild carnivores, such as minks and foxes (Truyen et al., 1998).

Few years after its successful spreading, the original form of CPV-2 was completely replaced by CPV-2a, a new type with the ability to efficiently infect and cause disease in both dogs and cats (Truyen et al., 1996a). CPV-2a differs from the original type 2 in five aa changes in the VP2 coat protein. The present evidence indicates that those aa are responsible for the antigenic and host-range viral properties (Parrish, 1991; Truyen et al., 1995).

In 1984, another antigenic variant emerged as a new CPV type, designated CPV-2b, which is currently cocirculating with the CPV-2a within dog populations around the world. The antigenic differences observed in CPV-2b are consequence of only one aa substitution (Asn426Asp) placed in the major antigenic site of the capsid (epitope A) (Parrish et al., 1991). A novel CPV mutant (Glu-426) produced by a Glutamate substitution in the same 426th residue was detected in year 2000 in Italy (Buonavoglia et al., 2001). As observed with the antigenic variant CPV-2b, the Asn/Asp426Glu substitution caused an antigenic change that could be detected using monoclonal antibodies (MAbs) in inhibition of haemagglutination assays (HI) (Nakamura et al., 2004). Accordingly, some author has named the Glu-426 mutant as a new type called CPV-2c (Decaro et al., 2005b, 2006a,b), a nomenclature that will be used throughout the present paper.

At present, CPV-2c is broadly distributed in Italy, where it is cocirculating with types 2a and 2b (Martella et al., 2004). Antigenic and genetic analysis of CPV isolates collected during three consecutive years have revealed that CPV-2c is progressively replacing other CPV types in the Italian dog population (Martella et al., 2005). CPV-2c has also been found in Vietnam (Nakamura et al., 2004), Spain (Decaro et al., 2006b), Germany (Shackelton et al., 2005) and in the United Kingdom (Decaro et al., 2007) although it has not been reported outside Eurasia yet.

The pathological and epidemiological consequences of CPV-2c are still unknown, but some differences have been reported in the clinical course and mortality rate of the infected dogs (Decaro et al., 2005a).

The emergence and spread of CPV variants with different epidemiological and antigenic properties of

CPV have not only evolutionary relevance, but also pose a considerable sanitary threat worldwide. Accordingly, monitoring of CPV field isolates has been fundamental to understand virus evolution and to develop preventive measures aimed to control the spread of the old and new CPV variants.

In the present paper we established the presence and prevalence of CPV-2c in Uruguayan dogs with haemorrhagic enteritis. Our findings support the worldwide spreading of this new type and provide new information to understand the evolution of antigenic variants of CPV.

## 2. Materials and methods

### 2.1. Samples

A total of 30 faecal samples from vaccinated and unvaccinated domestic dogs suspected of having CPV were collected from May to December of 2006 in Uruguay. The samples came from young dogs (from 1 to 11 months) of different races from Montevideo (capital city), Canelones (Las Piedras, 25 km from Montevideo), San José (90 km from Montevideo) and Lavalleja (Minas, 120 km from Montevideo) (Table 1).

### 2.2. DNA extraction, PCR amplification and DNA sequencing

Viral DNA extraction was performed according to the method described by Schunck et al. (1995). Lyophilized vaccines (CPV-2) were resuspended in 1 ml of PBS and then processed using the same methodology. A 583-bp fragment of the VP2 gene, from position 4003 to 4585, was amplified following Buonavoglia et al. (2001).

Amplicons were sequenced, using an ABI prism 377-Perkin Elmer automated sequencer, either directly or after being cloned using the InsT/Aclone PCR product cloning kit (Fermentas). Sequencing was carried out twice on both strands using the Ampli Taq FS (Perkin Elmer, USA) kit as recommended by the manufacturer.

Nucleotide sequences were submitted to the GenBank database (<http://www.ncbi.nlm.nih.gov>) and their accession numbers are displayed in Table 2.

Table 1  
Uruguayan CPV strains analysed in the present study

Sample	Vaccines	Course	Procedence	Race	CPV
Uy-1/06	Complete	Died	Montevideo	Rottweiler	2c
Uy-4/00	Complete	Died	Montevideo	Golden retriever	2c
Uy-5/06	Complete	NA	Lavalleja	Labrador retriever	2c
Uy-6/06	Incomplete	Died	Montevideo	Caniche	2a
Uy-12/06	Complete	Died	Montevideo	Short terrier	2c
Uy-15/06	Incomplete	NA	Canelones	Undefined	2c
Uy-17/06	Complete	Recovered	Lavalleja	Caniche	2c
Uy-19/06	Complete	Recovered	Montevideo	Rottweiler	2c
Uy-21/06	Complete	Recovered	Montevideo	Fila	2c
Uy-23/06	Complete	NA	Canelones	Undefined	2c
Uy-26/06	Incomplete	Recovered	Montevideo	Labrador retriever	2c
Uy-27/06	None	Recovered	Montevideo	Undefined	2c
Uy-28/06	None	NA	Santa Lucia	Undefined	2c
Uy-32/06	Incomplete	Recovered	San José	Rottweiler	2c
Uy-34/06	Incomplete	Died	Montevideo	Caniche	2c
Uy-37/06	None	NA	Montevideo	Undefined	2c
Uy-39/06	None	Died	Montevideo	Undefined	2c
Uy-41/06	None	Recovered	Canelones	Undefined	2c
Uy-42/06	Incomplete	Recovered	Montevideo	Undefined	2c
Uy-46/06	Incomplete	NA	Montevideo	Caniche	2c
Uy-47/06	None	Recovered	Montevideo	German shepherd	2c
Uy-51/06	None	Recovered	Lavalleja	Undefined	2c
Uy-52/06	None	NA	Canelones	Undefined	2c
Uy-53/06	Incomplete	Recovered	Montevideo	Undefined	2c
Uy-55/06	NA	NA	Montevideo	Cimarron	2c

Faecal samples were collected from vaccinated and unvaccinated puppies with haemorrhagic enteritis. Dogs were assisted in veterinary clinics located in different Uruguayan departments. Some dogs presented a complete vaccination plan; other had not received the complete series (at least three doses) of vaccines and other did not receive any vaccines at all. Genotype characterization was performed by DNA sequencing and/or restriction fragment length polymorphism (RFLP). NA: non-available data.

Table 2  
Nucleotide and amino acid differences in 583 bp of the VP2 sequences of Uruguayan isolates of CPV (type 2a: Uy-6/06, and type 2c: Uy-12/06, Uy-15/06 and Uy-17/06) with other CPV-2c isolates from Italy (56/00), Germany (U51) and Vietnam (HNI-4-1) and a CPV-2a from Brazil (BR8155-00)

Isolates	Country	VP2: codon position and amino acid						
		426	450	452	467	491	553	555
			(Thr)	(Gly)	(Gly)	(Gln)	(Ile)	(Val)
Uy 12/06 (EF375479), Uy 15/06 (EF375480)	Uruguay	GAA (Glu)	ACT	GGT	GGT	CAA	ATT	GTA
Uy 17/06 (EF375481)	Uruguay	...	...	..C	...	...	...	...
56/00 (AY380577)	Italy	...	...	...	...	...	...	...
U51 (AY742942)	Germany	...	...	...	...	...	...	...
HNI-4-1 (AB120727)	Vietnam	...	..G	...	..A	...	..C	...
BR8155-00 (DQ340434)	Brazil	AAT (Asn)	...	...	...	...	...	...
Uy 6/06 (EF375482)	Uruguay	AAT (Asn)	...	...	...	..G	...	...

Accession numbers are indicated in bracket. The only aa changes are observed at position 426 (Glutamic acid in CPV-2c and Asparagine in CPV-2a). Positions are referred to strain CPV-b (accession M38245).

### 2.3. RFLP analysis

All the samples, including the CPV-2 type vaccine, were subjected to RFLP assay. For restriction enzyme digestion, 15 µl of each amplicon were digested with five units of the *Mbo*II restriction enzyme (Fermentas) and assayed on 1.5% agarose gels to determine the cleavage pattern of the amplicons.

### 2.4. Nucleotide and aa sequence analysis

Nucleotide and aa sequence alignment was performed by Clustal method with BioEdit Sequence Alignment Editor (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>). For sequence comparison, the nucleotide sequences of CPV-2c (AY380577, AY742942 and BAD34656) and CPV-2a (DQ340434) isolates were retrieved from the GenBank.

## 3. Results

### 3.1. CPV diagnosis

A single DNA band of the expected size (583 bp), corresponding to the partial amplification of the VP2 gene, was observed by gel electrophoresis in 25 of the 30 field samples suspected of having CPV. A band of the same size was amplified from CPV-2 type vaccine and used as positive control. No amplified products were obtained from faecal samples of clinically healthy dogs used as negative controls.

### 3.2. Genotyping the samples: sequence and RFLP analyses

The sequencing of three samples (Uy-12/06, Uy-15/06, and Uy-17/06) revealed the presence of a GAA codon at position 426 of the VP2 protein. This GAA triplet codes for Glutamate that is indicating that these strains were type 2c (Table 2). They had identical DNA sequences, except for the presence of a single nucleotide polymorphism (SNP) at position 4142 in the Uy-17/06 sample. This SNP corresponds to a transition (T to C) in the third position of the 452nd codon that did not alter the aa sequence (Table 2).

In order to test for the presence of CPV-2c in the remaining samples, we performed a RFLP analysis

using the *Mbo*II enzyme as reported by Buonavoglia et al. (2001). Our analysis indicated that 24 samples had type 2c RFLP pattern and only one sample had a CPV-2/2a/b pattern (Table 1). The sequencing of this last sample revealed the presence of Asn at position 426, characteristic of the CPV-2a type (Table 2).

The comparative sequence analyses were performed using a 516-bp fragment (4023–4538) of the 583 amplicon that codes for 172 aa (413–584) of the VP2 protein.

The sequence alignment of the Uruguayan CPV-2c indicated that the strains Uy-12/06 and Uy-15/06 exhibited 100% nucleotide identity with the CPV-2c from Italy and Germany, whereas the Uy-17/06 had 99.8% identity as a consequence of the 4142 SNP. In comparison with the Vietnamese CPV-2c (HNI-4-1), the Uy-12/06 and Uy-15/06 Uruguayan strains had 99.4% (three differences), and the Uy-17/06 had 99.3% (four differences) nucleotide identity. All the nucleotides changes observed among CPV-2c were synonymous, therefore their aa sequence showed 100% identity (Table 2).

The nucleotide sequence of the Uruguayan sample Uy-6/06 presented high similarity with CPV-2a isolates described worldwide. This sequence showed a SNP at position 4259 that had not been previously detected in any other CPV-2 types. This SNP is an A to G transition in the third position of the 491 codon that does not modify its aa assignment (Table 2).

## 4. Discussion

As surprising as the emergence and sudden dispersion that CPV-2 experimented in the 1970s, were the changes, including an extended host range that the virus further underwent in a few years (Shackelton et al., 2005; Truyen, 2006). The great virus variability, in part consequence of its high mutation rate similar to the one observed in fast evolving RNA viruses (Shackelton et al., 2005), has raised concern about its potential negative impact on the health of domestic dogs and wildlife species.

CPV-2c is a current example of how a new viral variant could arise and evolve. This type has a short history that begins six years ago, when it was first described from two clinical cases collected in southern Italy (Buonavoglia et al., 2001). The recent detection

of this type in other European countries is an indicative of its expansion throughout this continent (Shackelton et al., 2005; Decaro et al., 2006b; Decaro et al., 2007). The spreading ability of the CPV-2c was here confirmed by its presence in clinical samples from Uruguayan dogs. Our finding adds the American continent to the distribution of CPV-2c, supporting the fact that it is reaching a worldwide distribution as occurred with 2a and 2b antigenic types.

More striking than the simple presence is the high frequency (24 out of 25) that CPV-2c achieved in Uruguay. This fact, together with its occurrence in different localities (see Table 1) is indicative that type 2c is currently the prevalent field CPV circulating in the country. Although we know that CPV appeared in Uruguay around 1980 (Luis Carretto, personal communication), there is no data about CPV genotypes existing in the country in previous years that allow the comparative analysis of the evolution of CPV variants over time. We can hypothesize that the Uruguayan CPV-2c replaced previous circulating CPV, as occurred in Italy. After its first description, the Italian CPV-2c increased its proportion in relation to other circulating types from 17% in the year 2000, to 60% in 2004 (Martella et al., 2005). This result, together with our own findings, indicates that the spreading ability of the type 2c is associated with an important replacement capacity that could eventually lead to the elimination of other CPV types.

Besides the CPV-2c, we identified in Uruguayan samples a single CPV-2a strain characterized by the presence of an Asn426 residue. Although CPV-2a was originally also characterized by the presence of a Isoleucine replacement at position 555 (Parrish, 1991), our sample has a Valine in that position as a consequence of a single transition (A/G) at nucleotide 4449 (Table 2). This change is considered a reversion to the original CPV-2 and it has been reported in most recent 2a isolates (Martella et al., 2006). However, this reversion also occurred in older isolates as observed in Brazilian samples from the 80s (Pereira et al., 2007). Probably, the CPV-2a strain represents a relic of the original types that were predominant in Uruguay before the CPV-2c appearance. In this sense, previous CPV population would be typical CPV-2a as described for neighbouring countries as Brazil (Pereira et al., 2007). It is evident that further studies will be needed to obtain additional evidence about the CPV-2c

behaviour in South America. In order to accomplish this objective, we have to look for its presence in other countries for a more conclusive hypothesis about its emergence and epidemiology importance. To our knowledge, no reports about South America isolates are available after year 2001. Actually, the only DNA sequences available of CPV in South America came from Brazil (Shackelton et al., 2005; Pereira et al., 2007). It is likely that a new and extended screening revealed a different scenario that the one described six years ago. Recent CPV isolates should be examined using techniques (e.g. sequencing, PCR-RFLP, minor groove binder probe assays) able to differentiate the current and eventually the novel viral variants (Buonavoglia et al., 2001; Desario et al., 2005; Decaro et al., 2006c). This is particularly important in the case of CPV-2c since it had been overlooked in antigenic analysis using the usual MAbs as well as PCR-base typing assays that consider only CPV2a/b variations (Nakamura et al., 2004; Martella et al., 2006).

Finally, from a clinical point of view, it is important to note that there is a report that described less severe clinical symptoms and lower mortality rate in CPV-2c-infected dogs than in reported outbreaks caused by type 2a and type 2b CPVs (Decaro et al., 2005a). However, most of the CPV-2c cases reported so far, had clinical symptoms associated with severe haemorrhagic enteritis and frequently experience fatal outcomes (Buonavoglia et al., 2001; Decaro et al., 2006b). The same disease course was observed in Uruguayan dogs infected with the CPV-2c since all of them presented severe haemorrhagic gastroenteritis and five of them even died (Table 1). It is worth to point out that the disease occurs even in dogs with a complete scheme of vaccination. These results raise several concerns on the efficiency of the current CPV-2 vaccines against this mutant, and reinforce the need of a specific CPV-2c vaccine as suggested by Decaro et al. (2006b).

## Acknowledgments

This work was partially supported by the “Programa de Desarrollo de las Ciencias Básicas” (PEDECIBA) from Uruguay. We are grateful to Dr. Luis Carretto and Diego Hernández for valuable

suggestions about CPV research. We thank the clinical practitioners from Uruguayan veterinaries for generously providing faecal samples for testing.

## 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.
- 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.
- 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., Campolo, M., Desario, C., Camero, M., Cirone, F., Lorusso, E., Lucente, M.S., Narcisi, D., Scalia, P., Buonavoglia, C., 2006a. Characterisation of the canine parvovirus type 2 variants using minor groove binder probe technology. *J. Virol. Methods* 133, 92–99.
- 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., Martella, V., Elia, G., Desario, C., Campolo, M., Buonavoglia, D., Bellacicco, A.L., Tempesta, M., Buonavoglia, C., 2006c. 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., Elia, G., Campolo, M., Lorusso, A., Mari, V., Martella, V., Buonavoglia, C., 2007. Occurrence of severe gastroenteritis in pups after canine parvovirus vaccine administration: A clinical and laboratory diagnostic dilemma. *Vaccine* 25, 1161–1166.
- 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.
- 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., Decaro, N., Elia, G., Buonavoglia, C., 2005. 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.
- 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., 1991. Mapping specific functions in the capsid structure of canine parvovirus and feline panleukopenia virus using infectious plasmid clones. *Virology* 183, 195–205.
- Parrish, C.R., Carmichael, L.E., 1986. Characterization and recombination mapping of an antigenic and host range mutation of canine parvovirus. *Virology* 148, 121–132.
- 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., Leal, E.S., Durigon, E.L., 2007. Selective regimen shift and demographic growth increase associated with the emergence of high-fitness variants of canine parvovirus. *Infect Genet Evol.* 7 (3), 399–409.
- Reed, A.P., Jones, E.V., Miller, T.J., 1988. Nucleotide sequence and genome organization of canine parvovirus. *J. Virol.* 62, 266–276.
- Schunck, B., Kraft, W., Truyen, U., 1995. A simple touch-down polymerase chain reaction for the detection of canine parvovirus and feline panleukopenia virus in feces. *J. Virol. Methods* 55, 427–433.
- 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.
- Strassheim, M.L., Gruenberg, A., Veijalainen, P., Sgro, J.Y., Parrish, C.R., 1994. Two dominant neutralizing antigenic determinants of canine parvovirus are found on the threefold spike of the virus capsid. *Virology* 198, 175–184.
- Truyen, U., 2006. Evolution of canine parvovirus: a need for new vaccines? *Vet. Microbiol.* 117, 9–13.
- Truyen, U., Evermann, J.F., Vieler, E., Parrish, C.R., 1996a. Evolution of canine parvovirus involved loss and gain of feline host range. *Virology* 215, 186–189.
- 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., Gruenberg, A., Chang, S.F., Obermaier, B., Veijalainen, P., Parrish, C.R., 1995. Evolution of the feline-subgroup parvoviruses and the control of canine host range in vivo. *J. Virol.* 69, 4702–4710.