Veterinary Research Communications, 29 (2005) 735–743

Genomic Typing of Canine Parvovirus Circulating in the State of Rio de Janeiro, Brazil from 1995 to 2001 Using Polymerase Chain Reaction Assay

A.P. Costa[1]*, J.P.G. Leite[1], N.V. Labarthe[2] and R.C.N. Cubel Garcia[3]
[1]Departamento de Virologia, Instituto Oswaldo Cruz, Fiocruz, RJ, Brazil; [2]Departamento de Patologia e Clínica Veterinária, Faculdade de Veterinária, [3]Departamento de Microbiologia e Parasitologia, Instituto Biomédico, Universidade Federal Fluminense, Niterói, RJ, Brazil
**Correspondence: Instituto Oswaldo Cruz, Departamento de Virologia, Laboratório de Virologia Comparada – Pavilhão Cardoso Fontes, Avenida Brasil, 4365 Manguinhos, Rio de Janeiro/RJ, CEP 21045-900, Brazil*
E-mail: alexpina@ioc.fiocruz.br

Costa, A.P., Leite, J.P.G., Labarthe, N.V. and Cubel Garcia, R.C.N., 2005. Genomic typing of canine parvovirus circulating in the State of Rio de Janeiro, Brazil from 1995 to 2001 using polymerase chain reaction assay. *Veterinary Research Communications*, **29(8)**, 735–743

ABSTRACT

In this study, the genomic types of canine parvovirus (CPV) circulating in the State of Rio de Janeiro, Brazil, from 1995 to 2001, were investigated using the polymerase chain reaction assay (PCR). A total of 78 faecal samples from gastroenteritic puppies, confirmed as positive for canine parvovirus by haemagglutination/haemagglutination inhibition tests or virus isolation in cell culture (MDCK), were examined. The viral DNA was extracted from faecal samples using a combination of phenol–chloroform and silica–guanidine thiocyanate methods. PCR was carried out with differential pairs of primers to distinguish the old (CPV-2) and new types of virus (CPv-2a or CPV-2b). Specific amplicons were observed for all samples using the primer pair P2ab, which detects CPV-2a and CPV-2b. Seventy-six from a total of 78 samples (97%) were considered as CPV-2b because of their reaction with the primer pair P2b. Thirty samples (30/78) were from previously vaccinated puppies and in 15 of them the enteritis symptoms began from 1 to 12 days after vaccination. PCR confirmed the infection by wild virus (CPV-2b) in 5 of these 15 puppies who had received old-type vaccines. Our results show that CPV-2b was the prevalent type circulating in the State of Rio de Janeiro from 1995 to 2001.

Keywords: canine parvovirus, genomic typing, polymerase chain reaction assay, Rio de Janeiro

Abbreviations: CPV, canine parvovirus; FPLV, feline panleukopenia virus; HA, haemagglutination; MDCK, Madin–Darby canine kidney cells; PCR, polymerase chain reaction

INTRODUCTION

Since it emerged in 1978, canine parvovirus has been considered an important pathogen of domestic dogs and has spread worldwide in domestic and wild canid populations (Appel *et al.*, 1979; Steinel *et al.*, 2000).

Soon after the appearance of CPV-2, new antigenic types, termed CPV-2a and CPV-2b emerged in 1979 and 1984, respectively (Parrish *et al.*, 1985). These variants may be distinguished from the original CPV-2 using monoclonal antibodies, restriction enzymes and, more recently, polymerase chain reaction assay (PCR) (Parrish *et al.*, 1985, 1988, 1991; Mochizuki *et al.*, 1993; Senda *et al.*, 1995; Pereira *et al.*, 2000). It has been demonstrated that CPV-2a differs from CPV-2 in three regions of the gene that encodes the VP2 capsid protein. The only significant difference between CPV-2a and CPV-2b variants is the substitution of one amino acid (aa) (Asn→Asp) at position 426 in VP2 protein (Parrish *et al.*, 1988, 1991).

Several reports showed that the original CPV-2 is no longer circulating; meanwhile the new variants CPV-2a and CPV-2b have become variously distributed in canine populations worldwide (Parrish *et al.*, 1988; De Ybañez *et al.*, 1995; Greenwood *et al.*, 1996; Hirasawa *et al.*, 1996; Sagazio *et al.*, 1998; Steinel *et al.*, 1998; Ganiere *et al.*, 2000; Truyen *et al.*, 2000).

Our results from a study conducted in the State of Rio de Janeiro, Brazil, showed that, in spite of vaccination, canine parvovirus infection is responsible for about 44% of the haemorrhagic enteritis in puppies (Cubel Garcia *et al.*, 2002). The present survey was conducted to characterize, for the first time, the types of CPV-2 circulating in the State of Rio de Janeiro from 1995 to 2001 using the PCR and nested-PCR assays.

MATERIALS AND METHODS

Faecal specimens and canine parvovirus detection

A total of 78 faecal samples from gastroenteritic puppies under 7 months of age were examined. All puppies lived in the State of Rio de Janeiro. Fourty-four samples arose from the outpatient office of the Universidade Federal Fluminense, and 12 samples from a privately owned animal hospital, both located in Niterói city (20 km from Rio de Janeiro city). Seven samples were from puppies that had had consultation with practitioners in Rio de Janeiro city and another one from Nova Iguaçu city (20 km far from Rio de Janeiro city). Eleven samples were from a private clinic from Teresópolis and the remaining three were from Petrópolis; both of these cities are located in the mountain region, approximately 91 and 68 km, respectively, from Rio de Janeiro city. These samples were collected during a 7-year period, from April 1995 to April 2001, with the following distribution: 1995 (10 samples), 1996 (12), 1997 (11), 1998 (10), 1999 (11), 2000 (15) and 2002 (9).

The samples were first tested for the presence of CPV-2 by haemagglutination (HA) test and confirmed as positive by haemagglutination inhibiton test or virus isolation in cell culture (Madin–Darby Canine Kidney, MDCK) (Cubel Garcia *et al.*, 2000).

Virus strains

Feline panleukopenia virus (FPLV, Felocell CVR, Pfizer, New York, NY), CPV type 2 (Vanguard, Pfizer), CPV type 2a (Vencomax, Vencofarma, Londrina, PR, Brazil) and CPV type 2b viruses (Galaxy, Fort Dogde, Iowa and Primodog Merial, Duluth, GA), were obtained from modified-live vaccines commercially available in Brazil and were used to test the specificity of the primers.

Sample preparation

Approximately 10% faecal supensions were prepared using Tris-Ca^{2+} (0.01 mol/L, pH 7.2) and digested with proteinase K (20 mg/ml) (Gibco, Carlsbad, CA) at 56°C for 15 min. After digestion, the viral DNA was extracted using a combination of phenol–chloroform–isoamyl alcohol (Invitrogen, Carlsbad, CA) and silica–guanidine thiocyanate, as described by Alfieri and colleagues (2004).

Primers for PCR assays

Differential primers for the capsid protein VP1/VP2 gene were used to distinguish the old and new types of viruses (Table I). The primer pairs P2 and P2ab, located at nt 3025–3045 and 3685–3706, respectively, which detect CPV type 2 and the new type strains (CPV-2a and 2b), have been already described by Senda and colleagues (1995). The primer pair P2b, located at positions 4043–4062 and 4449–4470, specific for CPV

TABLE I
PCR primers designed in the VP1/VP2 gene of canine parvovirus

Primer	Sequence	Position	Amplicon length (bp)
VPF	ATGGCACCTCCGGCAAAGA	(2285–2303)	2245
VPR	TTTCTAGGTGCTAGTTGAG	(4512–4530)	
P2S	GAAGAGTGGTTGTAAATAATA*	(3025–3045)	681
P2AS	CCTATATC*ACCAAAGTTAGTAG*	(3685–3706)	
P2abS	GAAGAGTGGTTGTAAATAATT*	(3025–3045)	681
P2abAS	CCTATATA*ACCAAAGTTAGTAC*	(3685–3706)	
P2bS	CTTTAACCTTCCTGTAACAG	(4043–4062)	427
P2bAS	CATAGTTAAATTGGTTATCTAC	(4449–4470)	

type 2b, has been described by Pereira and colleagues (2000). At first, each sample was tested with the primer pairs P2 and P2ab. Those found positive for P2ab were subjected to a new amplification assay with primer P2b to distinguish among types 2a and 2b. For the nested-PCR assay, a primer pair, VPF (2285–2303) and VPR (4512–4530), which amplifies FPLV as well as the old and new types of canine parvovirus, was used in the first amplification (Mochizuki *et al.*, 1993). The first amplicon product was then submitted to a nested-PCR assay using the primer pairs described above (P2, P2ab, P2b).

Polymerase chain reaction (PCR) assay

The PCR was performed as described previously (Mochizuki *et al.*, 1993; Senda *et al.*, 1995) with some modifications. The most sensitive concentration of $MgCl_2$ was found to be 1.5 mmol/L for primers P2, P2ab and VPF/VPR, and 2.5 mmol/L for primer P2b. Briefly, 10 µl of DNA was incubated at 94°C for 2 min with 1 µl of each primer (20 pmol) pair. After 2 min at 4°C, DNA amplification was performed in a 50 µl reaction mixture containing 50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 8.3) (Biotools), 1.5 mmol/L $MgCl_2$ (Invitrogen), 200 µmol of each dNTP (Invitrogen) and 0.5 U of *Taq* polymerase (Biotools). The PCR was performed in a thermal cycler (LabNet Multigene IR System, Edison, NJ) and consisted of 30 cycles of incubation at 94°C for 30 s, 55°C for 2 min and 72°C for 2 min and final extension of 10 min at 72°C. The PCR products were resolved on 1% agarose gel followed by ethidium bromide staining and the amplicons were visualized with UV light. Distilled milli-Q water was used as a negative control in all techniques, and recommended manipulations for PCR procedures were carried out as a precaution to avoid false-positive results (Kwok and Higuchi, 1989).

Restriction enzyme analysis of the PCR products after amplification with primer pair VPF/VPR

The DNA sequences corresponding to the VP1/VP2 genes of CPV-2 (GenBank M38245), 2a (Genbank AJ564427) and 2b (GenBank M74849) were searched for matches to the recognition sites of a large database of restriction endonucleases by computer-aided analysis (DNASIS v.6, Hitachi Software Co., San Francisco, CA, USA). The restriction enzyme *Hae*III was found to recognize selectively the sequence GG!CC at nt 2418 and 3837 of CPV type 2, 2a and 2b strains. Then, amplicons (10 µl) obtained by PCR assay using the primer pair VPR/VPF were digested at 37°C for 3 h with 20 units of *Hae*III restriction endonuclease as recommended by the manufacture (Promega, Madison, WI). The 1% agarose gels were photographed under UV light after ethidium bromide staining.

RESULTS

Specificity of the primers

The specificity of the four primer pairs used was evaluated with the amplification of the CPV-2 vaccine samples. The primer pair P2 reacted with FPV and CPV-2 samples, while P2ab detected the new types of canine parvovirus. Both primer pairs generated an expected 681 bp amplicon. A 427 bp amplicon was observed only with the P2b and CPV-2b vaccines. To show the specificity of PCR reaction using this set of primers, 11 positive samples and controls (vaccines) were first tested in a PCR using the primer pair VPF/VPR. As expected a 2.2 kbp amplicon was observed with FPV, CPV-2 (old type), CPV-2a and CPV-2b (new types) vaccines. The amplicons were then submitted to a nested-PCR using the primer pairs described above (P2, Pab and Pb). The results were similar to those obtained with a single PCR reaction. To attempt to confirm that the amplicons detected after the amplification using primer pair VPF/VPR were canine parvovirus-specific, the PCR products obtained with canine parvovirus vaccines and four clinical samples were digested with the restriction endonuclease *Hae*III. After digestion, three fragments of the expected sizes (1419 bp, 693 bp, 133 bp) were observed on 1% agarose gel for all samples tested (Figure 1).

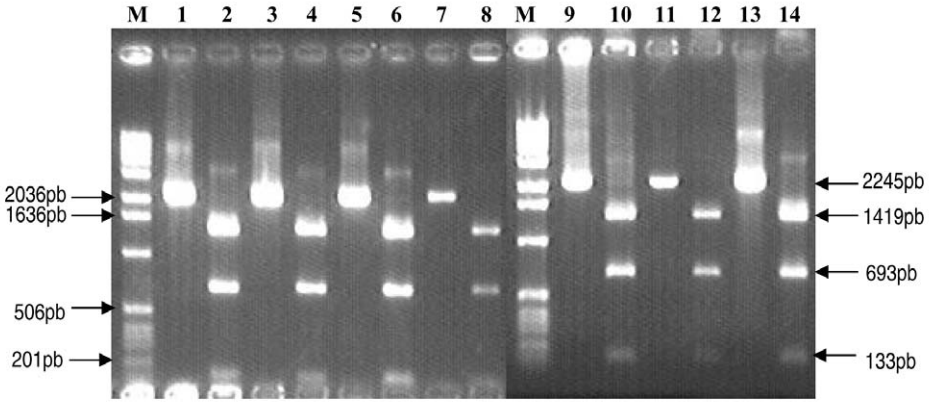

Figure 1. *Hae*III restriction patterns of the PCR products obtained with primer pair VPF/VPR. Lane M, 1 kb ladder (Invitrogen); lanes 1, 3, 5, 7, 9, 11, 13, undigested products; lanes 2, 4, 6, 8, 10, 12, 14, digested products. Lanes 1–2, CPV-2 vaccine; lanes 3 and 4; CPV-2a vaccine; lanes 5 and 6; CPV-2b vaccine; lanes 7, 8 and 9, 10, clinical samples that showed amplification with primer pairs P2ab and P2b; lanes 11, 12 and 13, 14, clinical samples that showed amplification only with primer pair P2ab

CPV-2 genotyping from clinical samples

None of the 78 samples tested showed an amplicon with the primer pair P2. All except two samples were amplified with the primer pair P2ab and, because they reacted with the primer pair P2b, were considered CPV-2b (Table II). The other two samples that did not show amplification with the primer pair P2b were tested in a new PCR reaction using the primer pair VPF/VPR. A specific 2.2 kbp amplicon was observed on 1% agarose gel and a nested-PCR was carried out using individual primers P2, P2ab and P2b; an amplicon of 681 bp was observed only with the primer pair P2ab.

According to the owners' information among the 78 studied samples, 63 puppies were from 2 to 4 months old. Thirty puppies received at least one dose of parvovirus vaccine and 40 puppies had not been vaccinated (Table III). Vaccination data were not available for the remaining 8 puppies.

TABLE II
Distribution of the faecal samples typed by PCR as CPV-2a or CPV-2b according to the cities where dogs lived in the State of Rio de Janeiro, Brazil

City	CPV-2a	CPV-2b	No. of samples tested
Niterói	1	55	56[a]
Rio de Janeiro	1	6	7
Teresópolis	0	11	11
Petrópolis	0	3	3
Nova Iguaçú	0	1	1
	2 (2.5%)	76 (97.5%)	78 (100%)

[a]56 samples: 44 from Universidade Federal Fluminense, 12 from private animal hospitals

TABLE III
Detection of CPV-2 in vaccinated puppies

No. of days between vaccination and onset of the symptoms	Puppies vaccinated with			
	1 dose	2 doses	3 doses	Total
1–3	3	2	0	5
4–9	5	3	0	8
10–12	2	0	0	2
>13	10	2	0	12
NA[a]	1	1	1	3
Total	21	8	1	30

[a]NA, not available

As shown in Table III, among the 30 vaccinated puppies, 21 received one dose, 8 received two doses and only one had completed the vaccination protocol. For 12 of these 30 puppies, the interval between the last vaccination and the onset of the disease was longer than 13 days; this information was not available for the other three puppies. For another 15 puppies, the enteritis symptoms began from 1 to 12 days after vaccination.

DISCUSSION

The results of the investigation of the 78 faecal samples indicated that CVP-2b was the prevalent type of canine parvovirus circulating in Rio de Janeiro State from 1995 to 2001. This paper reports the first molecular characterization of CPV-2 in Rio de Janeiro and the results are in agreement with those reported by Pereira and colleagues (2000), who detected CPV-2b in São Paulo, Brazil. Similar epidemiological patterns have been described in the United States, Japan, South Africa and Switzerland (Parrish *et al.*, 1988; Hirasawa *et al.*, 1996; Steinel *et al.*, 1998; Truyen *et al.*, 2000). In contrast, studies conducted in France, Austria, and Germany in the same period showed that CPV-2a was more common than CPV-2b (Ganiere *et al.*, 2000; Truyen *et al.*, 2000), while in United Kingdom and Spain the two types appear to be distributed about equally (De Ybañez *et al.*, 1995; Greenwood *et al.*, 1996).

The reasons for the difference in the worldwide distribution of the two variants are unclear and the coexistence of CPV-2a and CPV-2b in various populations in different countries as well as in different ratios shows that there seems to be no evolutionary advantage for one type or the other. CPV-2b differs from CPV-2a in only one amino acid change (Asn-426 to Asp) and it is believed to be unlikely that this difference arose from immunoselective pressure by vaccines (Parrish *et al.*, 1991; Steinel *et al.*, 1998).

Approximately 80% (63/78) of the characterized samples were from young puppies (2–4 months), probably because at this early age the vaccination programme had not been completed or puppies had not been vaccinated. As shown (Table III), 30 puppies (30/78) had received one, two or three doses of vaccine. These samples were considered positive for parvovirus by HA assay or virus isolation in cell culture (MDCK) (Cubel Garcia *et al.*, 2000, 2002). It is not possible to discriminate between wild or vaccine virus using these tests and it is known that both vaccine and wild CPV-2 can be detected in faecal samples by HA test from 3 to 9 days after oral inoculation (Carmichael *et al.*, 1981). In 12 of these 30 vaccinated puppies the interval between vaccination and the onset of disease was longer than 13 days, showing that it was, in fact, wild virus shedding. For another 5 puppies, the interval was 1–3 days, suggesting that they were probably vaccinated during the incubation period of the disease. For 10 samples the positive result could be due to detection of vaccine virus since the interval was 3–12 days post immunization.

Most of the vaccines available in Brazil during the study period were constituted of CPV-2 old type. Using the PCR with differential primer pairs it was possible to confirm the infection by wild virus (CPV-2b) in 5 of the 15 puppies in which gastroenteritis symptoms began from 1 to 12 days after vaccination with the old type. For another 10

puppies, data on the type of CPV-2 vaccine were not available.

Presently in Brazil, vaccines constituted of the old and new types of virus (CPV-2, CPV-2a and CPV-2b) are available in the market. Since the tests currently employed at animal hospitals are not able to distinguish among vaccine and wild virus, it is important now to know about the vaccination protocol that an animal has received for the appropriate diagnosis of CPV-2 (Carmichael *et al.*, 1981; Sagazio *et al.*, 1998; Cubel Garcia *et al.*, 2002).

The natural variation of CPV-2 has already had biological consequences for dogs and cats. Mochizuki and colleagues (1996) reported that about 5% of the FPV isolates from domestic cats in Germany and the United States were either CPV-2a or CPV-2b. In addition, Ikeda and colleagues (2000) demonstrated that both CPV-2a and CPV-2b are prevalent in cat populations in Southeast Asia. CPV strains were also isolated from peripheral blood mononuclear cells of apparently healthy Vietnamese leopard cats and were designed as LCPV. Three of these LCPV strains presented a specific amino acid substitution at residue 300 in the VP2 capsid protein and were denominated CPV-2c (Ikeda *et al.*, 2000). CPV-2 emerged suddenly in 1978 and it continues to evolve. The sequence analysis of the capsid protein gene of two CPV-2b isolates in Italy (Buonavoglia *et al.*, 2000) revealed two amino acid changes. One of these was at AA 426 (Asp→Glu) and this substitution seems not to be present in other CPVs or in closely related parvoviruses.

CPV-2 infection is still widespread among domestic dogs in the State of Rio de Janeiro, as has demonstrated before (Cubel Garcia *et al.*, 2002). By constantly monitoring the CPV-2 types circulating in the State of Rio de Janeiro it will be possible to detect future genetic changes as well as maintaining surveillance on infection by canine parvovirus in domestic or wild cats.

ACKNOWLEDGEMENTS

The authors thank the clinical practitioners from Policlinica Veterinária/UFF and other private hospitals who generously provided faecal samples for testing. This work was supported by Brazilian Research Council (CNPq), FAPERJ, and Instituto Oswaldo Cruz. A.P. Costa received CNPq fellowship due to his Graduate Program (MSc) at Instituto Oswaldo Cruz, FIOCRUZ.

REFERENCES

Alfieri, A.F., Alfieri, A.A., Barreiros, M.A.B., Leite, J.P.G. and Richtzenhain, L.J., 2004. G and P genotypes of group A rotavirus strains circulating in calves in Brazil, 1996–1999. *Veterinary Microbiology*, **99**, 167–173

Appel, M., Scott, F. and Carmichael, L.E., 1979. Isolation and immunization studies of a canine parvo-like virus from dogs with haemorrhagic enteritis. *Veterinary Record*, **105**, 156–159

Buonavoglia, C., Martella, V., Pratelli, A., Tempesta, M., Cavalli, A., Buonavoglia, D., Bozzo, G., Elia, G., Decaro, N. and Carmichael, L., 2000. Evidence for evolution of canine parvovirus type 2 in Italy. *Journal of General Virology*, **82**, 3021–3025

Carmichael, L.E., Joubert, J.C. and Pollock, R.V., 1981. A modified-live canine parvovirus strain with novel plaque characteristics I: viral attenuation and dog response. *Cornell Veterinary*, **71**, 784–791

Cubel Garcia, R.C.N., Pinto, A.M., Costa, A.P., Maciel, B.M., Oliveira, L.H.S., Nascimento, J.P., Santos, A.O., Castro, M.C.N., Willi, L.M.V. and Labarthe, N.W., 2000. Canine parvovirus infection in puppies with gastroenteritis in Niterói, Rio de Janeiro, from 1995 to 1997. *Brazilian Journal of Veterinary Research in Animal Science*, **37**, 132–135

Cubel Garcia, R.C.N., Leite, J.P., Xavier, M.T.P., Castro, T., Mertens, R., Lemos, M. and Labarthe, N., 2002. Infecção pelo parvovírus canino no Rio de Janeiro: um estudo de 5 anos. *Revista Brasileira de Ciência Veterinária*, **9**, 42–46

Ganiere, J.P., Ruvoen, N., Gueguen, S., Hege, T., Chazel, M. and Aubert, A., 2000. Identification des types de parvovirus canin circulant en France. *Revue Médicine Véterinaire*, **151**, 43–46

De Ybañez, R.R., Vela, C., Cortés, E., Simarro, I. and Casal, J.I., 1995. Identification of types of canine parvovirus circulating in Spain. *The Veterinary Record*, **136**, 174–175

Greenwood, N.M., Chalmers, W.S.K., Baxendale, W. and Thompson, H., 1996. Comparison of isolates of canine parvovirus by monoclonal antibody and restriction enzyme analysis. *Veterinary Record*, **138**, 495–496

Hirasawa, T., Yono, K. and Mikazuki, K., 1996. Detection and genomic analysis of canine parvovirus by the polymerase chain reaction. *Journal of Veterinary Medicine*, **43**, 545–554

Ikeda, Y., Mochizuki, M., Naito, R., Nakamura, K., Miyazawa, T., Mikami, T. and Takahashi, E., 2000. Predominance of canine parvovirus (CPV) in unvaccinated cat populations and emergence of new antigenic types of CPVs in cats. *Virology*, **278**, 13–19

Kwok, S. and R. Higuchi. 1989. Avoiding false positives with PCR. *Nature*, **339**, 237–238

Mochizuki, M., San Gabriel, M.C., Nakatani, H. and Yoshida, M., 1993. Comparison of polymerase chain reaction with virus isolation and haemagglutination assays for the detection of canine parvoviruses in faecal specimens. *Research in Veterinary Science*, **55**, 60–63

Mochizuki, M., Horiuchi, M., Hiragi, H., San Gabriel, M.C., Yasuda, N. and Uno, T., 1996. Isolation of canine parvovirus from a cat manifesting clinical signs of feline panleukopenia. *Journal of Clinical Microbiology*, **34**, 2101–2105

Parrish, C.R., O'Connell, P.H., Evermann, J.F. and Carmichael, L.E., 1985. Natural variation of canine parvovirus. *Science*, **230**, 1046–1048

Parrish, C.R., Have, P.D., Foreyt, W.J., Evermann, J.F., Senda, M. and Carmichael, L.E., 1988. The global spread and replacement of canine parvovirus strains. *Journal of General Virology*, **69**, 1111–1116

Parrish, C.R., Aquadro, C.F., Strassheim, M.L., Evermann, J.F., Sgro, J.Y. and Mohammed, H.O., 1991. Rapid antigenic-type replacement and DNA sequence evolution of canine parvovirus. *Journal of Virology*, **65**, 6544–6552

Pereira, C.A.D., Monezi, T.A., Mehnert, D.U., D'angelo, M. and Durigon, E.L., 2000. Molecular characterization of canine parvovirus in Brazil by polymerase chain reaction assay. *Veterinary Microbiology*, **75**, 127–133

Sagazio, P., Tempesta, M., Buonavoglia, D., Cirone, F. and Buonavoglia, C., 1998. Antigenic characterization of canine parvovirus strains isolated in Italy. *Journal of Virological Methods*, **73**, 197–200

Senda, M., Parrish, C.R., Harasawa, R., Gamoh, K., Muramatsu, M., Hirayama, N. and Itoh, O., 1995. Detection by PCR of wild-type canine parvovirus which contaminates dog vaccines. *Journal of Clinical Microbiology*, **33**, 110–113

Steinel, A., Venter, E.H., Van Vuuren, M., Parrish, C.R. and Truyen, U., 1998. Antigenic and genetic analysis of canine parvoviruses in Southern Africa. *Onderstepoort Journal of Veterinary Research*, **65**, 239–242

Steinel, A., Munson, L., Van Vuuren, M. and Truyen, U., 2000. Genetic characterization of feline parvovirus sequences from various carnivores. *Journal of General Virology*, **81**, 345–350

Truyen, U., Steinel, A., Bruckner, L., Lutz, H. and Möstl, K., 2000. Die verteilung der antigenen typen des caninen parvovirus in der Schweiz, Österreich und Deutschland. *Schweizer Archiv für Tierheilkunde*, **142**, 115–119

(Accepted: 17 July 2004)