Molecular characterization and phylogenetic analysis of a canine parvovirus isolate in India

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ABSTRACT: Canine parvovirus 2 (CPV-2) is the causative agent of acute hemorrhagic enteritis and myocarditis in dogs. In this study the nucleotide sequence of the VP1/VP2 gene of a CPV isolate from India was analyzed and the phylogenetic relationship with other CPV isolates was established. Out of 36 samples analyzed, 16 were found positive for CPV-2 by polymerase chain reaction (PCR). Among the 16 positive samples, five were inoculated in MDCK cells for isolation out of which one adapted successfully to the cell culture system. Phylogenetic analysis based on the nucleotide sequence of the VP-1/VP-2 gene revealed that the Indian isolate closely resembled a CPV-2b Italian strain, showing 98.4% nucleotide sequence homology indicating very little genetic divergence since it was first identified in 1978.

Keywords: canine parvovirus; PCR; MDCK; phylogenetic analysis

CPV-2 causes a highly contagious and often fatal disease in dogs. CPV-2 emerged in 1978 as a cause of new disease in dogs throughout the world, when it rapidly spread in domestic dog populations as well as wild dogs with high morbidity (100%) and frequent mortality up to 10% (Appel et al., 1978). CPV-2 was shown to be closely related, genetically and antigenically to the feline panleukopenia virus (FPLV) and FPLV-like parvoviruses from wild carnivores (Parrish et al., 1991) from which it presumably originated by host species shift and subsequent rapid adaptation. A few amino acid substitutions between CPV and FPLV determine the ability for each virus to replicate in dogs and cats both *in vivo* and *in vitro* (Parker et al., 2001). In the 1980s, two antigenic variants of CPV-2, distinguishable using monoclonal antibodies (MAbs), emerged almost simultaneously and were termed as CPV-2a and CPV-2b (Parker et al., 2001). Currently, CPV-2a is the major field strain in Italy and Germany, while CPV-2b is common in USA, Taiwan and Japan (Battilani et al., 2001; Martella et al., 2004). Mutations affecting important residues of the capsid protein of CPV, such as residues 300 and 426, have been described recently, suggesting that CPV is still in the process of evolution (Martella et al., 2004; Nakamura et al., 2004). Further, Ikeda et al. (2002) reported that the CPV-2c (a) and CPV-2c (b) type viruses emerged from CPV-2a and CPV-2b type viruses in domestic and leopard cats in Vietnam. CPV-2c with a change (Asp426Glu) occurring in an important residue on the VP2 protein responsible for the antigenicity of CPV-2b has been detected in Vietnam, Italy, Spain, Germany, United Kingdom and South America (Nakamura et al., 2004; Decaro et al., 2007).

However, very few reports on antigenic and genetic characterization of CPV isolates from India are available. The purpose of this study was to genetically characterize a CPV isolate from India and to compare it with other published CPV strains.

MATERIAL AND METHODS

Madin darby canine kidney (MDCK) cell line

The MDCK cell line was obtained from the National Centre for Cell Science, Pune. It was maintained at the Virus lab in Dulbecco's modified Eagle's medium (DMEM) (Life technologies) with 10% fetal calf serum (FCS) as growth medium.

Faecal sample preparation

Dogs suffering from diarrhea and vomition and suspected of CPV infection were selected for the study. Fecal samples were collected from the dogs presented to the Veterinary Polyclinic IVRI, Izatnagar Bareilly (U.P) and a total of 36 samples were collected, out of which 15 were Pomerian, 7 German Shepard, 3 Doberman and 11 were mixed breed. The faecal samples were collected in the form of a rectal swab in Hank's balanced salt solution (HBBS) in a ratio of 1 : 9, containing streptomycin (100 mg/l) and penicillin (1 lakh IU/l). These were filtered through a disposable syringe filter (0.45 µm) (Millex, Milipore) and then centrifuged at 10 000 rpm at 4°C for 3 min in a refrigerated centrifuge. A commercially available inactivated vaccine was used as a positive control of CPV and a stool sample from a healthy dog processed similarly was used as a negative control.

PCR for confirmation of CPV-2

The primer set pCPV-RT (F) 5'-CAT TGG GCT TAC CAC CAT TT-3' (20-mer) and pCPV-RT (R) 5'-CCA ACC TCA GCT GGT CTC AT-3' (20-mer) from position 3136-3155 to 3276-3295 of VP1/VP2 gene of CPV-2 was custom designed and synthesized to yield an amplicon of 160 bp in PCR. The PCR was performed in a thermocycler (Applied Biosystems) using a reaction volume of 50 µl which contained 5 µl of *Taq* DNA polymerase buffer (10×), 3 µl of $MgCl_2$ (25mM), 200µM dNTPs, 10 pmol of each primer, 5 µl of processed sample as source of template DNA and 1 µl of *Taq* DNA polymerase (1 IU/µl). The thermal conditions comprised of initial denaturation at 94°C for 3 min, 30 cycles consisting of denaturation at 94°C for 30 s, annealing at 52°C for 1 min and extension at 72°C for 30 s, and a final extension at 72°C for 5 min. The PCR products were electrophoresed along with a 100 bp DNA ladder in 1% agarose gel containing 0.5 µg/ml ethidium bromide and progress of the mobility was monitored by migration of dye.

Isolation of virus and DNA extraction

MDCK cells were grown in 25cm^2 cell culture plastic flasks containing DMEM medium with 10% fetal calf serum and were used to isolate the virus by the adsorption method. When the cell monolayer was grown to 70%, it was washed with DMEM without serum and 0.5 ml of processed faecal sample was added from each of five faecal samples found positive by PCR and incubated for 1 h at 37°C for adsorption. After incubation, the infected cell monolayer was washed three times with DMEM and 5 ml of DMEM medium with 2% fetal calf serum (FCS) was added. The infected cells were incubated at 37°C for 3–5 days. Then the virus was harvested and kept at –20°C for further use.

The genomic DNA of CPV-2 was extracted using DNAzol reagent from the CPV-2 infected MDCK cell lines and genomic DNA was used to amplify the entire VP1/VP2 gene of the CPV (Sambrook and Russel, 2001).

PCR amplification of the VP1/VP2 gene of CPV-2

The primer set used to amplify the entire VP1/VP2 gene of the CPV in PCR was custom designed to yield an amplicon of 2.2 kbp. The forward primer has a restriction site for *Eco*RI while the reverse primer is possesses a restriction site for *Xba*I in order to facilitate its cloning and sub-cloning.
pCPV forward primer-5' GGG GAA TTC ATG GCA CCT CCG GCA AAG AGA 3' (30-mer)
pCPV reverse primer-5' GGC TCT AGA TTA ATA TAA TTT TCT AGG TGC TAG 3' (33-mer).

PCR was performed in 200 µl thin layered PCR tubes (Axygen) with a reaction volume of 50 µl. The reaction mixture contained 200µM dNTPs, 10 pmol of each primer, 5 µl of 10× *Taq* DNA polymerase buffer containing 15mM $MgCl_2$, 5 µl of extracted DNA as template and 1 µl of DNA polymerase (1 IU/µl). The cycling conditions consisted of initial denaturation at 95°C for 5 min followed by 30 cycles of 95°C for 1 min, at 55°C for 2 min and 72°C for 3 min. Final extension at 72°C was extended to 10 min (Nandi et al., 2008). After PCR, the amplified products were analyzed on a 1.0% agarose gel containing ethidium bromide to a final concentration of 0.5 µg/ml. 10 µl of amplified product was mixed with 2 µl of bromophenol dye (6×), loaded into the well and run in 1× TAE electrophoresis buffer at 5 volts/cm^2. Progress of mobility was monitored by migration of the dye. At the end of the electrophoresis, the gel was visualized under the UV transillumiator. The PCR products were purified from the gel using the QIAquick gel extraction kit (QIAGEN Inc. Valentia, USA) as per the manufacturer's protocol. After purification

1 µl of purified product was checked by agarose gel electrophoresis.

Cloning of the PCR product

The PCR product was cloned into the TOPO TA vector using the TOPO TA cloning kit following the manufacturer's instructions. Briefly, 4 µl of purified PCR product (25 ng DNA), 1 µl of salt solution (NaCl) and 1 µl of TOPO TA vector was used. It was then mixed gently and incubated at room temperature for 30 min. Competent cells were prepared from overnight culture of *E.coli* DH5α cells and transformation was carried out using the calcium chloride method. Five µl of the cloning mixture was mixed with 200 µl of competent cells and chilled on ice for 30 min. A heat shock of 42°C was given for 90 s and the cells were rapidly transferred onto ice for 5 min. Eight hundred µl of the LB medium was added to each vial and incubated at 37°C for 45 min to facilitate the growth of bacteria. LB agar containing 100 µg/ml ampicillin was poured into a Petri dish. These plates were allowed to solidify and an appropriate volume (200 µl) of the transformed competent cells was plated onto the agar. The plates were incubated at 37°C for 16–20 h to allow the growth of the transformed bacteria (Sambrook and Russell, 2001).

Plasmid DNA isolation and confirmation of the recombinants

The plasmid DNA from recombinant bacterial colonies was extracted by alkaline lysis followed by phenol-chloroform extraction (Sambrook and Russell, 2001). The recombinant plasmid was checked for the presence of the desired insert by restriction endonuclease mapping. The RE mapping of plasmid DNA was carried out using restriction enzymes *EcoR*I and *Xba*I designed as a primer pair, in order to release the insert for confirmation of positive clone. The reaction mixture consist of 2.0 µl of 10 × RE universal buffer, 0.5 µl (10 IU/µl) each of *Xba*I and *Eco*RI, 4 µl of plasmid DNA and nuclease free water to make 20 µl.

Characterization of PCR products by restriction enzyme mapping

RE analysis of the released PCR product of the VP1/VP2 gene of 2.2 kbp was carried out using the restriction enzyme *Pvu*II (Life technologies) selected on the basis of sequence analysis using M/S DNASTAR Inc, USA Software. To 4 µl of PCR product in three separate Eppendorf tubes, 1 µl of restriction enzyme (*Pvu*II) (10 IU/µl) was added with 2.0 µl of 10× respective RE buffer and the volume was made up to 20 µl with nuclease free water and incubated at 37°C for 4–6 h. The enzyme activity was stopped by freezing at –20°C. The obtained RE digests were electrophoresed in 1.2% agarose gel at 80 volts for 1 h. The gel was visualized under a U.V. transilluminator/Gel documentation system.

Nucleotide sequencing of the cloned insert

One stab culture tube containing an insert of the 2.2 Kbp amplicon of the VP-1/ VP-2 gene in the TOPO TA Cloning Vector was sent for sequencing to the DNA Sequencing Facility, University of Delhi, South Campus, New Delhi using the M13 reverse primer in an automated DNA sequencer (ABI PRISM310, Perkin Elmer). The entire VP1/VP2 gene of CPV was sequenced with Sanger's dideoxy chain termination method using primer walking. The sequences were aligned against the other published CPV VP1/VP2 gene sequences using M/S DNASTAR Inc, USA Software. The amino acid sequence, phylogenetic maps and percentage homology were deduced and analyzed from the sequences using the same software.

RESULTS

In this study, out of 36 samples tested, an amplicon of 160 bp size was obtained using primer set (pCPV-2RT) in 16 samples indicating the presence of CPV in all of these samples. In the positive control, there was amplification of template DNA whereas, in the negative control, no amplification of template DNA was visualized on an agarose gel (Figure 1). A total of five positive faecal samples in PCR were selected as an inoculum to infect a monolayer of MDCK cells. After first passage there was no visible cytopathic change in MDCK cells. All samples were subjected to three or more blind passages in MDCK cells and, out of five samples, only one showed cytopathic changes including rounding of cells, granulation and aggregation of cells 72 h post-infection which increased subsequently and

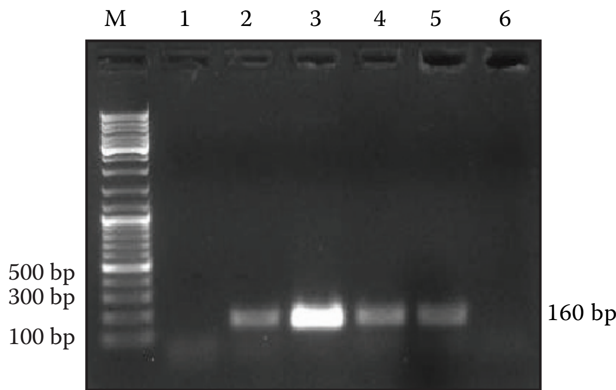

Figure 1. Agarose gel showing amplification of part of the VP-1/VP-2 gene (160 bp) using primer set pCPV-RT (F & R). Lane M = DNA marker 100 bp – 5 kbp, Lane 1 = faecal sample negative for CPV, Lane 2, 3 and 4 = faecal sample positive for CPV, Lane 5 = PCR product of positive control (Nobivac puppy DP vaccine), Lane 3 = no amplication in negative control (faecal sample of healthy dog)

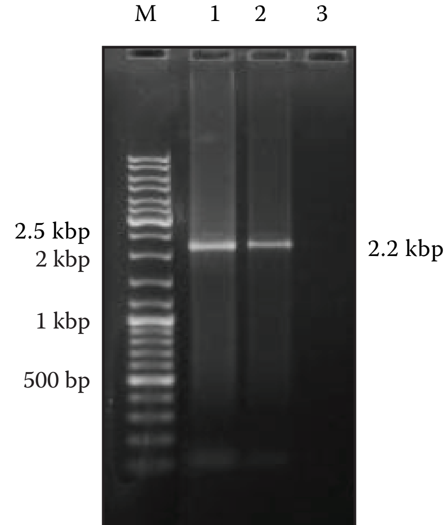

Figure 2. Agarose gel showing amplification of the entire VP-1/VP-2 gene (2.2 kbp) using primer set pCPV (F) and pCPV (R). Lane M = DNA marker 100 bp – 5 kbp, Lane 1 = 2.2 kbp PCR product of IVRI isolate, Lane 2 = 2.2 kbp PCR product of positive control (Nobivac puppy DP vaccine), Lane 3 = no amplication in negative control (faecal sample of healthy dog)

was widely distributed throughout the whole monolayer. Detachment of the cell monolayer from the surface was seen four days post-infection. Isolation of genomic DNA was carried out using the phenol-chloroform method from the cell culture supernatant of an adapted isolate (IVRI isolate) and PCR was carried out using pCPV primers. An amplicon of 2.2 kbp from the VP-1/VP-2 genes was observed on a 1% agarose gel from extracted DNA of the adapted isolate and from the positive control, but not in the fecal sample from a

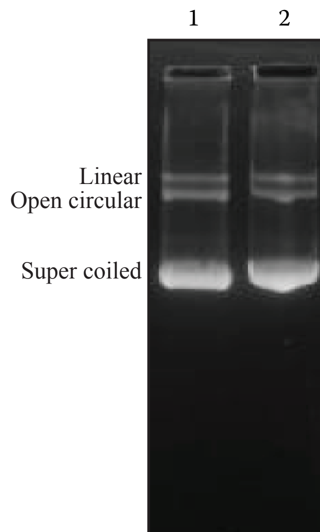

Figure 3. Recombinant TOPO TA plasmid DNA 2.2 kbp insert with three distinct bands. Lane 1 = recombinant plasmid DNA with 2.2 kbp insert with three distinct bands (IVRI isolate), Lane 2 = recombinant plasmid DNA with 2.2 kbp insert with three distinct bands (positive control; Nobivac puppy DP vaccine)

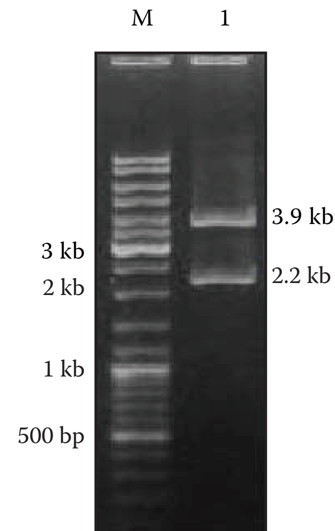

Figure 4. Double digestion of the cloned PCR product (2.2 kbp) in the TOPO TA vector with *Eco*RI and *Xba*I enzymes. Lane M = DNA marker 100 bp – 5 kbp, Lane 1 = release of 2.2 kbp PCR insert of IVRI isolate and TOPO TA vector

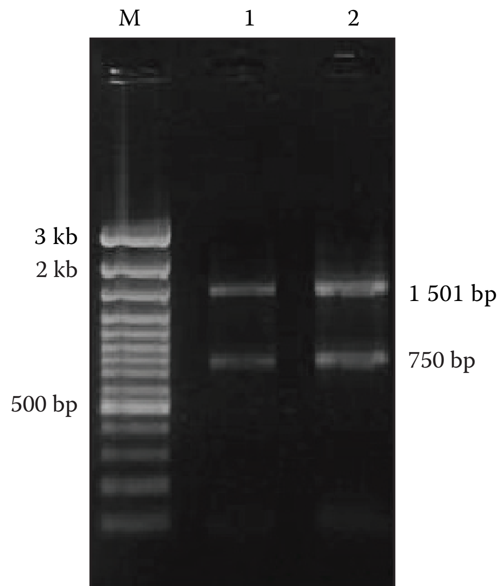

Figure 5. Digestion of the cloned PCR product (2.2kbp) in TOPO TA vector with *Pvu*II enzymes. Lane M = DNA marker 100 bp – 5 kbp, Lane 1 = release of 774 bp fragment from the VP-1/VP-2 gene of the IVRI isolate, Lane 2 = release of 774 bp fragment from the VP-1/VP-2 gene of the positive control

healthy dog (Figure 2). The purified product was rechecked and a distinct single band of 2.2 kbp was observed.

The purified product of 2.2 kbp from the VP-1/VP-2 genes was used for ligation into the TOPO TA cloning vector and transformation was carried out in *E. coli* DH5α cells. Three recombinant colonies were picked and grown in LB broth containing ampicillin and recombinant plasmid DNA was extracted. 3 μl of extracted plasmid DNA was electrophoresed on a 1% agarose gel and three bands (super coiled, open circular and linear) were observed on gel documentation (Figure 3). There was release of insert DNA (2.2 kbp) from the vector when recombinant plasmid DNA was digested with *Eco*RI and *Xba*I as visualized under UV with a transilluminator (Figure 4). The 2.2 kbp PCR product of the VP1/VP2 gene of CPV was digested with *Pvu*II and two fragments of size 1501 bp and 755 bp in length were obtained thus substantiating the results of PCR and confirming the authenticity of the product (Figure 5).

The recombinant plasmids containing the correct inserts of the field isolate were sequenced. The sequence of the entire VP1/VP2 structural gene (size 2.2 kbp) was obtained and sequence data also confirmed that our desired inserts were cloned in the correct orientation in the TOPO TA vector. The nucleotide sequencing data was aligned and was phylogenetically characterized and compared using the published nucleotide sequence data of different CPV-2 isolates, feline panleukopaenia virus (FPV) and mink enteritis virus (MEV) available on the NCBI, with the help of MegAlign DNASTAR Inc, USA software and a phylogenetic tree (Cladoram). These are depicted in Figure 6. Phylogenetic analysis, showing percent similarity in the upper triangle and percent divergence in the lower triangle of different isolates is shown in Table 1. The phylogenetic analysis, based on the nucleotide sequence of the VP-1/VP-2 gene, revealed that our virus isolate closely resembled a CPV-2b Italian strain (Accession number AY900660), with which it showed 98.4% homology and only 1.6% divergence. The IVRI isolate showed 98.3% homology and 1.7% divergence when compared with a Brazil CPV-2a isolate. The percent similarity between the IVRI isolate and FPLV at nucleotide level was 97.7 and the percent divergence was 2.3. The IVRI isolate showed 97.8% homology and 2.2% divergent with MEV (Table 1). Analysis of the VP1/VP2 gene sequence of the IVRI isolate at nucleotide positions 760, 803, 1400, 1414, 1 600, 1642 and 1777 (within 1 to 2.2) showed that it belongs to CPV type 2b.

Table 1. Phylogenetic analyses of the CPV-2 IVRI isolate showing, percent homology with other CPV-2 mutants, FPV, and MEV, in the upper triangle and percent divergence in the lower triangle

Types	IVRI isolate	Italy isolate 2b	Brazil isolate 2a	FPV	MEV	
IVRI isolate		98.4	98.3	97.7	97.8	IVRI isolate
Italy isolate 2b	1.6		99.2	98.5	98.8	Italy isolate 2b
Brazil isolate 2a	1.7	0.8		98.6	98.8	Brazil isolate 2a
FPV	2.3	1.5	1.4		99.4	FPV
MEV	2.2	1.3	1.3	0.6		MEV

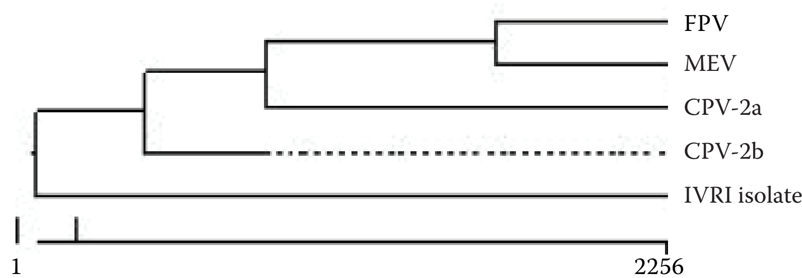

Figure 6. Phylogenetic analysis of CPV-2b (IVRI isolate) using other CPV-2 mutants, FPV and MEV

This was further confirmed by the presence of aspartate at position 426 in the deduced amino acid sequence of the IVRI isolate which was found to be CPV-2b mutant (sequence data unpublished). Phylogenetic analysis also revealed that CPV variants are not only closely related among themselves but also show minimum divergence from ancestors like FPV indicating very little divergence since it was first identified in 1978 (Appel et al., 1978). There was no appreciable variation within the various strains. The variations that were observed nevertheless played a crucial role in the evolution of different variants in several geographical regions of the world.

DISCUSSION

Currently, CPV-2a and CPV-2b along with CPV-2c are prevalent at different proportions in several countries. However, various studies have reported CPV-2b as the virus responsible for most outbreaks of CPV infection throughout the world. CPV-2b was found to be the most prevalent antigenic variant in Southern Africa (Steinel et al., 1998) and Brazil (Pereira et al., 2000; Costa et al., 2005). In contrast, both CPV-2a and CPV-2b were reported to be prevalent in equal proportion in the UK, Germany and Spain (Ybanez et al., 1995). CPV-2a was reported to be predominant in Taiwan and Italy (Sagazio et al., 1998). In India, it has been reported that CPV-2b is the major antigenic variant of CPV-2 along with CPV-2a at a smaller proportion in Bareilly region of U.P., India. The analysis of CPV strains detected in India revealed the evolution of an unusual CPV-2 mutant, with a change (Asp-426 to Glu) occurring in the strategic residue 426 and designated CPV-2c (Nandi et al., 2009a,b). However, these observations were in contrast with the findings of Chinchkar et al. (2006) who reported that CPV-2a is the major antigenic variant prevalent in Southern and Central India, based on VP2 gene sequences. Further, it was revealed that the Indian isolates formed a separate lineage distinct from the South East Asian isolates and the canine parvovirus isolates in India appear to have evolved independently without any distinct geographical patterns of evolution (Chinchkar et al., 2006). CPV-2 is closely related to feline panleukopenia virus (FPV) with less than 1% nucleotide sequence divergence and as few as six coding nucleotide differences in the VP1/VP2 protein (positions: 3025, 3065, 3094, 3753, 4477 and 4498) (Truyen et al., 1995; Parrish, 1999). The biological effects of these few genomic changes were sufficient for CPV-2 to acquire a canine host range, but it has lost the ability to replicate in a feline host (Truyen et al., 1995). The common differences in CPV-2a and CPV-2b compared to the original CPV-2 are confined to three coding nucleotides at positions 3045, 3685, and 3699. CPV-2a and CPV-2b differ in the presence of two single-nucleotide polymorphisms (SNPs) in the capsid protein gene sequences which determine amino acid changes in the major antigenic sites of the viral capsid. Furthermore, CPV-2a, -b acquired the ability to infect, and cause disease, in cats. A Single nucleotide polymorphism, i.e., (SNP) A (adenine) to T (guanine) occurring at position 4062 of the viral genome is responsible for the presence of the amino acid Asn (type 2a) or Asp (type 2b) at residue 426 of the VP2 protein whereas the SNP A (adenine) to G (cytosine) encountered at position 4449 determines the presence of the amino acid Ile (type 2a) or Val (type 2b) at residue 555 (Parrish et al., 1991). Further, the substitution of Asp 426 Glu is due to a change (T to A) in the third codon position at nucleotide 4064 of type 2b and has led to the emergence of the CPV type 2c (Buonavoglia et al., 2001). Mutations affecting important antigenic epitopes of CPV-2 are identifiable by a panel of MAbs used commonly to characterize CPV strains antigenically (Decaro et al., 2005). However, the emergence of new variants represents

a constant threat to domestic dogs. The reason for the worldwide distribution of these variants is still obscure. The coexistence of CPV-2a, CPV-2b and CPV-2c at different ratios in various countries shows that there is no evolutionary advantage of one type over other and that this coexistence has not evolved under immuno-selective pressure from vaccines (Parrish et al., 1991; Steinel et al., 1998). However, continued epidemiological surveillance and sequence analysis will help to uncover the presence of mutations and will provide insights into the prevalence of different antigenic variants of CPV.

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REFERENCES

Appel M.J.G., Cooper B.J., Greisen H., Carmichael L.E. (1978): Status report: canine viral enteritis. Journal of the American Veterinary Medicine Association, 173, 1516–1518.

Battilani M., Scagliarini A., Tisato E., Turilli C., Jacoboni I., Casadio R., Prosperi S. (2001): Analysis of canine parvovirus sequences from wolves and dogs isolated in Italy. Journal of General Virology, 82, 1555–1560.

Buonavoglia C.V., Martella A., Pratelli M., Tempesta A., Cavalli D., Bozzo G., Decaro N., Carmichael L.E. (2001): Evidence for evolution of canine parvovirus type-2 in Italy. Journal of General Virology, 82, 1555–1560.

Chinchkar S.R., Mohana Subramaniam B., Hanumantha Rao N., Rangarajan P.N., Thiagarajan D., Srinivasan V.A. (2006): Analysis of VP2 gene of canine parvovirus in India. Archives of Virology, 151, 1881–1887.

Costa A.P., Leite J.P.G., Labarthe N.V., 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. Veterinary Research Communication, 29, 735–743.

Decaro N., Elia G., Campolo M., Desario C., Lucente M.S., Bellacicco A.L., Buonavoglia C. (2005): New approaches for the molecular characterization of canine parvovirus type 2 strains. Journal of Veterinary Medicine B, 52, 316–319.

Decaro N., Desario C., Addie D.D., Martella V., Vieira M.J., Elia G., Davis C., Thompson G., Truyen U., Buonavoglia C. (2007): The study of molecular epidemiology of canine parvovirus, Europe. Emerging Infectious Diseases, 13, 1222–1224.

Ikeda Y., Nakamura K., Miyazawa T., Tohya Y., Takahashi E., Mochizuki M. (2002): Feline host range of canine parvovirus: recent emergence of new antigenic types in cats. Emerging Infectious Disease, 6, 341–346.

Martella V., Cavalli A., Pratelli A., Bozzo G., Camaro M., Buonavoglia D., Narcisi D., Buonavoglia C. (2004): A canine parvovirus mutant is spreading in Italy. Journal of Clinical Microbiology, 42, 1333–1336.

Nakamura M., Tohya Y., Miyazawa T., Mochizuki M., Phung H.T., Huynh L.M., Nguyen L.T., Nguyen N.P., Akashi H. (2004): A novel antigenic variant of canine parvovirus from a Vietnamese dog. Archives of Virology, 149, 2261–2269.

Nandi S., Pandey A.B., Sharma K., Chauhan R.S. (2008): Polymerase chain reaction for the detection of canine parvoviral DNA in vaccines. Indian Journal of Virology, 19, 9–11.

Nandi S., Chidri S., Kumar M., Chauhan R.S. (2009a): Occurrence of canine parvovirus type 2c in the dogs with haemorrhagic enteritis in India. Research in Veterinary Science, (Accepted, article in press; doi:10.1016/j.rvsc.2009.05.018).

Nandi S., Anbazhagan R., Kumar M., Chauhan R.S. (2009b): Molecular characterization of canine parvo virus strains in vaccines by polymerase chain reaction and restriction endonuclease analysis. Indian Journal of Virology, 20, 12–15.

Parker J.S., Murphy W.J., Wang D., O'Brien S.J., Parrish C.R. (2001): Canine and feline parvoviruses can use human or feline transferrin receptors to bind, enter, and infect cells. Journal of Virology, 75, 3896–3902.

Parrish C.R. (1999): Host range relationship and the evolution of canine parvovirus. Veterinary Microbiology, 69, 29–40.

Parrish C.R., Aquadro C.F., Strassheim M.L. (1991): Rapid antigenic-typereplacement and DNA sequence evolution of canine parvovirus. Journal of Virology, 65, 6544–6552.

Pereira C.A., Monezi T.A., Mehnert D.U., D'Anglo M., 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., Buonavoglia C. (1998): Antigenic characterization of canine parvovirus strains isolated in Italy. Journal of Virological Methods, 73, 197–200.

Sambrook J., Russell D.W. (2001): Molecular cloning; A laboratory Manual. 3rd ed. Cold Spring Harbor Laboratory Press, New York. 1.31–1.38, 1.116–1.118.

Steinel A., Venter E.H., Van Vuuren M., Truyen U. (1998): Antigenic and genetic analysis of canine parvo virus in Southern Africa. Ondersteport Journal of Veterinary Research, 65, 239–242.

Truyen U., Gruneberg S.F., Chang B., Obermaier P., Veijalainen A., Parrish C.R. (1995): Evolution of the feline-subgroup of parvoviruses and the control of canine host range *in vivo*. Journal of Virology, 69, 4702–4710.

Ybanez R.R., Vela C., Cortes E., Simarro I., Casal J.I. (1995): Identification of types of canine parvovirus circulating in Spain. Veterinary Record, 136, 174–175.

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