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Isolation and characterization of feline panleukopenia virus from a diarrheic monkey

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ABSTRACT

A feline panleukopenia virus (FPV) mutant, monkey/BJ-22/2008/CHN, was isolated from intestinal contents of a diarrheic monkey in Beijing, China. The virus was identified by morphology and physicochemical characteristics, and specific fragments were obtained by PCR using consensus primers of parvovirus and specific primers of FPV. Sequence of the full-length VP2 gene of the isolated FPV was determined and analyzed by comparison with reference FPV and canine parvovirus (CPV) isolates, showing high homology with FPV (98.75%) and CPV (98.15%). Phylogenetic analysis indicated that the isolated FPV formed a monophyletic branch in FPV cluster which differed from the other 11 FPV isolates from China and other countries. The isolated virus caused typical clinical symptoms of FPV in cats. This is the first report on isolation of FPV from a monkey.

1. Introduction

Animal parvoviruses, belonging to the genus *Parvovirus* within the family *Parvoviridae*, are usually responsible for acute gastroenteritis and leukopenia in young animals. Within the genus *Parvovirus*, there are several closely related viruses including feline panleukopenia virus (FPV), mouse minute virus (MMV), mink enteritis virus (MEV) and canine parvovirus (CPV) (Hueffer and Parrish, 2003; Steinel et al., 2001). FPV has been known since the 1920s, whereas CPV only emerged as a dog pathogen in the late 1970s (Appel et al., 1979; Carmichael and Binn, 1981). The origin of CPV is still not clear but it is arguable that CPV originated from FPV or a very closely related carnivore parvovirus (Barker et al., 1983; Horiuchi et al., 1998; Hueffer et al., 2004).

The important difference between these two parvoviruses is that FPV varies at a slow rate by random genetic drift, whereas CPV shows genomic substitution rates similar to those of RNA viruses, with values of about 10^{-4} substitutions per site per year under selection pressure (Hoelzer et al., 2008; Shackelton et al., 2005). Evidence suggests that the original type 2 (CPV-2) came from FPV infecting only cats in 1978, and gave rise to the antigenic variant CPV-2a through five or six amino acid mutations in the major capsid protein VP2 during 1979–1981 (Parrish et al., 1988; Parrish et al., 1985). A second variant, CPV-2b, was identified in 1986, which naturally infects dogs and cats (Parrish et al., 1991). A third variant, CPV-2c, was discovered in Italy in 2000, displaying an exceptional ability to spread rapidly through the canine population in that country (Buonavoglia et al., 2001; Martella et al., 2005), as well as in other European countries, Asia (Nakamura et al., 2004) and America (Kapil et al., 2007). Although the first reports suggested a low pathogenicity of CPV-2c, experimental data and field observations now indicate a more severe clinical course and higher mortality rates associated with CPV-2c infection, as well as an ability to infect and cause disease in adult dogs, even if repeatedly vaccinated (Decaro et al., 2008a).

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Acute infectious diarrhea was observed in monkeys in the Experimental Animals Center in Beijing, China in 2008, with clinical symptoms similar to canine/feline parvovirus enteritis. While there have not previously been any reports of monkeys infected with CPV or FPV, an FPV mutant was isolated from the intestinal contents of one of the affected monkeys. We report here the nucleotide sequence of the capsid protein gene of the new isolate and compare it with those of reference FPV and CPV strains.

2. Materials and methods

2.1. Clinical case

In June 2008, a severe outbreak of haemorrhagic enteritis occurred in monkeys from an Experimental Animals Center located in Beijing, China, where there were about 2000 monkeys, belonging to rhesus monkey (*Macaca mulatta*) and crab-eating monkey (*Macaca fascicularis*). The bloody purulent stool first emerged in some rhesus monkeys. Three weeks later, about 200 (70%) 2-year-old young monkeys in a building displayed haemorrhagic diarrhoea, fever and anorexia, and underwent a fatal outcome with a mortality of 50%, within 3–5 days after the onset of the clinical signs. There were also about 300 adult monkeys presented mild clinical signs, among which 10% died. The HI titers of healthy, died, and recovered monkeys were 2^{7-9}, 2^{6-7}, and 2^{10-11}, respectively. All these monkeys did not directly contact with cats and dogs, whose disease was probably related to some monkeys recently bought from GuangXi Provine, China.

2.2. Virus isolation

The intestinal contents of a diarrheic monkey were collected from diarrheic monkeys, and virus was isolated as described elsewhere (Kapil et al., 2007). Briefly, the faecal sample was homogenized (10%, w/v) in phosphate buffered saline (PBS, pH 7.2) and subsequently clarified by centrifugation at 12,000 × *g* for 15 min. The samples were treated with penicillin and streptomycin at 4 °C overnight and were then inoculated into F81 cells, Vero cells and DK cells, respectively, which were purchased from China Institute of Veterinary Drug Control. Inoculated cell cultures showing cytopathic effects were used for morphology, physical and chemical properties. Hemagglutination (HA) assay was performed according to the protocol described elsewhere (Carmichael et al., 1980).

2.3. PCR amplification and sequence analysis

Consensus primers of parvovirus (P1: 5′-GGATGGGTGGAAATCACAGC-3′; P2: 5′-ATAACCAACCTCAGCTGGTC-3′) were used as described previously (Pereira et al., 2000) to identify the FPV isolated from monkey. PCR amplification was performed with 30 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s, and extension at 72 °C for 45 s with a recombinant Taq DNA polymerase. Specific primers of FPV (P3: 5′-AAAGAGTAGTTGTAAATAATT-3′; P4: 5′-CCTATATAACCAAAGTTAGTAG-3′) were also used to amplify the isolated virus. The PCR assays were performed with 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 45 s, and extension at 72 °C for 45 s.

The full-length VP2 gene of the isolated FPV was cloned and sequenced using primers (P5: 5′-CTC*GGATCC*CCAATGAGTGATGGAGCAGTTCAACCAGAC-3′; P6: 5′-AAC*CTCGAG*CTAGGTGCTAGTTGATATGTAATAAAC-3′) in routine methods (Spitzer et al., 1997). The sequences of gene and deduced protein from VP2 were analyzed using DNASIS and DNAstar software, and compared with other sequences of VP2 from other parvovirus isolates.

2.4. Phylogeny

The phylogenetic relationships were evaluated with Mega version 4.0 (http://www.megasoftware.net/). A phylogenetic tree was constructed by the neighbor-joining method and a bootstrap analysis with 1000 replicates was done to assess the confidence level of the branch pattern. Bootstrap values >70% were considered to be significant.

2.5. Artificial infection of cats

Twenty 3-month-old healthy cats were purchased from the Center of Experimental Animals, Jilin University, China. All cats had undetectable levels of FPV serum neutralizing (SN) antibodies (titer < 1:2). They were randomly divided into two equal groups. One group was injected orally with 3 ml medium containing 2.9×10^6 virus/ml, the other served as negative control, received orally the same dose of media without virus. Treated cats were observed for clinical symptoms for 14 days, and their peripheral white blood cells (WBC) were counted daily for 5 days. At day 4 post-infection, their feces were collected and assayed for virus by PCR.

3. Results

3.1. Virus isolation

Typical CPE of parvovirus appeared in F81 cells inoculated with the isolated virus, and 20–24 nm virus particles with cubic symmetry, typical of parvovirus, were observed. However, no CPE was present in Vero or DK cells. The virus was highly resistant to heat (50° for 30 min), 20% ether, acid (pH 3.0), but was susceptible to 5-IUDR (data not shown). HA assay indicated that the highest HA titer of the virus was 1:512 with porcine erythrocytes, and <1:2 for chicken, guinea pig, rat and human erythrocytes. All the characteristics were consistent with those of a parvovirus. The isolated virus strain was designated as monkey/BJ-22/2008/CHN.

3.2. PCR amplification and sequence analysis

Consensus primers of parvovirus were used to distinguish parvovirus from other viruses, and a gene fragment of 845-bp was amplified. Moreover, a gene fragment of 618-bp was amplified using specific primers for FPV, indicating that the isolated virus is an FPV strain.

The full-length VP2 gene of the isolated FPV was cloned and sequenced, and a 1755-bp gene encoding for a 584-amino acid VP2 protein was identified (GenBank

Table 1
Host-specific sites in VP2 genes of FPV and CPV strains (nucleotide position).

Name	Genetic type	GenBank number	239	259	279	308	899	913	967	1276	1691	1703
BJ-22	FPV	FJ231389	A	A	A	T	C	G	A	A	G	C
PLI-IV	FPV	D88287	A	A	A	T	C	G	G	A	A	C
CPV-b	CPV-2	M38245	G	A	C	C	C	G	A	A	G	G
V154	CPV-2a	AB054217	G	T	C	C	G	T	A	A	G	G
V139	CPV-2c(a)	AB054221	G	T	C	C	A	T	A	A	G	G
V203	CPV-2c(b)	AB054224	G	T	C	C	A	T	A	G	G	G

Table 2
Host-specific amino acids in the VP2 protein of FPV and CPV strains (amino acid position[a]).

Name	Genetic type	80	87	93	103	300	305	323	426	564	568
BJ-22	FPV	K	M	K	V	A	D	N	N	S	A
PLI-IV	FPV	K	M	K	V	A	D	D	N	N	A
CPV-b	CPV-2	R	M	N	A	A	D	N	N	S	G
V154	CPV-2a	R	L	N	A	G	Y	N	N	S	G
V139	CPV-2c(a)	R	L	N	A	D	Y	N	N	S	G
V203	CPV-2c(b)	R	L	N	A	D	Y	N	D	S	G

[a] The highlight colors black and grey denote FPV- and CPV-specific amino acids, respectively.

accession number FJ231389). Sequence comparison showed nucleotide identities of 98.75% with FPV, and 98.15% with CPV.

The obtained sequence was compared using DNAstar with parvovirus reference strains from GenBank (Table 1). Results showed that there were eight nucleotides identical to FPV, and two nucleotides identical to CPV among the 10 important nucleotides encoding host-specific amino acids of FPV and CPV (Table 1), with resulting amino acid changes (Table 2).

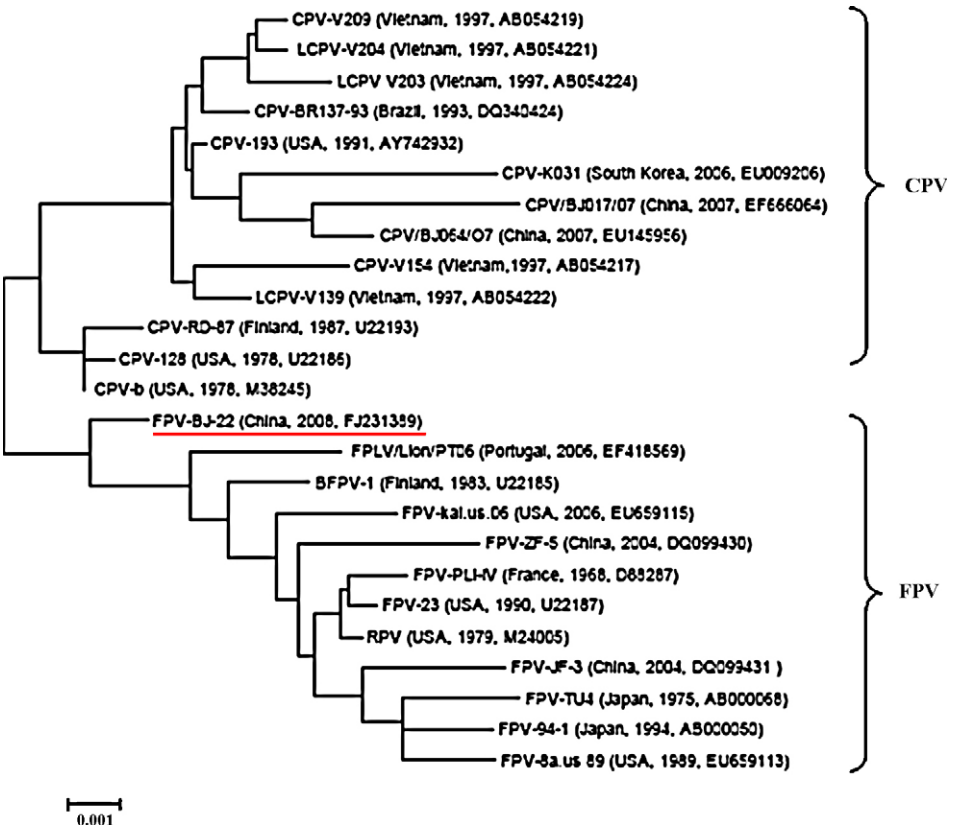

Fig. 1. Phylogenetic relationships among the different parvovirus isolates based on VP2 nucleotide sequences. The tree was inferred by the MEGA4 program using the neighbor-joining method. For each strain, the country and year of isolation and the GenBank accession number are indicated.

3.3. Phylogeny

A phylogenetic tree was constructed from the full-length VP2 nucleotide sequence obtained here and additional sequences retrieved from the GenBank database. It was evident that the isolated FPV from monkey formed a monophyletic branch in the FPV cluster, separating it from the other 11 FPV isolates from China and other countries (Fig. 1).

3.4. Artificial infection of cats

Cats given the isolated virus intraperitoneally and orally all presented with fever, anorexia, dehydration, depression, vomiting, and leukopenia, and all died between days 9 and 14 post-infection. Leukocyte counts in infected cats ranged from 3000 to 5000/mm^3 (normal 15,000–20,000/mm^3), and serious pathological changes were apparent. However, no clinical symptoms were observed in the control groups administered medium without virus.

Intestinal mucous membranes from dead cats were collected and DNA was extracted. The FPV VP2 gene was amplified from all cats infected with the isolated virus by PCR and sequenced (data not shown), indicating that the VP2 was identical to that of the challenged virus.

4. Discussion

FPV and CPV-2, which are very closely related viruses with a genome homology with 98%, are significant pathogens for domestic cats and dogs as well as for various wild carnivore species (Steinel et al., 2000). However, these two closely related viruses differ antigenically and in their host ranges. FPV replicates only in feline cells *in vitro* and in cats *in vivo* whereas CPV-2 replicates in canine and feline cells *in vitro*, but does not infect cats *in vivo* (Truyen and Parrish, 1992).

The host range difference between FPV and CPV-2 is determined by six nucleotides (positions 3025, 3065, 3094, 3753, 4477 and 4498) in the open reading frame (ORF) encoding structural proteins VP1 and VP2 (Steinel et al., 2000). These amino acid changes affect the viral surface structure facilitating replication in canine cells and leading to loss of the feline host range (Parker and Parrish, 1997). Amino acids at positions 80, 93, 103, 323, 564 and 568 of VP2 are conserved among all CPV and FPV viruses (Steinel et al., 2000; Decaro et al., 2008a,b). CPV sequences at amino acid positions 93, 103 and 323 are critical for the ability to replicate in the dog, whereas the FPV sequence at amino acids 80, 564 and 568 are required for replication in the cat. The feline host range of the new antigenic types CPV-2a and CPV-2b is most likely determined by amino acid changes at positions 87, 300 and 305 (Truyen et al., 1996; Parrish, 1999). Amino acid differences at positions 87, 300 and 305 define the new antigenic types CPV-2a and -2b. Amino acid 426 is conserved in FPV, CPV-2 and CPV-2a viruses but is different in CPV-2b virus (Parrish et al., 1991; Steinel et al., 2000). The amino acids at positions 323 and 564 of VP2 from the isolated parvovirus are identical with those of CPV, and the other eight positions are identical to FPV (Table 2). The amino acid changes at positions 323 and 564 of VP2 from FPV may alter the host range of FPV, gaining the ability to infect monkeys, but this remains to be determined. The parvovirus HI titers in the monkeys indicated that these monkeys have certain anti-FPV antibody titers, which did not effectively protect against FPV infection. However, the recovered monkeys can produce higher anti-FPV antibody titers and can protect against FPV infection.

It is well established that certain important human infectious diseases, such as severe acute respiratory syndrome (SARS) and highly pathogenic avian influenza (H5N1, H1N1), have origins in non-human hosts. Gene mutation, deletion or arrangement of some key sites in these viruses have been responsible for changes of their host range and pathogenicity. The isolation of an FPV mutant from a primate, therefore, provides some cause for concern, and indicates the need to conduct further studies of its pathogenesis and differential diagnosis.

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References

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

Barker, I.K., Povey, R.C., Voigt, D.R., 1983. Response of mink, skunk, red fox and raccoon to inoculation with ink virus enteritis, feline panleukopenia and canine parvovirus and prevalence of antibody to parvovirus in wild carnivores in Ontario. Can. J. Comp. Med. 47, 188–197.

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.

Carmichael, L.E., Binn, L.N., 1981. New enteric viruses in the dog. Adv. Vet. Sci. Comp. Med. 25, 1–37.

Carmichael, L.E., Joubert, J.C., Pollock, R.V., 1980. Hemagglutination by canine parvovirus: serologic studies and diagnostic applications. Am. J. Vet. Res. 41, 784–791.

Decaro, N., Desario, C., Elia, G., Martella, V., Mari, V., Lavazza, A., Nardi, M., Buonavoglia, C., 2008a. Evidence for immunisation failure in vaccinated adult dogs infected with canine parvovirus type 2c. New Microbiol. 31, 125–130.

Decaro, N., Desario, C., Miccolupo, A., Campolo, M., Parisi, A., Martella, V., Amorisco, F., Lucente, M.S., Lavazza, A., Buonavoglia, C., 2008b. Genetic analysis of feline panleukopenia viruses from cats with gastroenteritis. J. Gen. Virol. 89, 2290–2298.

Hoelzer, K., Shackelton, L.A., Parrish, C.R., Holmes, E.C., 2008. Phylogenetic analysis reveals the emergence, evolution and dispersal of carnivore parvoviruses. J. Gen. Virol. 89, 2280–2289.

Horiuchi, M., Yamaguchi, Y., Gojobori, T., Mochizuki, M., Nagasawa, H., Toyoda, Y., Ishiguro, N., Shinagawa, M., 1998. Differences in the evolutionary pattern of feline panleukopenia virus and canine parvovirus. Virology 249, 440–452.

Hueffer, K., Parrish, C.R., 2003. Parvovirus host range, cell tropism and evolution. Curr. Opin. Microbiol. 6, 392–398.

Hueffer, K., Truyen, U., Parrish, C.R., 2004. Evolution and host variation of the canine parvovirus: molecular basis for the development of a new virus. Berl. Munch. Tierarztl. Wochenschr. 117, 130–135 (in German, with English abstract).

Kapil, S., Cooper, E., Lamm, C., Murray, B., Rezabek, G., Johnston 3rd, L., Campbell, G., Johnson, B., 2007. Canine parvovirus types 2c and 2b circulating in North American dogs in 2006 and 2007. J. Clin. Microbiol. 45, 4044–4047.

Martella, V., Decaro, N., Elia, G., Buonavoglia, C., 2005. Surveillance activity for canine parvovirus in Italy. J. Vet. Med. 52, 312–315.

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.

Parker, J.S., Parrish, C.R., 1997. Canine parvovirus host range is determined by the specific conformation of an additional region of the capsid. J. Virol. 71, 9214–9222.

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.

Parrish, C.R., Burtonboy, G., Carmichael, L.E., 1988. Characterization of a nonhemagglutinating mutant of canine parvovirus. Virology 163, 230–232.

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

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.

Shackelton, L.A., Parrish, C.R., Truyen, U., Holmes, E.C., 2005. High rate of viral evolution associated with the emergence of carnivore parvovirus. PNAS 102, 379–384.

Spitzer, A.L., Parrish, C.R., Maxwell, I.H., 1997. Tropic determinant for canine parvovirus and feline panleukopenia virus functions through the capsid protein VP2. J. Gen. Virol. 78, 925–928.

Steinel, A., Munson, L., van Vuuren, M., Truyen, U., 2000. Genetic characterization of feline parvovirus sequences from various carnivores. J. Gen. Virol. 81, 345–350.

Steinel, A., Parrish, C.R., Bloom, M.E., Truyen, U., 2001. Parvovirus infections in wild carnivores. J. Wildl. Dis. 37, 594–607.

Truyen, U., Evermann, J.F., Vieler, E., Parrish, C.R., 1996. Evolution of canine parvovirus involved loss and gain of feline host range. Virology 215, 186–189.

Truyen, U., Parrish, C.R., 1992. Canine and feline host ranges of canine parvovirus and feline panleukopenia virus: distinct host cell tropisms of each virus *in vitro* and *in vivo*. J. Virol. 66, 5399–5408.