Evidence of feline panleukopenia infection in cats in India

Manoharan Parthiban · Komandur Seshadri Aarthi ·
Mani Balagangatharathilagar ·
Kathaperumal Kumanan

Received: 5 June 2014 / Accepted: 8 October 2014 / Published online: 1 November 2014
© Indian Virological Society 2014

Abstract The samples collected from cats showing clinical signs suspected for feline panleukopenia infection were confirmed using various molecular techniques and virus isolation. The suspected samples were confirmed using feline parvovirus specific primers. The partial VP₂ gene was submitted to GenBank for the first time in India (Accession number JQ684660.1). The PCR positive samples were further amplified using full length FPV VP₂ gene specific primers and sequenced. The blast analysis revealed that the local field isolates of FPV showed 99 % homology with other FPV sequences available in the GenBank. The evidence for occurrence of feline panleukopenia infection in cats in Tamil Nadu, India was further confirmed by host specific nucleotides present in the VP₂ gene region as well as virus isolation in A72 cell line.

Keywords Feline panleukopenia · VP₂ gene · Polymerase chain reaction · Phylogenetic analysis · Host specific nucleotides

Feline panleukopenia is caused by the feline parvo or panleukopenia virus; a linear single stranded DNA of about 5 kb lengths with unique hairpin structures at both ends belonging to the Parvoviridae family [14]. Feline parvovirus (FPV) has been known since 1920s, whereas canine parvovirus (CPV) only emerged as a dog pathogen in the late 1970s [1]. Feline panleukopenia is a highly contagious viral disease of cats characterized by its sudden onset, fever, loss of appetite, dehydration, depression, vomiting,

decreased numbers of circulating white blood cells (leukopenia), and often a high mortality rate [9]. Feline panleukopenia virus (FPV) is most commonly transmitted by direct contact of susceptible animals with infected cats or their secretions. It is shed from all body secretions during the active stages of the diseases but is most consistently recovered from intestine and feces [4].

All members of the cat family (Felidae) are susceptible to infection with FPV. Many excellent vaccines are available to protect cats against panleukopenia. In unvaccinated populations, however panleukopenia remains the most severe and destructive disease of cats [3]. Rapid diagnosis is especially important in order to isolate infected cats and prevent secondary infections. Many laboratory techniques have been developed like haemagglutination test, enzyme linked immunosorbent assay, immunofluorescence antibody test and polymerase chain reaction since clinical diagnosis is not definitive [8].

There are lot of research work has been done in India regarding the prevalence of CPV [5, 7, 11] and however, no scientific evidence for prevalence of FPV in India. Hence in this study, attempts were made to confirm the presence of FPV by molecular methods followed by virus isolation and also phylogenetic analysis was done to find out the relationship of our local FPV strains with other FPV strains. The host specific nucleotides present in VP₂ region of our isolates were compared with reference strains to confirm the prevalence of FPV in Tamil Nadu, India.

A total of sixty samples (eight intestinal samples from dead cats and fifty-two faecal samples) were collected from animals showing clinical symptoms such as diarrhea, persistent vomiting and hemorrhagic enteritis in clinics ward, Madras Veterinary College, Tamil Nadu, India during the year 2012 and 2013. Fecal samples were directly suspended in 2 ml of phosphate buffer saline (PBS) pH 7.2

M. Parthiban (✉) · K. S. Aarthi · M. Balagangatharathilagar ·
K. Kumanan
Department of Animal Biotechnology, Madras Veterinary
College, Chennai 600007, India
e-mail: drparthiban66@gmail.com

whereas intestinal samples were also suspended similarly after grinding. These samples were centrifuged at 10,000 rpm for 10 min. The supernatant of each sample was filtered using 0.45 µm filter (Millipore, USA) and stored at -20 °C until use. The filtered samples were used for PCR and virus isolation studies.

Haemagglutination test was performed using pig erythrocyte as per the method described earlier [6]. The FPV vaccine virus (Biofel PCHR) and the fecal samples from healthy dogs were used as positive and negative controls respectively. DNA was isolated with 200 µl of filtered field samples along with positive and negative control using QIAamp DNA isolation kit (Qiagen, Netherlands) as per the manufacturer's instructions. Consensus primers of Parvovirus (P1: 5'-GGA TGG GTG GAA ATC ACA GC-3', P2: 5'-ATA ACC AAC CCT CAG CTG GGT C-3') designed by Pereira et al. [10] were used in this study 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. The final elongation was done at 72 °C for 7 min. FPV specific primers (P3: 5'-AAA GAG TAG TTG TAA ATA ATT-3', P4: 5'-CCT ATA TAA CCA AAA GTT AGT AG-3') designed by Yang et al. [17] were used in this study and the temperature profile is same as above except annealing temperature at 55 °C for 45 s.

The full length VP₂ gene of FPV primers were designed [13] and were used in this study (P5: CTC GGA TCC CCA ATG AGT GAT GGA GCA GTT CAA CCA GAC; P6: AAC CTC GAG CTA GGT GCT AGT TGA TAT GTA ATA AAC). The temperature profile includes 30 cycles of initial denaturation 94 °C for 4 min, annealing at 55 °C for 45 s., and extension at 72 °C for 7 min. After amplification, 10 µl of each PCR products were loaded onto a 1 % Agarose gel stained with ethidium bromide and electrophoresis was performed at 100 V for 40 min.

The positive PCR products were purified using gel extraction kit (Bio Basic, Canada). The purified PCR products were sequenced using genetic analyzer (3130 Applied Biosystems, USA). The obtained sequences were subjected to BLAST analysis to find out the homology percentage of our isolates with other reference isolates available in the GenBank. The strains with similar homology were selected for multiple sequence alignment using MEGA software 6 version and phylogenetic analysis was done to analyze the homology between our strains with other FPV isolates using DNA Star software. The local FPV VP₂ nucleotide sequences were compared with reference FPV and CPV strains to find out the feline host specific nucleotides. The filtered field sample was used as inoculum for infecting A72 cell line and the virus was passaged 15 times. DNA was isolated from infected culture fluid and subjected to PCR amplification using FPV specific primers. Out of 60 samples screened, 22 samples were positive by HA with titre ranging from 256 to 1,052 and 17 samples were found positive by

Fig. 1 Molecular confirmation of FPV using PCR. **a** PCR amplification using parvovirus genus specific primers (845 bp). **b** PCR amplification using feline parvovirus specific primers (618 bp). Lane M 100 bp ladder, Lane 1–3 field samples, Lane 4 negative control

Table 1 The host specific nucleotide sites in VP₂ gene of local FPV isolates were compared with reference FPV isolates and also canine parvovirus isolates belongs to various antigenic types viz 2, 2a and 2c

Name	Genetic type	239	259	279	308	899	913	967
TN isolate 1	FPV	A	T	A	T	C	G	G
TN isolate 2	FPV	A	T	A	T	C	G	G
Ref. strain 1	FPV	A	A	A	T	C	G	A
FJ231389								
Ref. strain 2	FPV	A	A	A	T	C	G	G
D88287								
Ref. strain 1	CPV-2	G	A	C	C	C	G	A
M38245								
Ref. strain 2	CPV-2a	G	T	C	C	G	T	A
AB054221								
Ref. strain 3	CPV-2c(a)	G	T	C	C	A	T	A
AB054224								

PCR assay. The expected amplicon size of 845 and 618 bp were observed in 1.5 % Agarose gel electrophoresis for parvovirus genus specific primers and feline specific primers respectively (Fig. 1a, b).

The amplification of full length VP₂ gene yielded 1755 bp PCR product. Based on sequencing data, our local FPV isolates showed 99 % homology with other FPV isolates and 98 % homology with CPV isolates available in GenBank. Blast analysis revealed that our isolates showed 99 % homology with China, Italy and USA strains of FPV and 98 % homology with Germany, China and Indian strains of CPV. The phylogenetic analysis also revealed that our isolates are closely related to Japan, Italy, USA and China isolates of FPV. The host specific nucleotides in VP₂ gene of FPV and CPV were presented in Table 1. The characteristic cytopathic effect of parvovirus was observed after 5th passage onwards in A72 cell line. The infected culture fluid showed PCR amplification using FPV specific primers.

Out of 60 samples, more number of samples were positive in HA test than PCR due to non specific

agglutination reaction [11]. On the other hand, PCR was highly specific assay which avoid non specific reaction because samples were analyzed at nucleic acid level [11]. Initially parvovirus consensus primers were used to distinguish parvo virus from other viruses as reported by [10]. The feline specific primers amplified only clinical samples collected from suspected cats and no amplification was observed in CPV which clearly indicates the specificity of the primers as reported by [17]. The appearance of expected size product in agarose gel is not sufficient to confirm the presence of viral genome in clinical samples. Hence, PCR needs to be further confirmed by any one of the methods viz. nested PCR, southern blot hybridization and sequencing [12]. In this study, sequencing was done to confirm the PCR products further and it showed 99 % homology with other FPV strains. The VP₂ full length sequencing data and phylogenetic analysis revealed that our local FPV isolates are very closer to reference FPV strains rather than CPV strains.

Virus isolation is a gold standard method of confirmation of etiological agent. Hence virus was passaged in A72 cell line and based on the characteristic cytopathic effect and molecular method, the presence of virus in the infected culture fluid was confirmed.

Gene mutation, deletion and arrangement of some key nucleotides or amino acids were responsible for changes for their host range and pathogenecity. The hyper variable region of nucleotide sequence of VP₂ gene of infectious bursal disease virus was used for differentiation of IBDV pathotypes viz classical, very virulent, and variant subtypes [15]. Salmonella serovars *Enteritidis* and *Gallinarum* are closely related, but their host ranges are very different due to the genomic variation [16]. In our study also, the nucleotide at position 239, 279, 308, 899, 913 and 976 of VP₂ of our FPV isolates are similar to that of other reference FPV strains and different from other CPV strains. Hence, this nucleotide determines the host specificity and is responsible for multiplication of FPV in cats as reported by [2].

At present in India, imported vaccines are available in the market; hence an ideal cell culture adapted candidate vaccine strain would be useful for proper control of diseases.

References

1. Appel MJG, Scott FW, Carmichael LE. Isolation and immunisation studies of a canine parvo-like virus from dogs with haemorrhagic enteritis. *Vet Rec.* 1979;105:156–9.
2. Decaro N, Desario C, Parisi A, Martella V, Lorusso A, Miccolupo A, Mari V, Colaianni ML, Cavalli A, Di Trani L, Buonavoglia C. Genetic analysis of canine parvovirus type 2c. *Virology.* 2009;385(1):5–10.
3. Greene CE. Feline panleukopenia. In: Greene CE, editor. *Infectious diseases of the dog and cat.* 2nd ed. Philadelphia: W.B. Saunders Co; 1998. p. 52–7.
4. Inada S, Mochizuki M, Izumo S, Kuriyama M, Sakamoto H, Kawasaki Y, Osame M. Study of hereditary cerebellar degeneration in cats. *Am J Vet Res.* 1996;57:296–301.
5. Kumar M, Nandi S. Development of a SYBR Green based real-time PCR assay for detection and quantization of canine parvovirus in faecal samples. *J Virol Methods.* 2010;169:198–201.
6. Senda M, Norio H, Osamu I, Hisashi Y. Canine parvovirus: strain difference in haemagglutination activity and antigenicity. *J Gen Virol.* 1988;69:349–54.
7. Mohan Raj J, Mukhopadhyay HK, Thanissalass J, Antony PX, Pillai RM. Isolation, molecular characterization and phylogenetic analysis of canine parvovirus. *Infect Genet Evol.* 2010;10(8):1237–41.
8. Mosallanejad B, Avizeh R, Ghorbanpoor Najafabadi M. Antigenic detection of feline panleukopenia virus (FPV) in diarrhoeic companion cats in Ahvaz area. *Iran J Vet Res.* 2009;10:289–93.
9. Parrish CR. Parvoviruses: cats, dogs and mink. In: Webster RG, Granoff A, editors. *Encyclopedia of virology.* London: Academic Press; 1994. p. 1061–7.
10. Pereira CAD, Monezi TA, Mehnert DU, D'Angelo M, Durigon EL. Molecular characterization of canine parvovirus in Brazil by polymerase chain reaction assay. *Vet Microbiol.* 2000;75:127–33.
11. Parthiban M, Saranya R, Kurunchi C, Divya K, Kumanan. Detection of antigenic variation of canine parvovirus strains of Tamil Nadu using differential PCR. *Indian J Anim Sci.* 2012;82(3):237–9.
12. Schwarz TF, Jager G, Gilch S, Nitschko H. Nested RT-PCR for detection of sandfly fever virus, serotype Toscana, in clinical specimens, with confirmation by nucleotide sequence analysis. *Res Virol.* 1995;146(5):355–362(8).
13. Spitzer AL, Parrish CR, Maxwell IH. Determinant for canine parvovirus and feline panleukopenia virus functions through the capsid protein VP₂. *J Gen Virol.* 1997;78:925–8.
14. Steinel A, Parrish CR, Bloom ME, Truyen. Parvovirus infections in wild carnivores. *J Wildl Dis.* 2001;37:594–607.
15. Wu Z, Miller E, Agbandje-McKenna M, Samulski RJ. Alpha2, 3 and alpha2, 6 N-linked sialic acids facilitates efficient binding and transduction by adeno-associated virus types 1 and 6. *J Virol.* 2006;80:9093–103.
16. Feng X, DelSole T, Houser P. Comparison of statistical estimates of potential seasonal predictability. *J Geophys Res.* 2013;118(12):6002–16.
17. Zhang R, Yang S, Zhang W, Zhang T, Xie Z, Feng H, Wang S, Xia X. Phylogenetic analysis of the VP₂ gene of canine parvoviruses circulating in China. *Virus Genes.* 2010;40(3):397–402.