RESEARCH ARTICLE

Genomic Sequence and Phylogenetic Analysis of Canine Parvovirus New 2a Variant Strain in Indian Dogs

Yuvachandran Viva Vimalan[1], Raja Paramasivam[1], Parthiban Manoharan[1]*, Maha Prabhu Ramalingam[2], Parthiban Sivamurthy[1], Sathish Gopal[1], Sarathchandra Ghadevaru[3]

Abstract

In this study, a virulent field canine parvovirus type - 2a variant (CPV-new 2a) strain, ABT/CPV/MVC02, was identified from the faeces of a dog with severe haemorrhagic gastroenteritis in Chennai, Tamil Nadu, India. The genomic sequence of ABT/CPV/MVC02 (MN661243) shares 99.33% nucleotide identity with those of CPV new 2a variant strains. Based on the VP2 capsid protein amino acid variation (Ser297Ala), the ABT/CPV/MVC02 strain was classified as a new CPV 2a variant strain. Two VP2 mutations Tyr324Ile and Thr440Ala were reported in most of the CPV-2a stains also present in ABT/CPV/MVC02.The phylogenetic analysis of the reported strain revealed that it was distinct from existing CPV 2a strains from India and other countries. This complete genomic analysis revealed that the CPV-2a new variant strain circulating in India is evolving rapidly with unique antigenic variations between field CPV-2a and vaccine strains which may be the major cause for vaccine failure in vaccinated dogs.

Key words: Canine parvovirus (CPV), CPV-2a Strain, CPV-2b, CPV-2c and Genomic Sequence.

Ind J Vet Sci and Biotech (2023): 10.48165/ijvsbt.19.2.09

Introduction

Canine parvovirus 2 (CPV2) is a member of the genus *Protoparvovirus* within the family Parvoviridae (Mira *et al.,* 2018). CPV-2 is one of the fatal viral agents that causes severe haemorrhagic gastroenteritis and lymphopenia in domestic dogs, particularly in young, unvaccinated puppies.The CPV 2 has a linear single-stranded DNA (ssDNA) genome with two major open reading frames (ORFs). They encode two gene cassettes: a non-structural replicase gene (NS1 and NS2) that encodes the enzymes required for replication and a capsid (VP1 and VP2) gene encoding structural proteins (Shackelton *et al*., 2005).

The original CPV was designated as CPV type 2 (CPV-2) to distinguish it from canine minute virus or CPV type 1. CPV2 infections were first described in 1982. Subsequently, widespread outbreaks of canine haemorrhagic enteritis occurred throughout the country and CPV2 infection emerged as an important infectious disease of dogs because of the high morbidity and mortality associated with the virus (Zhao *et al*., 2013; Zhao *et al*., 2016; Zhao *et al*., 2017). It is well-established that the emergence of CPV-2 resulted from the site-specific mutation of FPV or FPV-like virus (Truyen *et al.,* 1995). Later, as a consequence of mutations on the viral capsid surface of CPV, the cross-species transmission of CPV occurs by altering the species-specific binding of viral capsid to the host receptor, transferrin receptor (TfR), with dynamic motion (Lee *et al.,* 2019). In 2015, new CPV-2a and new CPV-2b were discovered with full-length genome analysis in Gansu province (Zhao *et al.,* 2016).

VP2 is a major capsid protein that plays an important role in the determination of antigenicity and host range of CPV. It is also known that the mutations which affect the VP2 gene are mainly responsible for evolving different antigenic variants of CPV (Phromnoi *et al*., 2010). Increasing incidences of CPV in the vaccinated dog population is a matter of concern with regard to the prevailing CPV strains showing possible mutational escape from the vaccinal strains.

It's very difficult to identify the CPV genotypes based on PCR and RFLP methods and partial sequencing of the VP2 region, since it undergoes frequent mutation over a short

[1]Department of Animal Biotechnology, Faculty of Basic Sciences, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai-600 007.

[2]Department of Veterinary Pathology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai-600 007.

[3]Dean, Faculty of Basic Sciences, Madras Veterinary College Campus, Tamil Nadu Veterinary and Animal Sciences University, Chennai-600 007.

Corresponding Author: Yuvachandran Viva Vimalan, Department of Animal Biotechnology, Faculty of Basic Sciences, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai-600 007, e-mail: drparthiban66@gmail.com

How to cite this article: Vimalan, Y. V., Paramasivam, R., Manoharan, P., Ramalingam, M. P., Sivamurthy, P., Gopal, S., & Ghadevaru, S. (2023). Genomic Sequence and Phylogenetic analysis of Canine Parvovirus New 2a Variant Strain in Indian Dogs. Ind J Vet Sci and Biotech. 19(2), 47-53.

Source of support: Nil

Conflict of interest: None

Submitted: 12/03/2022 **Accepted:** 20/02/2023 **Published:** 10/03/2023

time. Although, the PCR coupled with high-resolution melt (HRM) curve analysis provides a low-cost, rapid, and robust genotyping alternative without the need for sequencing (Houriuchi *et al*., 1998). However, the HRM analysis is effective and applicable only when the genome is completely characterized to determine point mutations that could specify antigenic variants (Houriuchi *et al*., 1998; Decaro *et al*., 2007).

There have been reports of CPV 2 infection after vaccination which poses a challenge to veterinarians and vaccine producers. There is concern that the vaccines used currently to prevent CPV infection in dogs may fail to protect effectively pups against the new canine parvovirus type 2 antigenic variants (Truyen, 2006). In spite of the fact that the original CPV 2 type was completely replaced by the antigenic types CPV-2a, 2b, and 2c, it is still used in most commercial vaccines. Various studies have, however, demonstrated that the CPV 2 vaccine is still effective in inducing protection against CPV 2 variants (Yule *et al*., 1997; Spibey *et al*., 2008; Larson *et al*., 2008).

The aim of the present study was to study the complete genome sequence and phylogenetic analysis of the canine parvovirus new CPV 2a variant strain in dogs from India.

Materials and Methods

The faecal swab sample was collected from the suspected dog with severe haemorrhagic gastroenteritis and brought to the Small Animal Clinics, Madras Veterinary College Hospital, Chennai, Tamil Nadu, India. The faecal swab was resuspended in 1 mL sterile phosphate buffer solution (1X PBS) and subjected to DNA extraction using QIAamp virus isolation kits as per the manufacturer's instructions and subjected to PCR using a specific primer for identification of CPV-2. The primers CPV F (5'AGCTATGAGATCTGAGACAT-3') and CPV R (5'AGTATGTTAATATAATTTTCTAGGTGC-3') with the product size of 1198 bp targeting VP2 gene (Mizak and Rzezutka, 1999) were used in this study. The PCR amplification reactions were performed in a 25 µL reaction mixture containing 12.5 µL of Pfu DNA Polymerase Master Mix (GOLDBIO, USA), 1 µL of each forward and reverse primers, and 2 µL of DNA template, and the final volume was adjusted to 25 µL by adding nuclease-free water. The cycle condition for PCR amplification consists of initial denaturation of 95 ˚C for 5 min; 35 cycles of denaturation of 95˚C for 30 sec; annealing of 55˚C for 1 min; extension of 72 ˚C for 1 min and final extension of 72 ˚C for 5 min. The final PCR amplified products were electrophoresed on 1.5% agarose gel and examined under gel documentation.

A total of 10 sets of primer pairs for the amplification of the whole genome were designed using Primer 3 plus software and custom synthesized (IDT). All 10 sets of primers were optimized for PCR with the annealing temperature of 55˚C (Fig. 1) and complete genome sequencing was carried out with the final reaction volume of 25 µL each primer as shown in Table 1.

The PCR amplified products were purified using PCR gel purification kit (Bio Basic Inc, Canada) and sequencing was performed using both forward and reverse primers at M/s. Shrimpex Biotech, Chennai-600019 (India). The nucleotide sequence data were subjected to BLAST analysis (www.ncbi.nlm.nih.gov), assembled and analyzed using Seqman and MegAlign programs of Laser gene package (version 7.1.0) (DNA Star Inc. Madison, WI). Nucleotide sequence alignment was performed by the ClustalW method with the MegAlignTM program (DNA Star Inc), and the predicted amino acid sequence was analyzed by the ProteanTM program of Laser gene (DNA Star Inc). Phylogenetic analysis of CPV 2a new strain was performed using the Neighbor-joining method with 1000 bootstrap replication in the MEGA software version 10.

Results and Discussion

In the present study a virulent field strain, ABT/CPV/MVC02, was identified from a faecal sample of a dog with severe haemorrhagic gastroenteritis in Chennai, Tamil Nadu, India. The genome sequence of ABT/CPV/MVC02 was determined and analyzed to study the CPV-2 antigenic variant currently circulating in the field and sequencing was performed using both forward and reverse primers at M/s. Shrimpex Biotech, Chennai-600019 (India).

The genomic sequence of ABT/CPV/MVC02 comprises 4495 nucleotides (nt) containing two open reading frames (ORFs). ORF1 (nt 1-2007) encoded two non-structural proteins (NS1 and NS2), and ORF2 (nt 2014-4270) encoded two structural proteins (VP1 and VP2) through alternative splicing of mRNAs. The genome sequence of ABT/CPV/MVC02 shares 99.33% nucleotide identity with those of CPV new 2a variants (MH476591 and MH476580) strains and shares 99.20% and 98.98% nucleotide identity with JQ268284 (CPV 2b) and KU508407 (CPV 2c) strains respectively. The genome sequence of the new CPV-2a strain ABT/CPV/MVC02 has been deposited in GenBank under the accession number MN661243.

There was one insertion site at the VP1 region (nt- 2113) and two deletion sites were found at the 3' untranscribed region (UTR) (nt-4478 and 4481)(Fig 2). Compared with the existing CPV new 2a strain (MH476591), the ABT/CPV/MVC02 strain has 28 nucleotide variations, of which 5 variant loci were identified in the VP2 gene. Based on the VP2 capsid protein amino acid variation (Ser297Ala), the ABT/CPV/MVC02 strain was classified as a new CPV 2a variant strain. Martella *et al*., (2005) stated that German CPV isolates had an additional amino acid difference in CPV 2a and CPV 2b at the 297th amino acid position (Ser297Ala) and the mutants were designated as New CPV 2a/2b. In our study, the additional amino acid difference Ser297Ala was also observed (Table 2). Nookala *et al*. (2016) analyzed the full-length sequence analysis of VP2 gene of five Indian CPV isolates from different geographical locations and were identified as new CPV 2b type based on amino acid residues (Ala 297 & Asp 426). The amino acid

change Ser297Ala, which was under strong positive selection pressure, played an important role in host cell adaption (Pereira *et al*., 2007).

The phylogenetic analysis of the ABT/CPV/MVC02 strain indicated that it was distinct from existing CPV new 2a variant strains from India (MH545963) and other countries of CPV 2a variant strains (MH476591 and MH476589) Figure 2. There were five specific nucleotide mutations observed in the VP2 region compared to other CPV 2a variants at position 2551(A-G), 2575 (A-T), 3736 (G-A), 3802 (A-G), and 4015 (G-A) (MN661243).The specific nucleotide mutation in the VP2 region for the new CPV- 2a variant strain is shown in Table 2 and the deduced amino acid variation is shown in Table 3.

Two VP2 mutations Tyr324Ile and Thr440Ala were reported in most of the CPV-2a stains also present in ABT/CPV/MVC02 but not in the vaccine strains. Houriuchi *et al*., (1998) revealed that residue 324 was prone to strong positive selection in all carnivorous CPV isolates. Residue 324 is responsible for TfR binding and together with residue 93, determined the canine host range. Therefore, mutation at amino acid residue 324 is likely to have an impact on the parvovirus host range. It was also documented that Tyr-324 to Ile mutation was also found in our ABT/CPV/MVC02 strain and this substitution might directly influence viral biology. The next important amino acid change Thr440Ala was observed in CPV 2a types of Indian isolates (Nookala*et al.,*2016) as well

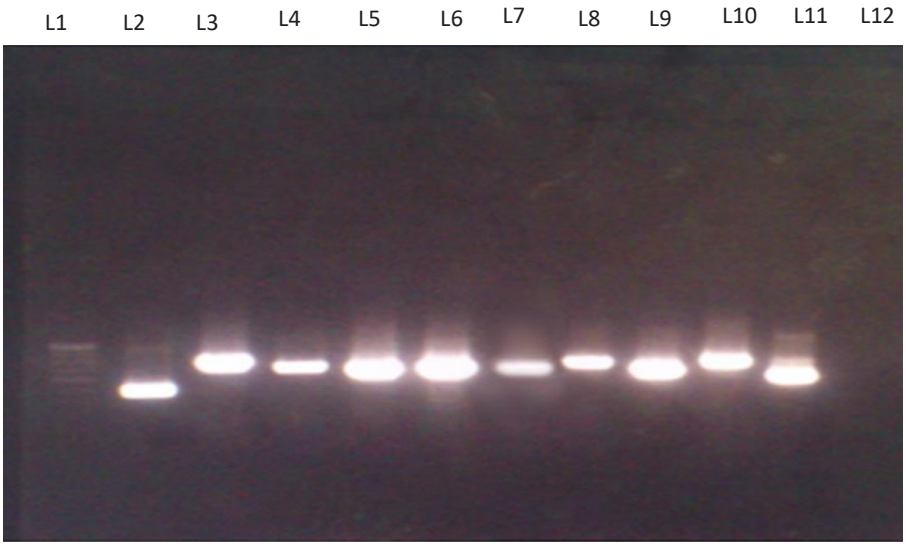

Fig. 1: Complete genome of CPV 2a new variant amplified by PCR, 1.2% Agarose gel electrophoresis of PCR products
Lane (L)- L1-100 bp DNA marker, L2-507 bp, L3-812 bp, L4-745 bp, L5-702 bp, L6-701 bp, L7-716 bp, L8-784 bp, L9-689 bp, L10-999 bp, L11-612 bp, L12-NTC.

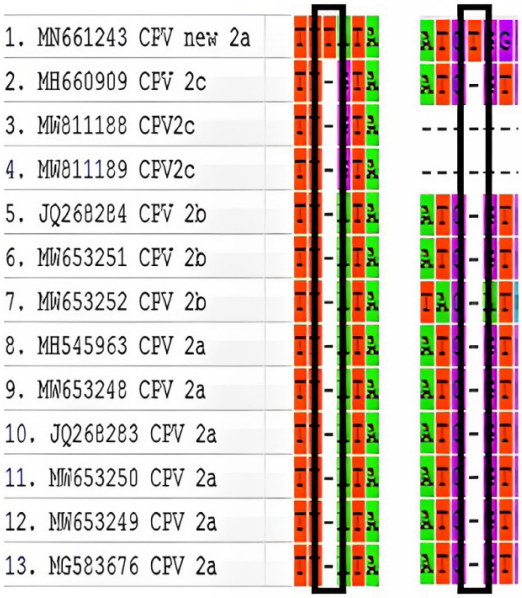

Fig. 2: Two insertion sites at VP1 region (nt- 2113 (T)) and 3' untranscribed region (UTR) (nt-4478 (T)) in the CPV new 2a variant (highlighted in black colour.)

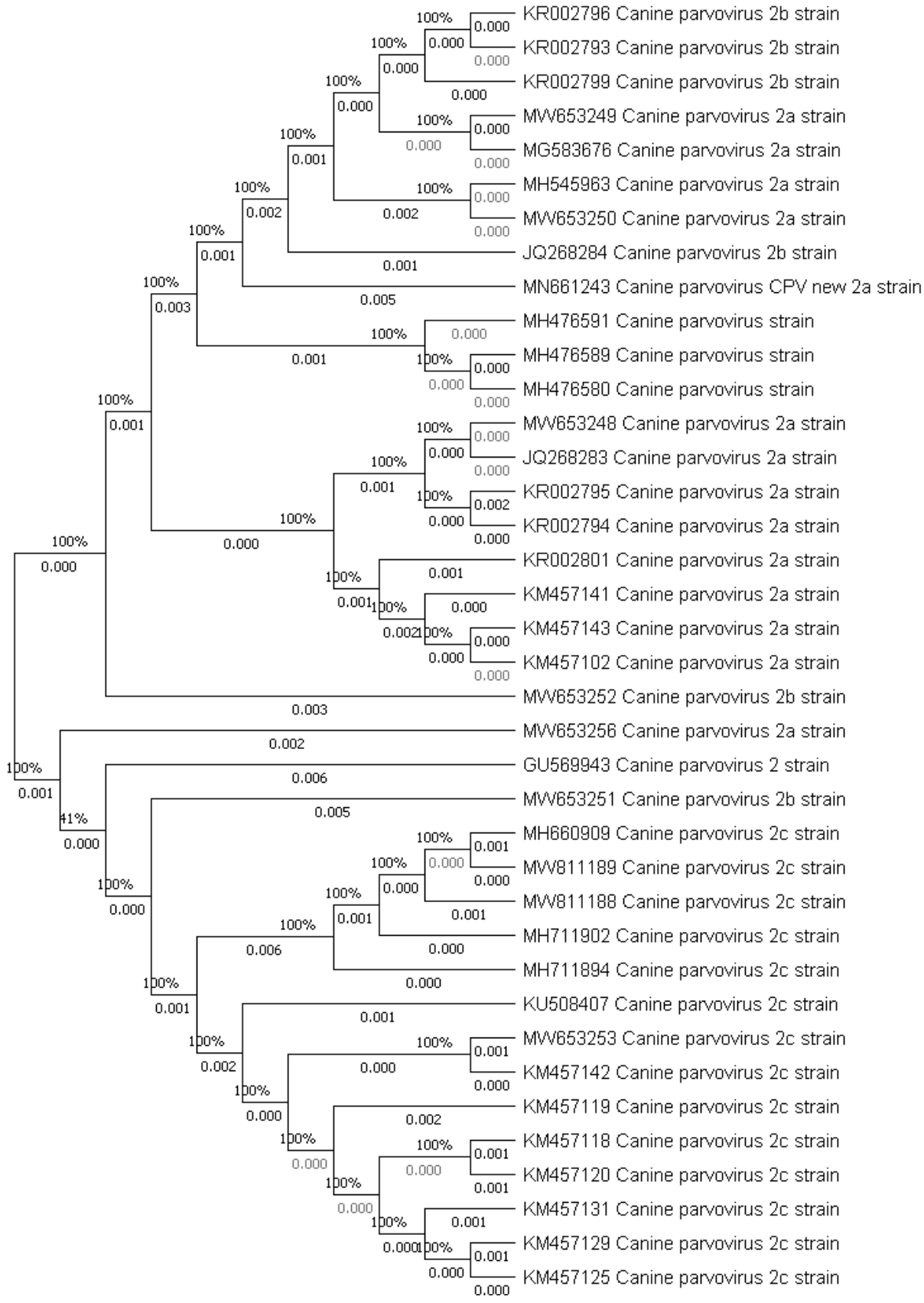

Fig. 3: Phylogenetic analysis of CPV new 2a strain.

Table 1: List of primers used for complete genome amplification of CPV

S.No	Primer	Sequence	Gene position	Product size (bp)
1.	CPV-F1	5′-CTGAGGAAGTTATGGAGGGAGT-3′	1-24	507
	CPV-R1	5′TGAGCTTAATCTTTTCAGTTGGTG-3′	709-730	
2.	CPV-F2	5′-AGAAGAAGAAATGGACTGGGAATC-3′	484-507	812
	CPV-R2	5′-AATGCTGTTTTTGTTCTTGCTAA-3′	1274-1296	
3.	CPV-F3	5′-TGAACAAATGAAACCAGAAACC-3′	1033-1054	745
	CPV-R3	5′-GTTCAGGTCTTTCTTCACATCCA-3′	1756-1778	
4.	CPV-F4	5′-AAGCTGTGGGTAATGTTTGGTTGTT-3′	1515-1538	702
	CPV-R4	5′-TCTGCCTCTATTTCGGACCAC-3′	2197-2217	
5.	CPV-F5	5′-AGAGACACAAGCGGCAAGCAATCC-3′	2017-2040	701
	CPV-R5	5′-ATATGAGGTGGTGGTTTACTTCTT-3′	2695-2718	
6.	CPV-F6	5′-TACGCTGCTTATCTTCGCTCTGGT-3′	2492-2515	716
	CPV-R6	5′-AAAACTAACTAAATGCAACTTCACT-3′	3185-3208	
7.	CPV-F7	5′-AATCACAGCAAACTCAAGCAGACT-3′	2965-2988	784
	CPV-R7	5′-TATTTCCCATTTGAGTTACACCAC-3′	3726-3749	
8.	CPV-F8	5′-AGAACAGGTGATGAATTTGCTACA-3′	3551-3574	689
	CPV-R8	5′-ATTTACATGAAGTCTTGGTTTTA-3′	4218-4240	
9.	CPV-F9	5′-AAACTACCACAACAGGAGAAACAC-3′	3945-3968	999
	CPV-R9	5′-AACCACCCACACCATAACAACATA-3′	4921-4944	
10.	CPV-F10	5′-CAGCAGATGGTGATCCAAGA-3′	4645-4667	612
	CPV-R10	5′-TTTCATATACAATTTTCATACCTCCAA-3′	5300-5323	

Table 2: Specific nucleotide mutation in the VP2 region for new CPV 2a variant strain
The specific nucleotide mutation in the VP2 region compared between CPV-2a from this study and other CPV-2 variants strain from NCBI.

Gene	**VP2 region**				
Amino acid position	2551	2575	3736	3802	4015
MH476591 2a	A	A	G	A	G
MN661243 2a	G	T	A	G	A
MG583676 2a	A	A	G	.	G
JQ268284 2b	.	T	.	.	.
MH660909 2c	.	.	.	A	.
MH545963 2a	.	.	.	.	.

A – Adenine; **T** – Thymine; **G** – Guanine.
The nucleotide mutation in the VP2 region compared between CPV-2a from this study and other CPV-2 variants strainfrom NCBI.

as our ABT/CPV/MVC02 strain. The amino acid residue 440 is important because it is located at the top of the 3-fold spike (GH loop) of the VP2 protein on the surface of the capsid, the main antigenic site of the virus. This residue was undergoing positive selection for the past few years and had evolved independently in different populations, which explained its worldwide mutation in unrelated CPV 2 populations. Decaro *et al.* (2007) reported an Asp434Val mutation in certain Italian CPV types; however, this study did not observe the mutation. Phylogenetic analysis revealed that our TN CPV 2a strain is very close to CPV 2a reference strains from China and our findings are in agreement with earlier findings of Nookala *et al.* (2016).

Conclusions

The present study reveals that the ABT/CPV/MVC02 CPV-2a new variant strain circulating in India is evolving rapidly with unique variations at the nucleotide level. This study provides insight and knowledge on the occurrence of CPV infection in vaccinated dogs due to the antigenic variations between field CPV-2a and vaccine strains which may be important reasons for vaccine failure in India. Comparative epidemiological and pathogenic investigations of the new antigenic strains of CPV-2 are needed to effectively control CPV infection worldwide, which helps control CPV infection in dogs.This is the first report of complete genome sequencing

Table 3: Amino acid variation for new CPV 2a variant strain
The amino acid comparison between CPV2a from this study and CPV2a, CPV2b and CPV2c strain from NCBI

Gene	**NS 1**											**NS 2**						**VP 1**							**VP2**					
Amino acid position	19	60	108	351	544	545	572	583	596	616	630	19	60	92	94	125	116	125	131	148	513	569	583	5	297	324	370	426	440
MH476591 2a	K	I	N	N	Y	E	K	E	V	D	L	K	I	R	A	R	R	L	A	A	Q	N	A	A	S	I	Q	N	A
MN661243 2a	.	.	.	K	F	.	E	.	.	N	.	.	.	.	.	K	.	.	.	.	.	.	.	.	.	.	.	.	.
MG583676 2a	R	.	.	.	F	V	E	K	A	.	.	R	.	K	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.
JQ268284 2b	R	.	.	.	.	V	E	.	.	.	.	R	.	.	T	.	.	.	.	.	.	D	.	.	A	Y	.	D	.
MH660909 2c	.	V	.	.	F	V	E	.	.	.	P	.	V	.	T	.	K	.	T	G	R	E	T	G	S	Y	R	E	T
MH545963 2a	.	.	K	.	F	V	E	K	.	.	.	.	.	K	T	.	.	F	.	.	.	.	.	.	A	I	.	.	.

G – Glycine; **A –** Alanine; **L –** Leucine; **M –** Methionine; **F –** Phenylalanine; **W –** Tryptophan; **K –** Lysine; **Q –**Glutamine; **E -** Glutamic Acid; **S –** Serine; **P –** Proline; **V –** Valine; **I –**Isoleucine; **C –**Cysteine; **Y –**Tyrosine; **H –** Histidine; **R –** Arginine; **N –** Asparagine; **D -** Aspartic Acid; **T–**Threonine

The table revealed the amino acid difference in the different region of CPV2a, CPV2b and CPV2c strain. The unique amino acid changes on the VP2 region of CPV2a new variants were reported on the Ser297Ala, Tyr324Ile and Thr440Ala.

of a new CPV-2a variant from India, which would act as an epidemiological benchmark for this rapidly evolving virus. This study demonstrated the importance of continue surveillance of emerging viruses in canine populations.

Acknowledgment

This work was supported by a self-finance scheme on "Training on Human Resource Development" [Scheme code. 18006] at Department of Animal Biotechnology, Faculty of Basic Sciences, Madras Veterinary College, Chennai - 600 007.

References

Decaro, N., Desario, C., Addie, D.D., Martella, V., Vieira, M.J., Elia.,Davis, C., Thompson, G., Truyel, U., &Buonavoglia, C. (2007).Molecular epidemiology of canine parvovirus, Europe. *Emerging Infectious Diseases*, *13*(8), 1222.

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*(2), 440-452.

Larson, L.J., & Schultz, R.D. (2008). Do two current canine parvovirus type 2 and 2b vaccines provide protection against the new type 2c variant?.*Veterinary Therapeutics*, *9*(2), 94.

Lee, H., Callaway, H.M., Cifuente, J.O., Bator, C.M., Parrish, C.R., &Hafenstein, S.L. (2019). Transferrin receptor binds virus capsid with dynamic motion. *Proceedings of the National Academy of Sciences ofthe United States of America*, *116*(41), 20462–20471.

Martella, V., Decaro, N., Elia, G., &Buonavoglia, C. (2005). Surveillance activity for canine parvovirus in Italy. *Journal of Veterinary Medicine, Series B*, *52*(7-8), 312-315.

Mira, F., Dowgier, G., Purpari, G., Vicari, D., Di Bella, S., Macaluso, G., &Guercio, A. (2018). Molecular typing of a novel canine parvovirustype 2a mutant circulating in Italy. *Infection Genetics and Evolution,61,* 67–73.

Mizak, B.E.A.T.A., &Rzezutka, A.R.T.U.R. (1999). Application of nested PCR for the detection of canine parvovirus in faeces. *Bulletin-Veterinary Institute in Pulawy*, *43*, 19-24.

Nookala, M., Mukhopadhyay, H.K., Sivaprakasam, A., Balasubramanian, B., Antony, P.X., Thanislass, J., Srinivas M.V & Pillai, R.M. (2016). Full-length VP2 gene analysis of canine parvovirus reveals emergence of newer variants in India. *ActaMicrobiologicaetImmunologicaHungarica*, *63*(4), 411-426.

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. *Infection. Genetics and Evolution.7*(3), 399-409.

Phromnoi, S., Sirinarumitr, K., &Sirinarumitr, T. (2010). Sequence analysis of VP2 gene of canine parvovirus isolates in Thailand. *Virus genes.41*(1), 23-29.

Shackelton, L.A., Parrish, C.R., Truyen, U., & Holmes, E.C. (2005). High rate of viral evolution associated with the emergence of carnivore parvovirus. *Proceedings of the National Academy of Sciences*, *102*(2), 379-384.

Spibey, N., Greenwood, N.M., Sutton, D., Chalmers, W.S.K., &Tarpey, I. (2008). Canine parvovirus type 2 vaccine protects against virulent challenge with type 2c virus. *Veterinary Microbiology*, *128*(1-2), 48-55.

Truyen, U. (2006). Evolution of canine parvovirus - a need for new vaccines?. *Veterinary Microbiology*, *117*(1), 9-13.

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*. *Journal of Virology*, *69*(8), 4702–4710.

Yule, T.D., Roth, M.B., Dreier, K., Johnson, A.F., Palmer-Densmore, M., Simmons, K., &Fanton, R. (1997). Canine parvovirus vaccine elicits protection from the inflammatory and clinical consequences of the disease. *Vaccine*, *15*(6-7), 720-729.

Zhao, H., Wang, J., Jiang, Y., Cheng, Y., Lin, P., Zhu, H., & Cheng, S. (2017). Typing of canine parvovirus strains circulating in north-east China. *Transboundary and Emerging Diseases, 64,* 495–503.

Zhao, Z., Liu, H., Ding, K., Peng, C., Xue, Q., Yu, Z., &Xue, Y. (2016). Occurrence of canine parvovirus in dogs from Henan province of China in 2009-2014. *BMC Veterinary Research, 12,* 138.

Zhao, Y., Lin, Y., Zeng, X., Lu, C., & Hou, J. (2013). Genotyping and pathobiologic characterization of canine parvovirus circulating in Nanjing. *China Virology Journal, 10*, 272.