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Original Article

Molecular Phylogenetic Analysis of Canine Parvovirus Strains Circulating in Iran

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Abstract

Background and Aims: Canine parvovirus type 2 (CPV 2) suddenly appeared as a pathogen in dogs in the late 1970s, causing a severe and often fatal gastrointestinal illness. The original CPV 2 was replaced by three types of variants, CPV 2a, CPV 2b and CPV 2c, which to date have achieved global distribution in varying proportions. All previous studies in Iran were based on partial VP2 gene sequence. The aim of this study was to provide genome analysis to describe CPV strains collected in Tehran, Iran.

Materials and Methods: Rectal swab samples were collected in 2019 and tested using serological methods. Out of forty positive samples, nine samples were selected for further analysis using various molecular methods.

Results: The results revealed a high prevalence of CPV-2a, CPV-2b and CPV2-C variants. Phylogenetic analysis showed that in Tehran the CPV 2b, CPV 2a and CPV-2 C strains were related to a cluster of specimens. Thus, the results suggested that CPV 2 sequences is not the results potential recombination events here.

Conclusion: Continuous monitoring and molecular characterization of CPV2 samples is necessary not only to identify possible genetic and antigenic changes that may interfere with the effectiveness of vaccines, but also to better understand the mechanisms of CPV 2 evolution in Iran.

Keywords: CPV-2; Dog; PCR; Phylogenetic analysis; DNA virus

Introduction

Canine parvovirus type 2 (CPV 2) was first isolated during 1978 as one of the new diseases worldwide, when it spread rapidly among the domestic population as well as in wild dogs [1]. However, antibody testing in sera collected from dogs or related canids showed that the first positive titers in Europe were present in dogs between 1974 and 1976 [2-3]. At present, the original CPV 2 has been completely replaced by three main genetic and antigenic variants, called CPV 2a [2], CPV 2b [3] and CPV 2c [4], respectively, identified in 1980, 1984 and 2000, respectively. The virus has a single-stranded DNA (ssDNA) linear genome that is about 5 KB long with two main open reading frames (ORF). They encode two gene cassettes, a nonstructural replicase gene (NS1 and NS2) that encodes enzymes needed for replication, and a capsid gene (VP1 and VP2) that encodes structural proteins. [5]. This CPV 2 is a species of *carnivorous proparo-virus 1* that belongs to the genus *Protoparo-virus*, family Parvoviridae. According to the International Committee for Classification of Viruses, this species includes Feline panleuko-penia virus, Mink enteritis virus and Raccoon Parvovirus [6,7]. This virus is one of the most important intestinal pathogens in dogs.

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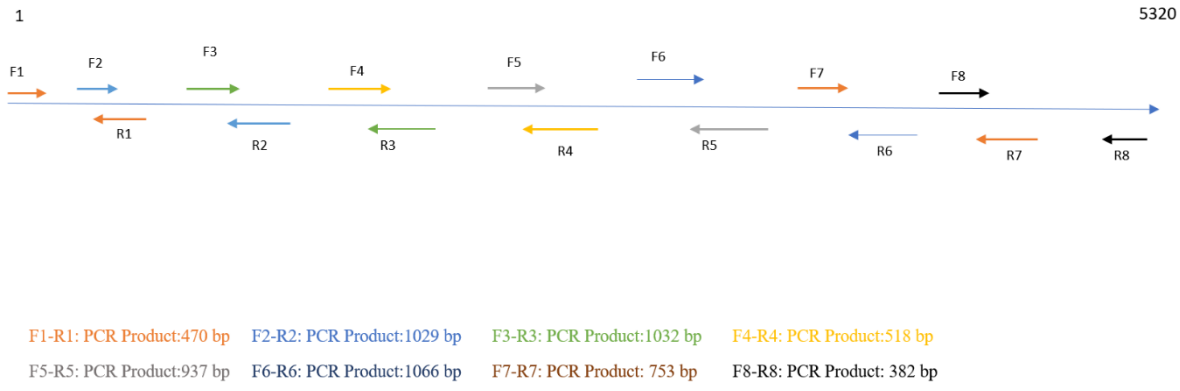

Fig. 1. Complete coverage of the virus genome with primers

The virus is highly contagious and increases the incidence of the disease by increasing the prevalence in shelters, pet stores and orphanages. [8].

The cause of hemorrhagic gastroenteritis is mainly attributed to CPV 2 compared to other microorganisms, often in dogs less than 6 months of age. [9]. The objectives of this study were to identify and describe CPV 2 in dogs in Tehran, Iran, by molecular analysis based on phylogenetic analysis in CPV-2 types.

Methods

Sample, ssDNA extraction, and PCR: 40 fecal samples were collected from dogs unvaccinated to CPV-2 with vomiting and diarrhea symptoms from the Veterinary Hospital in Tehran, Iran. The ssDNA was extracted using the DNP Sinaclon Kit and testing by PCR. First, the extracted samples were examined with specific primers of this virus. Then primers used for PCR detection based on Fig. 1 that amplified whole genome of virus, as previously described and designed in this study (table 1). For the amplification, the following conditions were applied: 94°C for 4 min35 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s and extention at 72°C for 40 s and 72°C 5 min, F1 with R1, F2-R2, F3-R3, F4-R4, F5-R5, F6-R6, F7-R7 and F8 with R8, they were tested together and the results were reviewed, followed by electrophoresis on a 2% agarose gel with Safe stain.

DNA sequencing and phylogeny: The PCR products were purified from the gel by using Sinaclon PCR Purification Kit (Sinaclon Co., Iran) and purified PCR products were used as a template for sequencing on an Applied Biosystems 373S automated DNA sequencer using dye terminator cycle sequencing chemistry (Perkin Elmer/Applied Biosystems, CA).

Purified PCR products were sequenced bidirectionally Sequence assembling, editing and analyses were made using CLC Main work bench version 7 BLAST homology analysis of the conserved regions were done to identify reference sequences for inclusion in multiple sequence alignments that were prepared using Clustal W software. The phylogenetic tree was constructed with the Neighbor-joining method using the Kimura-two-parameter model in Mega version 10 [10]. Bootstrap re-sampling and reconstruction was performed 1,000 times to confirm the reliability of the phylogenetic tree. Sequences used for comparison or phylogenetic analysis in this study were obtained from the GenBank database [11]

Results

Forty dog feces samples were amplified and the presence of specific CPV-2 primer was confirmed by PCR (Fig. 2). Nine samples were randomly selected for complete sequencing.

Each of these samples was reacted with primers that completely covered the entire virus sequence. Which was observed with F1 with R1, F2-R2, F3-R3, F4-R4, F5-R5, F6-R6, F7-R7 and F8 with R8 primers 470, 1029,

1032, 518, 927, 1066, 753, 382 bp on gel, respectively (data was not showed).

Table 1. Sequence and order of primers

Primer name	Primer sequence	TM	Size band
VP2-F	5'- GAAACCAACCATACCAACTCC - 3'	50.1°C	485 bp
VP2-R	5'- CCCGTCCTGCTGCAATAG -3'	51.4°C	
F1	5'- ATTCTTTAGAACCAACTGACCAAG-3′	50.7°C	470 bp
R1	5'- CATTGCTGTTTGTGCTCCTC-3'	50.4°C	
F2	5'- CCGTTACTGACATTCGCTTC-3'	50°C	1029 bp
R2	5′- AAGATTTTCACCTCCTGGTTG-3′	50°C	
F3	5′- CCTGAAGACTGGATGATGTTACAAC-3′	53.6°C	1032 bp
R3	5′- CCACGTCGGACTCGCTTG -3′	54°C	
F4	5′- GACCTTGCACTGGAACCG -3′	50.4°C	518 bp
R4	5′- TGTCCTATTTTCCCCCCC -3′	50.9°C	
F5	5′- AACACGACGAAGCTTACGC-3′	50.5°C	927 bp
R5	5′- GAGTTGGTATGGTTGGTTTCC -3′	50.1°C	
F6	5′- CTGAGACATTGGGTTTTTATCC -3′	50°C	1066 bp
R6	5′- GGATTCCAAGTATGAGAGGCT -3′	50°C	
F7	5′- CCAATTGGAGGTAAAACAGG -3′	50°C	753 bp
R7	5′- GACAGTATACGAGGCCATTTAG -3′	50°C	
F8	5′- GGTGGTTGGTTGGTTTGC -3′	50.6°C	382 bp
R8	5′- GAGATGTTAATATAATTTTCTAGGTGC -3′	49.1°C	

The sequence in results of PCR products were confirmed. Nucleotide sequences of isolated viruses in this study is deposited at GenBank with accession numbers MW653248, MW653249, MW653250, MW653251, MW653252, MW653253, MW653254, MW653255, MW653256.

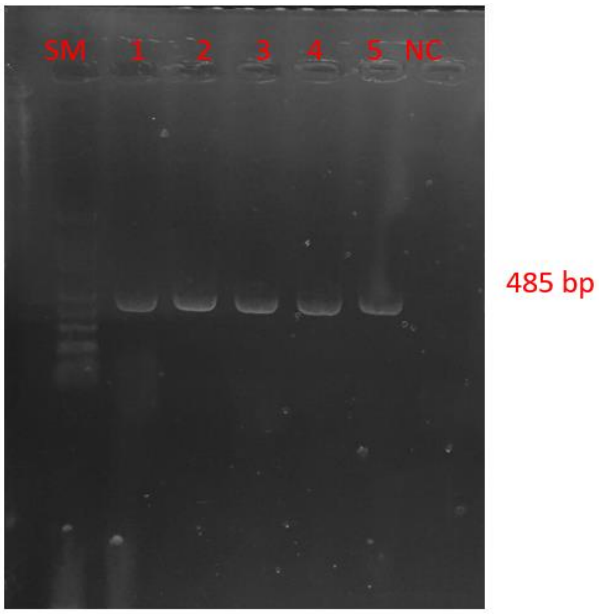

Fig. 2. PCR results on agarose gel (2%) electrophoresis of CPV-2 gene positive samples by primers of vp2 gene: Lane SM) Molecular marker (100-bp ladder); Lane 1-5) CPV-2 Suspected samples; NC) Negative control, Numbers on the left are molecular sizes (in base pairs).

Phylogenetic analysis: Nine complete sequences of the virus were identified in the study, Phylogenetic studies showed that there were different types of this virus in this study in Tehran province and were compared with the recorded sequences, they had a 99% similarity.

The CPV-2 virus is very deadly Dog disease. It is seen in many surrounding countries. This study conducted in Tehran showed that all three types of CPV-2 genetics (CPV 2a, 2b and 2c) are circulating in dogs.

The Virus sequences formed six distinct clusters. Nine virus sequences from this study clustered within each branch, together with the CPV-2 type2a, 2b and 2c (Fig. 3).

The similarities and divergences of nine CPV 2 sequences from Tehran and other CPV 2 sequences were compared with fourteen CPV 2 sequences of different types of viruses.

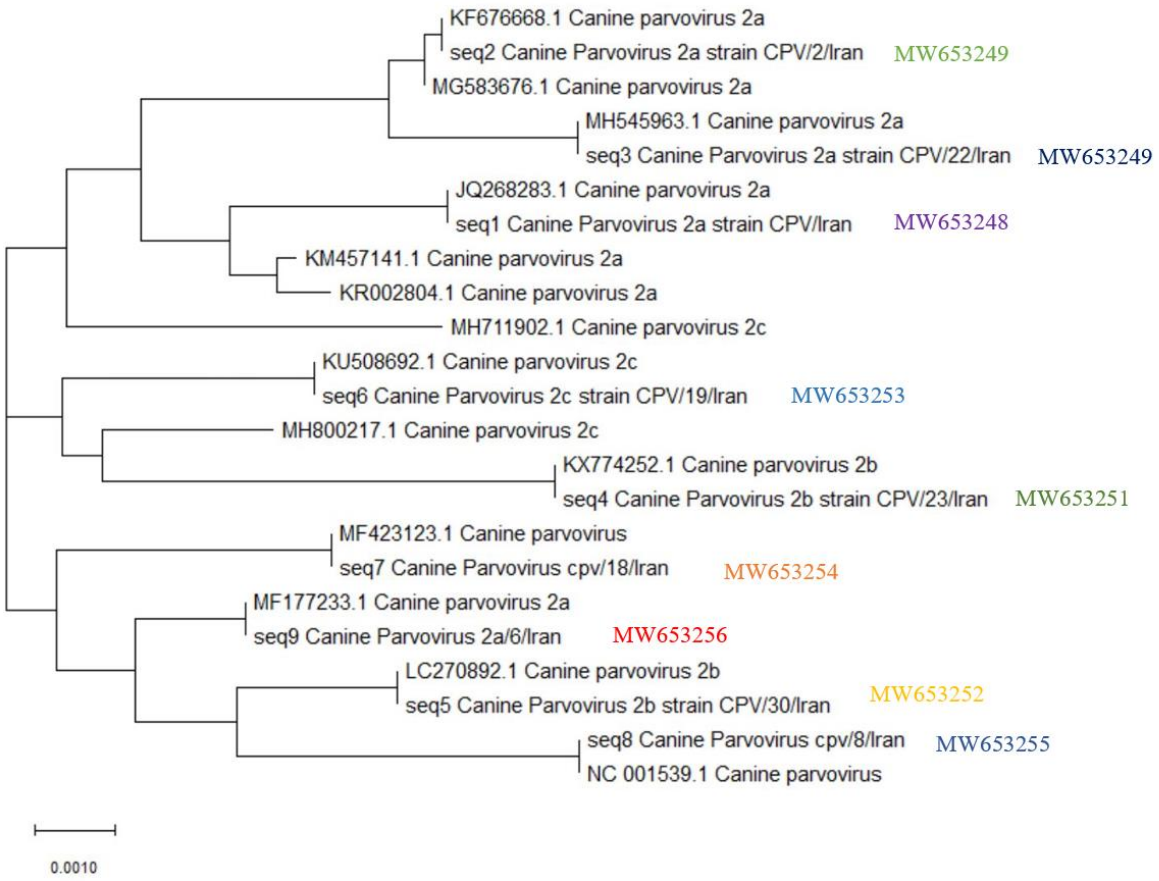

Fig. 3. Phylogenetic analysis of CPV-2 based on full sequence, the tree was generated by the neighbor-joining method with Mega 10 software, number below branches indicate bootstrap value from 100 replicates, bootstrap value.

Table 2. Estimates of Evolutionary Divergence between Sequences. The number of base differences per site from between sequences are shown. This analysis involved 23 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There was a total of 5333 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1. JQ268283.1 Ca																						
2. KF676668.1 Ca	0.01																					
3. KM457141.1 C	0.00	0.01																				
4. KR002804.1 Ca	0.00	0.01	0.00																			
5. KU508692.1 Ca	0.01	0.01	0.01	0.01																		
6. KX774252.1 Ca	0.01	0.01	0.01	0.01	0.01																	
7. LC270892.1 Ca	0.01	0.01	0.01	0.01	0.01	0.01																
8. MF177233.1 Ca	0.01	0.01	0.01	0.01	0.01	0.01	0.01															
9. MF423123.1 Ca	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01														
10. MG583676.1 (	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01													
11. MH545963.1 C	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00												
12. MH711902.1 C	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01											
13. MH800217.1 (	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01										
14. seq9 Canine P	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01									
15. seq8 Canine P	0.03	0.01	0.01	0.01	0.01	0.03	0.01	0.00	0.01	0.01	0.02	0.01	0.01	0.00								
16. seq7 Canine P	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01							
17. seq6 Canine P	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01						
18. seq5 Canine P	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01					
19. seq4 Canine P	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.03	0.01	0.01	0.01				
20. seq3 Canine P	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01			
21. seq2 Canine P	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00		
22. seq1 Canine P	0.00	0.01	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.03	0.01	0.01	0.01	0.01	0.01	0.01	
23. NC 001539.1 (	0.03	0.01	0.01	0.01	0.01	0.03	0.01	0.00	0.01	0.01	0.02	0.01	0.01	0.00	0.00	0.01	0.01	0.01	0.03	0.02	0.01	0.03

Discussion

Canine parvovirus infection is worldwide and is a significant fatal disease in puppies.
Unvaccinated or irregularly vaccinated dogs are more susceptible to infection than vaccinated dogs [12]. In addition, the isolated genetic diversity of CPV 2 can be used to further classify viruses into four genotypes (2, 2a, 2b, and 2c) that differ in amino acid sequence and phylogenetic relationships of the VP2 gene. Currently, CPV 2a and CPV 2b along with CPV 2c are prevalent in several countries in different proportions. However, various studies have reported CPV 2b as the virus responsible for the higher prevalence of CPV infection worldwide. CPV 2b is the most common type of antigen in South Africa and Brazil [13].
It has a high rate of genetic mutation, similar to that seen for RNA viruses, which are responsible for the continuous evolution of antigens and the rapid displacement of old species by new types of antigens [14].
The results of this study indicated the existence of three genetic types of this virus in dogs in Tehran province. All these studies show that CPV 2a, 2b and 2c are the predominant strains in the dog population in Iran. The study by Nikbakht et al. showed similar results based on the vp gene sequence [15].

Conclusion

However, continued epidemiological surveillance and sequence analysis will help to detect mutations and provide insight into different types of CPV antigens. It is also expanding to other parts of the world, thus providing insight into the mechanisms of CPV 2 evolution. In addition, it is important to continuously monitor the emergence of a new type of CPV 2 in the dog population in Iran.

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Conflict of interest

The authors declare that no conflict of interests lies among them.

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