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Detection of a new canine parvovirus mutant in Iran

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Abstract:

BACKGROUND: Canine Parvovirus (CPV) is one of the major causes of fatal gastroenteritis in young dogs. After emergence of CPV-2, two new variants were reported and named CPV-2a and CPV-2b. In 2001 the newest variant was reported and named CPV-2c. The difference between CPV strains is related to amino acid changes in viral capsid protein (VP2). **OBJECTIVES:** The aim of the present study was genetic characterization of common CPV-2 strains which are prevalent in Iran. **METHODS:** Total of 50 fecal samples were examined by specific PCR primers for VP2 gene which codes virus capsid protein. PCR products were bidirectionally sequenced. **RESULTS:** Sequence analysis results showed that based on the nucleotides encoding for residue 426 (nt 4062 to 4064) all previously assumed CPV-2b samples were true CPV-2b, except for strain Ir28 which showed substitution of Asp (GAT) with Gly (GGT). The sequence alignment also showed that strains Ir3 and Ir19 are similar to the reference strain M74849.1 (CPV-2b) but other strains differed by exhibiting further synonymous substitutions. **CONCLUSIONS:** A novel mutation in CPV-2 gene of Ir28 strain may lead to emergence of a new variant. This finding also emphasizes the previous study results which indicate the high evolution rate of CPV.

Introduction

Canine Parvovirus (CPV) was first reported in 1978 and soon after became one of the major causes of fatal gastroenteritis in young dogs (Miranda &Thompson 2016; Duque-García et al. 2017). Virus belongs to feline parvovirus subgroup and the genus parvovirus (Decaro et al. 2005, 2012). Parvoviral enteritis is caused by canine parvovirus type 2 (CPV-2) which is a small, non-enveloped, single stranded DNA virus (Nakamura et al. 2004). Genomic substitution rate of CPV-2 is similar to those of RNA viruses with value 10-4 substitutions per site per year (Zhou et al. 2017). After emergence of CPV-2, two new variants were reported and named CPV-2a and CPV-2b.In 2001 the newest variant was reported and named CPV-2c (Parrish et al. 1985, Buonavoglia et al. 2001). The difference between CPV strains is related to amino acid changes in viral capsid protein (VP2). Substitutions at positions 87 (Met to Leu), 300 (Gly to Ala), 305 (Tyr to Asp) and 555 (Val to Ile) caused the evolution of CPV2 to CPV-2a and changes in positions 426 (Asn to Asp) and 555 (Ile to Val) caused the evo-

lution of CPV-2a to CPV-2b (Parrish et al. 1991, Truyen et al. 1995). The newest detected strain (CPV-2c) has in position 426 a Glutamic acid, whereas CPV-2a and 2b have Asn and Asp in the same position, respectively (Martella et al. 2004). Since the virus has experienced multiple mutations during a short period of time, it is necessary to monitor the virus contineously to detect any new change. The aim of the present study was the genetic characterization of CPV-2 strains in Iran.

Materials and Methods

Sample collection: Total of 50 fecal samples from young dogs, referred to Small Animal Teaching Hospital of University of Tehran from September 2011 to September 2012, with clinical signs of acute gastroenteritis were collected using sterile swabs soaked with sterile water. Samples were stored at -70 ◦C prior to performing PCR reaction. A commercial polyvalent vaccine (Biocan, Czech Republic) was used as positive control and stool sample from a healthy dog was used as the negative control.

PCR reaction: Genomic DNA was extracted from fecal specimens and commercial vaccine using AccuPrep stool DNA extraction kit (Bioneer Co, Korea) based on manufacturer's instructions.Three different pairs of primers were used in this study. Primer pairs P2 and Pb which detect CPV2 and CPV-b respectively, were designed by Pereira (Pereira et al. 2000). Third primer pair (Pab) which detects CPV-2a and CPV-2b was designed by Senda (Senda et al. 1995). These primers were selected from different regions of VP2 gene which codes virus capsid protein. The sequences of primer pairs were as follows: Pb sense: 5'_CTTTAACCTTCCTGTAACAG_3', Pb antisense: 5'_CATAGTTAAATTGGTTATCTAC_3', P2 sense: 5'_GAAGAGTGGTTGTAAATAATA_3', P2 antisense: 5'_CCTATATCACCAAAGTTAGTAG_3' and Pab sense: 5'_GAAGAGTGGTTGTAAATAATT_3', Pab antisense: 5'_CCTATATAACCAAAGTTAGTAC_3'. Primer pairs Pb amplifies the region between nucleotides 4043 and 4470, and the length of PCR product is 427 bp. Primer pairs P2 amplifies the region between nucleotides 3025 and 3706, and the length of PCR product is 681 bp. Primer pairs Pab amplifies the same region as primer pairs P2 and the difference between these 2 primers is restricted to one base at the 3′ end of each primer. These 3 pairs of primers can differentiate parvovirus strains from each other. Original CPV-2 virus could be recognized by primer pair P2, while CPV-2a could be recognized only by primer pair Pab and CPV-2b with primer pairs Pab and Pb.

PCR amplification was performed using high fidelity Taq polymerase (CinaClon, Iran) with an initial denaturation step at 95 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 1min, and extension at 72 °C for 1min. A final extension step was performed at 72 °C for 5 min. PCR products were electrophoresed on 1.2% agarose gel and then were stained with ethidium bromide and visualized by Gel Doc.

For further analysis, the PCR products of CPV-2b positive samples were purified and sequenced bi-directionally by Macrogen Inc. (Seoul, South Korea) in an ABI 3730 XL automatic DNA sequencer.

Sequence analysis: Sequences were analyzed by BLAST through the National Center for Biotechnology Information (http://

www.ncbi.nlm.nih.gov) (Altschul et al, 1997). The Sequence alignments were made using Clustal software and also protein prediction was done using the BioEdit version 7.0.5.3 software package (http://jwbrown.mbio.ncsu.edu/Bioedit/bioedit.html).

The sequences of the reference strains with the following accession numbers: M38245.1 (CPV2), M24003.1 (CPV 2a isolate CPV-15), M74849.1 (CPV 2b isolate 39), AY380577.1 (CPV 2c isolate 56/00) were retrieved from the NCBI Genomes database and were chosen for phylogenetic analysis, which was conducted using MEGA6Beta (http://www.megasoftware.net/mega41.html). A phylogenetic tree was constructed by the neighbor-joining method and bootstrapping over 1000 replicates was done to assess the confidence level of the branch pattern.

The nt sequence of the VP2 gene of the analyzed CPV strains were deposited in GenBank under accession numbers KJ676838 to KJ676846.

Results

Virus strains: Three pairs of differential primers were used in our study which can determine CPV2a and CPV2b strains. Based on PCR results, 18 out of 50 (36%) samples were positive for CPV-2a and 32 (64%) were positive for CPV-2b. Vaccine strain was also positive for CPV-2 which is the original type (Fig. 1).

Sequence analysis: Sequence analysis results showed that based on the nucleotides encoding for residue 426 (position 4062 to 4064) all previously assumed CPV-2b samples were true CPV-2b, except for strain Ir28 which showed substitution of Asp (GAT) with Gly (GGT). This mutation

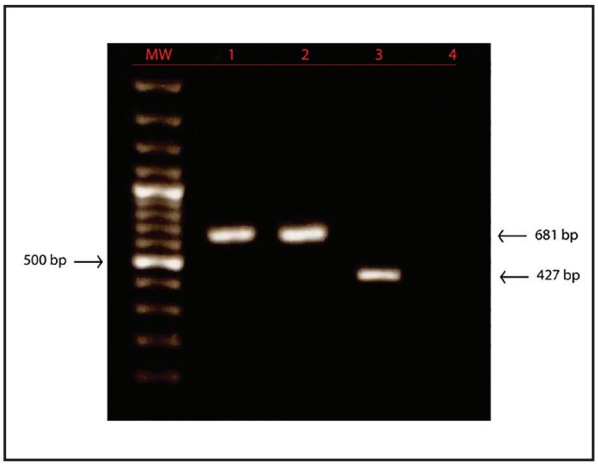

Figure 1. Representatives of PCR results. MW is molecular weight ladder (100 bp, Fermentas, Germany). Lane 1 is PCR-product 681bp using primer pairs of Pab, Lane 2 is PCR-product 681bp using primer pairs of P2 (positive control, commercial vaccine), Lane 3 is PCR-product 427bp using primer pairs of pb, Lane 4 is negative control (stool of healthy dog).

is novel and has not been reported before. The sequence alignment also showed that strains Ir3 and Ir19 are similar to the reference strainM74849.1(CPV-2b) but other strains differed by exhibiting further synonymous substitutions: C at nucleotide 4121 and A at nucleotide 4430 (Ir13);C at nucleotide 4265 (Ir35, 26 and 45); C at nucleotide 4268 (Ir15, 20, 47 and 45); C at nucleotide 4445 (Ir21, 22, 27, 24, 48 and 26) (Fig. 2).Strains Ir21, 22, 27, 24, 48 and 26 are the most identified strains in Iran and that have a unique substitution (C) at position 4445.

Sequence comparison and phylogenetic analysis: A phylogenetic tree was constructed from all CPV-2b positive samples (4043-4470 nt) and an additional 35 sequences were retrieved from the GenBank database. Sequences M38245.1 (CPV2), M24003.1 (CPV 2a isolate CPV-15), M74849.1 (CPV 2b isolate 39), AY380577.1 (CPV 2c isolate 56/00) were chosen as reference sequences (Fig. 2).

Iranian isolates 3, 5, 19, 26, 35, and 45 clustered together with the European and

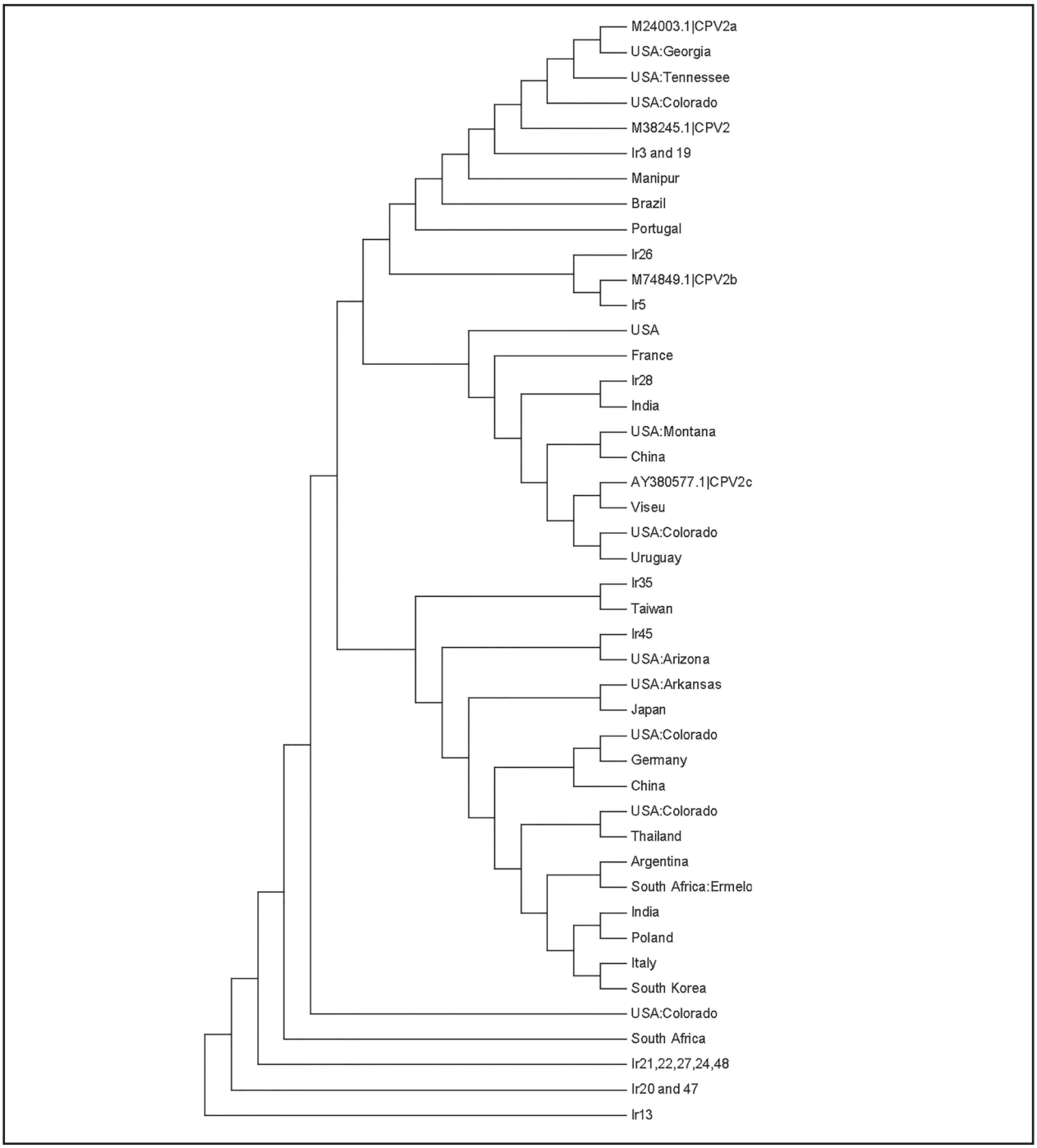

Figure 3. Neighbour-joining tree based on the partial VP2 gene sequences (4043-4470 nt) of canine parvoviruses. Iranian CPV isolates are indicated by Ir. The following reference strains were used for phylogenetic tree construction: M38245.1 (CPV2), M24003.1 (CPV 2a isolate CPV-15), M74849.1 (CPV 2b isolate 39), AY380577.1 (CPV 2c isolate 56/00). The sequences from regions outside Iran were retrieved from GenBank and for each country one representative sequence was selected for the phylogenetic tree. A statistical support was provided by bootstrapping over 1000 replicates.

some American and East Asian strains. Among these, Ir26 and Ir5 segregated together with M74849.1 (CPV 2b) reference isolate whereas Ir28, 35 and 45 segregated with Indian, Taiwan and Arizona strains, respectively. Other Iranian CPV-2b isolates formed separate cluster, with South Africa strain and the Ir13 being separated by a long branch which accounts for distinct aa substitution detected by sequence analysis.

Discussion

Since its first detection in 1978, canine parvovirus has experienced multiple mutations which lead to the emergence of new variants CPV-2a, 2b and 2c. There are many reasons for development of new antigenic variants. Vaccination programs and increased immunity against CPV has decreased the mortality and spread of the virus, however, host immunity pressure may be a cause of emergence of new antigenic variants (Decaro and Buonavoglia 2011, 2012). Additionally, because some viral variants replicate over others, the virus population changes over time (Hoelzer at al. 2008). Antigenic variants possess different biological and antigenic properties such as the improved ability of CPV-2a and CPV-2b to bind to the transferrin receptor. Therefore, as a consequence of a further adaptation, new CPV variants may cause a more severe disease (Carmichael, 2005; Hueffer & Parrish 2003).

According to PCR results, in Iran CPV-2b (64%) is more prevalent than CPV-2a(36%). Replacement of original CPV-2 with new variants CPV-2a and 2b within a few years is an example of how CPV has evolved and spread. At the moment the original CPV-2 is not circulating in canine population and is only used in vaccine production whereas variants 2a, 2b and 2c have different prevalence among different countries. A peculiarity of parvoviruses of the feline subgroup is that single nucleotide substitutions may determine drastic phenotypic changes affecting antigenicity, host range in vivo and in vitro, and hemagglutination (Buonavoglia et al. 2001). CPV-2a and 2b are different in five or six aa in viral capsid protein in comparison with the original CPV-2. Also, only 2 aa changes in VP2 has caused the evolution of CPV-2a to CPV-2b (Parrish et al. 1991; Truyen et al. 1995). The most recently reported strain is which named CPV-2c has only one aa change in important residue 426 of VP2 in comparison with CPV-2b (Chiang 2016). In addition, recent studies have reported a new CPV-2a variant which is only different from CPV-2b and 2c in position 426 of the VP2 protein. This mutation restricts the differences among the antigenic variants 2a, 2b and 2c to only one aa at position 426, that is Asn in CPV-2a, Asp in CPV-2b and Glu in CPV-2c. These findings show how few aa changes can profoundly change the antigenic characterization of the virus and also emphasizes the importance of changes in residue 426 which has led to emergence of new CPV variants. Our study shows a novel mutation in this important position which may lead to emergence of a new variant. However, this mutation was observed in only one of our samples and more studies are needed to determine the prevalence of this mutation in the dog population. This finding also emphasizes the previous study results which indicate the high evolution rate of CPV (Vannamahaxay et al. 2017). The newest variant CPV-2c was reported to cause more severe disease in most of the cases and in adult dogs which were vaccinated annually (Decaro and Bounavoglia 2011). Therefore, continuing monitoring of virus types will help to detect any new or potentially more pathogenic variant. In addition, it is necessary to evaluate the current vaccines ability in protecting dog population against newer CPV-2 variants.

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References

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J. (1997) Gapped BLAST and PSI- LAST: a new generation of protein database search programs. Nucl Acids Res. 25: 3389-3402.

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. (2005) An annotated historical account of canine parvovirus. J Vet Med B Infect Dis Vet Public Health. 52: 303-311.

Chiang, S.Y., Wu, H.Y., Chiou, M.T., Chang, M.C., Lin, C.N. (2016) Identification of a novel canine parvovirus type 2c in Taiwan. Virol J. 13: 160.

Decaro, N., Buonavoglia, C. (2011) Canine parvovirus-A review of epidemiological and diagnostic aspects, with emphasis on type 2c. Vet Microbiol. 155: 1-12

Decaro, N., Buonavoglia, C. (2012) Canine parvovirus--a review of epidemiological and

diagnostic aspects, with emphasis on type 2c. Vet Microbiol. 155: 1-12.

Decaro, N., Desario, C., Campolo, M., Elia, G., Martella, V., Ricci, D., Lorusso, E., Buonavoglia, C. (2005) Clinical and virological findings in pups naturally infected by canine parvovirus type 2 Glu-426 mutant. J Vet Diagn Invest. 17: 133-138.

Duque-García, Y., Echeverri-Zuluaga, M., Trejos-Suarez, J., Ruiz-Saenz, J. (2017) Prevalence and molecular epidemiology of Canine parvovirus 2 in diarrheic dogs in Colombia, South America: A possible new CPV-2a is emerging? Vet Microbiol. 201: 56-61.

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.

Hueffer, K., Parker, J.S., Weichert, W.S., Geisel, R.E., Sgro, J.Y., Parrish, C.R. (2003) The natural host range shift and subsequent evolution of canine parvovirus resulted from virus-specific binding to the canine transferrin receptor. J Virol. 77: 1718-26.

Martella, V., Cavalli, A., Pratelli, A., Bozzo, G., Camaro, M., Buonavoglia, D., Narcisi, D., Tempesta, M., Buonavoglia, C. (2004) A canine parvovirus mutant is spreading in Italy. J Clin Microbiol. 42: 1333-1336.

Miranda, C., Thompson, G. (2016) Canine parvovirus: the worldwide occurrence of antigenic variants. J Gen Virol. 97: 2043-57.

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.

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

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.

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.

Senda, M, Parrish, C.R., Harasawa, R., Gamoh, K., Muramatsu, M., Hirayama, N., Itoh, O.

(1995) Detection by PCR of wild-type canine parvovirus which contaminates dog vaccines. J Clin Microbiol. 33: 110-113.

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

Vannamahaxay, S., Vongkhamchanh, S., Intanon, M., Tangtrongsup, S., Tiwananthagorn, S., Pringproa, K., Chuammitri, P. (2017) Molecular characterization of canine parvovirus in Vientiane, Laos. Arch Virol. 162: 1355-1361.

Zhou, P., Zeng, W., Zhan,g X., Li, S. (2017) The genetic evolution of canine parvovirus - A new perspective. PLoS One. 31;12: e0175035.