

Genetic Characterization of Porcine Parvovirus 7 (PPV7) Isolates in Fujian, China

Ru-Jing CHEN¹ Ting-Ting LAI² Qiu-Yong CHEN¹ Xue-Min WU¹ Yong-Liang CHE¹
Shan YAN¹ Chen-Yan WANG¹ Long-Bai WANG¹ Lun-Jiang ZHOU¹ 

¹Institute of Animal Husbandry and Veterinary Medicine, Fujian Academy of Agriculture Sciences/Fujian Animal Disease Control Technology Development Center, Fuzhou 350013, CHINA

²FuZhou Da Bei Nong Biotech, Fuzhou 350013, CHINA

Article Code: KVFD-2017-19202 Received: 11.12.2017 Accepted: 09.03.2018 Published Online: 09.03.2018

How to Cite This Article

Chen RJ, Lai TT, Chen QY, Wu XM, Che YL, Yan S, Wang CY, Wang LB, Zhou LJ: Classification tree method for determining factors that affecting hatchability in chukar partridge (*Alectoris chukar*) eggs. *Kafkas Univ Vet Fak Derg*, 24 (3): 473-477, 2018. DOI: 10.9775/kvfd.2017.19202

Abstract

In this study, we sequenced and analyzed the complete genomes of the porcine parvovirus 7 (PPV7) isolated in Fujian, Southeast China. Genomic comparison revealed that PPV7 isolates in Fujian shared higher (more than 98.5%) nucleotide homology and closer relationship with other PPV7 strains. Phylogenetic analysis based on NS indicated that PPV7 formed a distinct cluster (proposed *Chapparvovirus*) closer to subfamily *Parvovirinae*. However, phylogenetic analysis based on genome and VP showed that PPV7 isolates formed a distinct cluster closer to subfamily *Densovirinae*. No recombination event observed suggesting recombination did not contribution to the genetic diversity of PPV7. These findings demonstrated that PPV7 continuous circulating in China, which may help us to understand the evolution diversity of porcine parvoviruses.

Keywords: *Porcine parvovirus 7, Genomic comparison, Phylogenetic analysis*

Çin'in Fujian Eyaletinde Domuz Parvovirus 7 (PPV7) İzolatlarının Genetik Karakterizasyonu

Öz

Bu çalışmada, Güneydoğu Çin'in Fujian Eyaletinde elde edilen domuz parvovirus 7 (PPV7) izolatlarının tüm genomu sekanslandı ve analiz edildi. Genomik karşılaştırmada Fujian'dan elde edilen PPV7 izolatlarının diğer PPV7 suşları ile yüksek nükleotid benzerlik (%98.5'den daha fazla) göstererek yakın ilişkili olduğu gözlemlendi. NS temelli filogenetik analiz PPV7'nin Parvovirinae alt familyasına yakın farklı topluluk oluşturduğunu (*Chapparvovirus* olabileceği önerilen) gösterdi. Ancak, genom ve VP temelli filogenetik analiz PPV7 izolatlarının *Densovirinae* alt familyasına daha yakın topluluk oluşturduğunu gösterdi. Rekombinasyonun gözlemlenmemesi nedeniyle PPV7'nin genetik farklılıklarının oluşmasında rekombinasyonun katkısının olmadığı belirlendi. Bu bulgular, Çin'de PPV7'nin halen mevcut olduğunu göstermiş ve domuz parvovirusunun kalıtsal farklılaşmasını anlamamıza yardımcı olabilir.

Anahtar sözcükler: *Domuz parvovirus 7, Genomik karşılaştırma, Filogenetik analiz*

INTRODUCTION

The family *Parvoviridae* consists of many small, non-enveloped, single-stranded DNA viruses which can infect a wide variety of animal species. Parvoviruses genomes are approximately 5.0 kb in size, with two major open reading frames (ORFs), ORF1 and ORF2. The ORF1 encodes the non-structural protein (NSP) which has replicase (REP) activity, while the ORF2 encodes the structural or capsid protein

(VP) ^[1]. Many different parvoviruses species had been identified in swine herds, including the PPV1 (also namely classic PPV), PPV2, PPV3 (also namely PARV4, hokovirus or partetravirus), PPV4, PPV5, PPV6, and porcine Bocaviruses (PBoV). Although these recently identified emerging PPV genotypes have been found in many pig breeding countries, but the clinical significance of PPV2 through porcine Bocaviruses infections remains unclear ^[2-4]. Moreover, no commercial vaccines are available at present.



İletişim (Correspondence)



+86 591 87572301



lunjiang@163.com

Currently, the family *Parvoviridae* includes two subfamilies, *Densovirinae* and *Parvovirinae*. The subfamily *Densovirinae* contains six genera: *Ambidensovirus*, *Brevidensovirus*, *Hepandensovirus*, *Iteradensovirus*, *Penstyldensovirus* and an unassigned genus. Meanwhile, the subfamily *Parvovirinae* contains eight genera: *Amdoparvovirus*, *Aveparvovirus*, *Bocaparvovirus* (including PBoV), *Copiparvovirus* (including PPV4, PPV5 and PPV6), *Dependoparvovirus*, *Erythroparvovirus*, *Protoparvovirus* (including PPV1) and *Tetraparvovirus* (including PPV2 and PPV3)^[1]. Additionally, there are a number of unassigned members in the subfamily *Parvovirinae*.

Recently, a novel genus parvoviruses (proposed *Chapparvovirus*) has been discovered from turkeys^[5], *Eidolon helvums*^[6], wild rats^[7] and pigs (namely porcine parvovirus 7, PPV7)^[8,9]. Previously study also demonstrated that *Chapparvovirus* still have unclear species. PPV7 was firstly discovered by metagenomic sequencing from porcine rectal swabs of healthy adult pigs in the USA, in 2016^[8], then was identified in Guangdong Province in southern China^[9] with no significant clinical signs observed. In this study, we sequenced and analysis the PPV7 isolates from lung tissues in Fujian, which help us to understand the genetic diversity of porcine parvoviruses in China.

MATERIAL and METHODS

Ethics Statement

The animal protocol used in this study was approved by the Research Ethics Committee of the Institute of Animal Husbandry and Veterinary Medicine, Fujian Academy of Agriculture Sciences (Permit Number AHVMFAAS20170012). All samples were handled in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals approved by the State Council of China.

Collection of Samples

The samples (lung tissues) were collected from healthy pigs (with the age about 180 d) after slaughtered from three commercial slaughterhouses in October, 2017. These three slaughterhouses located in Fuzhou city, Zhangzhou city and Longyan city in Fujian province (Southeast China), respectively. The total 30 lung tissues (10 samples for each slaughterhouse) were collected and kept at -80°C until processing.

DNA Extraction and PCR

These samples were homogenized in sterile phosphate-buffered saline (PBS, pH 7.2) and centrifuged at 4 000 rpm for 15 min at room temperature. The supernatants of each sample were used for DNA extraction with the MagicPure Viral DNA/RNA Kit (Transgen Biotek, Beijing,

China) according to the manufacturer's instructions. The eluted DNAs were stored at -20°C until PCR detection. The presence of PPV7 DNA was initially detected by PCR with primers (PPV7-3434-F and PPV7-3654-R, see [Table 1](#)) targeting a 241 bp fragment of the VP gene as described previously^[9]. The amplifications were performed using 2×TransTaq-T PCR SuperMix (+dye) (Transgen Biotek, Beijing, China) in a 50 µL PCR reaction containing 25 µL of 2X TransTaq-T PCR SuperMix, 1 µL PPV7-3434-F and PPV7-3654-R primer (10 µM each), 2 µL of the extracted DNA and 21 µL of PCR-grade water under the following conditions: denaturation for 5 min at 94°C followed by 35 cycles of 94°C for 30 sec, 56°C for 30 sec and 72°C for 30 sec, then with a final extension step at 72°C for 10 min. The PCR products were visualised by 1.0% agarose gel electrophoresis.

DNA Sequencing

Primers for genome sequencing ([Table 1](#)) were designed according to the similar strategy described previously^[9] after optimized, with overlapped fragments encompassed the PPV7 genome. All the PCR positive samples' genomes were amplified by PCR technology. Amplicons were purified by QIAquick PCR Purification Kit (QIAGEN, Germany) and inserted into the pEASY-Blunt Zero Cloning Kit (Transgen Biotek, Beijing, China). In each case, five positive clones were randomly selected and sent to Sangon (Sangon Biotech, Shanghai, China) for sequencing.

Genomic Sequence Alignment and Phylogenetic Analysis

The sequences were assembled by the SeqMan program of Lasergene 7.0 software (DNASTAR Inc., Madison, Wisconsin, USA)^[10] in order to obtain the PPV7 genome, and submitted to GenBank. Sequence comparison and genomic homology was determined using the ClustalW method^[8]. Phylogenetic trees based on the genome (nucleotide sequences), NS (amino acid sequences), and VP (amino acid sequences) were constructed by using the neighbor-joining method implemented in MEGA v6.0^[11] with other reference sequences downloaded from GenBank. Support for individual nodes was determined by 1000 bootstrap replicates (Bootstrap=1000). *Chapparvovirus*, which were discovered from turkeys (turkey parvovirus, TuPV, GenBank No. KF925531), *Eidolon helvums* (*Eidolon helvums* parvovirus 2, GenBank No. JX885610, only had NS gene which had not used for analysis in this study) and wild rats (rat parvovirus 2, RPV2, GenBank No. KX272741) were used for analysis. Additionally, *Ambidensovirus* (*Galleria mellonella* densovirus, GmDENV, GenBank No. NC004286), *Brevidensovirus* (*Anopheles gambiae* densovirus, AgDENV, GenBank No. NC011317), *Hepandensovirus* (*Decapod* hebandensovirus 1, DhDENV, GenBank No. KT316242), *Iteradensovirus* (*Bombyx mori* densovirus 5, BmDENV, GenBank No. NC004287) and *Penstyldensovirus* (*Decapod* penstyldensovirus 1, DpDENV, GenBank No. KT316259) in the subfamily *Densovirinae* also used as out-group.

Table 1. Primers used in this study

Primers	Sequences(5'→3')	Position *	Length (bp)
PPV7-3434-F	CCTCCATCAGCAGCGACCAGT	3434-3454	241
PPV7-3654-R	ACCAGGGTCCGTTTTCGTCT	3654-3674	
PPV7-F1	ATATGCACACCCTAGGCCAC	448-467	1375
PPV7-R1	GTCCCAGATAAATATGAACA	1803-1822	
PPV7-F2	TGCCGAGAACCCCATCATCA	1719-1739	1205
PPV7-R2	TGTGGTACCCGGTCTCGTA	2905-2923	
PPV7-F3	GTTCAATCCCATCCCATCACA	2802-2823	851
PPV7-R3	TGAGTGGTATTCTTTGATGAAGT	3629-3652	
PPV7-F4	GAACACCAACAAACCGTATCC	3102-3122	943
PPV7-R4	TAACAGGAAGGGCCAAAGATAGTT	4021-4044	

* Postion reference strain PPV 7 isolate 42 (GenBank number KU563733)

Recombination Analysis

Recombination analysis is a major evolutionary mechanism which has been observed in several porcine parvovirus species. We performed a recombination analysis using the Simplot 3.5.1 and the Recombination Detection Program (RDP 4.0 software). Porcine parvovirus into the subfamily *Parvovirinae*, such as PPV1, PPV2, PPV3, PPV4, PPV5, PPV6, PBoV and PPV7 were used for recombination analysis.

Characteristic Amino Acids Motifs of PPV7 VP Gene

Phospholipase A₂ (PLA₂) motif resembling the catalytic site of secretory phospholipase A₂ (sPLA₂) founded in parvoviruses, which located on the N-terminal of the capsid protein^[12]. The capsid protein of PPV7s were used to analysis based on the conserved motif in Ca²⁺ binding loop (YXGXG) and catalytic site of sPLA₂ (HDXXY), compared with PPV1 (GenBank No. NC001718).

RESULTS

Genomic Comparison

Three samples in Fuzhou, none in Zhangzhou and two samples in Longyan were detected positive by PCR, with the positive rate of 30%, 0 and 20%, respectively. The five positive samples were sequenced and assembled; the genomic comparison revealed that the three samples collected in Fuzhou city shared 100% genomic nucleotide identity (designated as PPV7 FJFZ2017). Meanwhile, two samples collected in Longyan city also shared 100% genomic nucleotide identity (designated as PPV7 FJLY2017). The sequenced PPV7 genome has a NS coding region of 2019 nucleotides (672 amino acids) and a VP coding region of 1401 nucleotides (466 amino acids). The complete genome of PPV7 strains FJFZ2017 and FJLY2017 were entered in to the NCBI GenBank under the accession number MG696111 and MG696112.

Based on sequence analysis of complete coding regions, the sequenced sequences (PPV7 FJFZ2017 and PPV7 FJLY2017) shared 99.7% nucleotide similarity with each other, shared 98.8% and 99.0% with PPV7 strain 42^[6] which was first identified in the US swine, shared 98.5% to 99.0% with PPV7 strains (GD-2014-1, GD-2014-2 and GD-2014-3)^[9] which were first identified in Southern China. Phylogenetic analysis based on genome sequences under the family *Parvoviridae* (subfamily: *Densovirinae* and *Parvovirinae*) indicated that all the PPV7 isolates, together with TuPV and RPV2, were formed a distinct cluster (*Chapparvovirus*), different with other porcine parvoviruses. Moreover, the *Chapparvovirus* cluster is closer to subfamily *Densovirinae* rather than subfamily *Parvovirinae* (Fig. 1A).

NS Comparison and Phylogenetic Analysis

The PPV7 NS shared 98.7%-99.9% and 96.6%-99.7% similarity with each other at the nucleotide and amino acid (aa) levels, respectively. Phylogenetic analysis based on NS aa sequences under the family *Parvoviridae*, the results demonstrated that all the PPV7 isolates shared a distinct cluster with TuPV and RPV2, formed a distinct *Chapparvovirus* cluster. Meanwhile, based on the NS evolution, the *Chapparvovirus* cluster shared the similar relationship with other porcine parvoviruses, under the subfamily *Parvovirinae* (Fig. 1B).

VP Comparison and Phylogenetic Analysis

The PPV7 VP shared 98.0%-99.9% and 94.6%-99.1% similarity with each other at the nucleotide and aa levels, respectively. Phylogenetic analysis based on VP aa sequences under the family *Parvoviridae* suggested that the PPV7 isolates together with TuPV and RPV2, shared a distinct cluster (*Chapparvovirus*). Similar like the genome evolution relationship, the *Chapparvovirus* cluster is closer to subfamily *Densovirinae* rather than subfamily *Parvovirinae* (Fig. 1C).

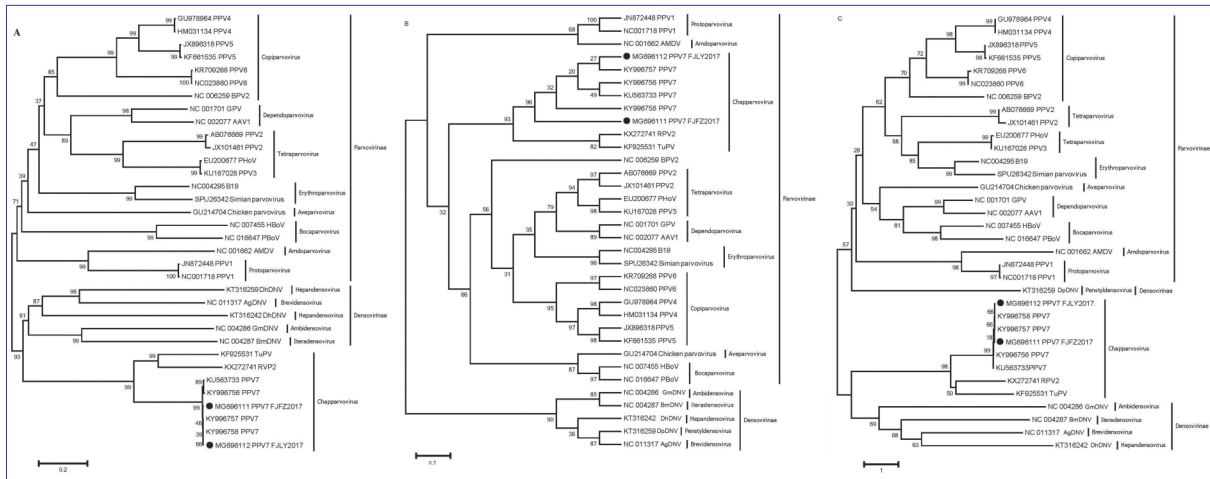


Fig 1. Phylogenetic relationship of PPV7s within family *Parvoviridae*. A- Phylogenetic tree constructed based on the genome nucleotide sequences, B- Phylogenetic tree constructed based on the NS protein sequences, C- Phylogenetic tree constructed based on the VP protein sequences. Phylogenetic trees were constructed by using the neighbor-joining method implemented in MEGA v6.0. Support for individual nodes was determined by 1000 bootstrap replicates (Bootstrap =1000). The evolutionary distance scale is shown at the bottom. The PPV7 sequenced in this study were indicated by filled circle (●). Reference sequences obtained from GenBank are indicated by accession number

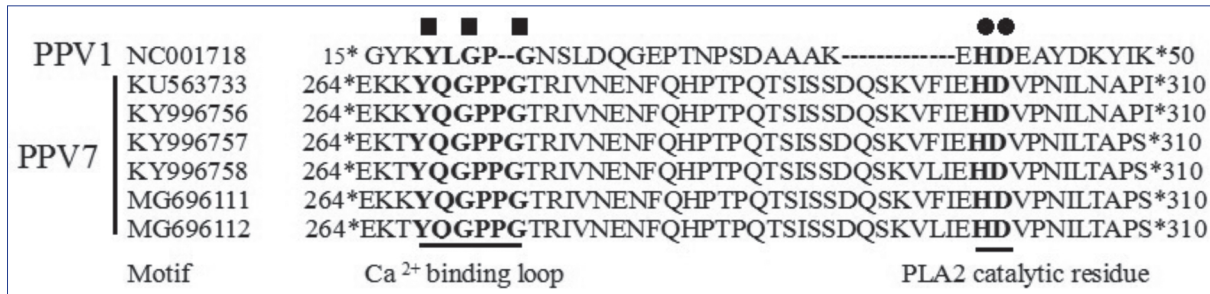


Fig 2. Sequence alignment of the putative phospholipase A₂ motif of PPV7s. The Ca²⁺ binding loop is indicated by filled squares (■) and the catalytic residues by filled circle (●)

Recombination Analysis

Using Simplot 3.5.1 and RDP 4.0 software, PPV7s shared no potential recombination signal with other parvoviruses discovered in pigs, including PPV1, PPV2, PPV3, PPV4, PPV5, PPV6 and PBoV. These data indicated that PPV7 is not a recombination of other PPVs and also suggesting recombination did not contribution to the genetic diversity for PPV7.

Identification of A Phospholipase A₂ Motif

In the alignments of the predicted VP protein sequence of PPV7 with PPV1, the conserved motif of Ca²⁺ binding loop (YXGXG) was different due to the single aa insertion at 271 aa (P) changed as (YXGXG). Moreover, the catalytic residues (HDXXY) of Phospholipase A₂ (PLA₂) were different due to the single aa mutation at 304 aa (Y to N) (Fig. 2).

DISCUSSION

Recent investigations demonstrated that the age of the family *Parvoviridae* may exceed 40 to 50 million years [13].

Besides the long history for parvoviruses, the genomes were exhibit similar high mutation and recombination rates as RNA viruses [14-16]. These data may explain for the high diversity and vast genetic divergence under the family *Parvoviridae* [17].

Genomic comparison revealed that PPV7 isolates analysed in this study shared higher (more than 98.5%) nucleotide homology with other PPV7 strains reported previously. Further genomic comparison analysis revealed that PPV7 FJFZ2017 and PPV7 FJLY2017, similar like the GD-2014-2 and GD-2014-3 which were identified in a co-infection with porcine circovirus 2 (PCV2), possess a consecutive 9-nt deletion (ATGTCTGTA) near the 5' end of the VP gene which leads to a three aa deletion. These PPV7 positive samples also tested positive with PCV2 by PCR [9].

The NS protein encode by the diversity PPV genome is commonly used to classify PPV genotype [1]. Phylogenetic analysis based on NS protein showed PPV7 isolates sharing closer relationship, which formed a closer genetic cluster, different with other eight genera under the subfamily

Parvovirinae. But phylogenetic analyses express the differences between genome (also the VP protein) and NS protein based phylogenetic analysis. Though based on genome and VP protein also demonstrated that PPV7 isolates together with TuPV and RPV2, shared a distinct cluster (*Chapparovirus*). But the relationship shared closer to subfamily *Densovirinae* rather than subfamily *Parvovirinae*. Until now, the PPV7 had not been classified by International Committee on Taxonomy of Viruses (ICTV), genomic comparison and phylogenetic analysis revealed that the PPV7s (together with TuPV and RPV2) may be classified in to a novel genus (proposed *Chapparovirus*), these data suggesting that *Chapparovirus* may be classified under subfamily *Densovirinae* rather than subfamily *Parvovirinae*.

Previously research indicated that potential recombination events can be detected both in domestic and wild boar origin recombinant PPVs [18]. But in this study, no potential recombination event can be observed with other parvoviruses discovered in swine herds. These data suggested that recombination did not contribution to the genetic diversity for PPV7. Although mutation of critical amino acids strongly reduce both PLA₂ activity and proportionally, viral infectivity, but cell surface attachment, entry and endocytosis by PLA₂-deficient virions were not affected [12]. Compared with PPV1, the PPV7 Ca²⁺ binding loop and catalytic residues were different due to the single aa mutation. Similar as turkey parvovirus, which also lack the PLA₂ domain [1,8]. Whether these changes can affect the PLA₂ activity needs further study.

In summary, this report confirms the PPV7 continuous circulating in China. Regardless of how the PPV7 generated, the PPV genomic characterization provide new sights for different PPVs in swine herds, which may help us to understand the evolution diversity of porcine parvoviruses.

ACKNOWLEDGEMENTS

This work was supported by the Fujian Agriculture Research Project (2017No.129), Fujian Public Welfare Project (2018R1023-9) and Fujian Nature Science Foundation (2018J01115).

REFERENCES

- Cotmore SF, Agbandje-McKenna M, Chiorini JA, Mukha DV, Pintel DJ, Qiu J, Soderlund-Venermo M, Tattersall P, Tijssen P, Gatherer D, Davison AJ:** The family Parvoviridae. *Arch Virol*, 159, 1239-1247, 2014. DOI: 10.1007/s00705-013-1914-1
- Streck AF, Canal CW, Truyen U:** Molecular epidemiology and evolution of porcine parvoviruses. *Infect Genet Evol*, 36, 300-306, 2015. DOI: 10.1016/j.meegid.2015.10.007
- Cui J, Biernacka K, Fan J, Gerber PF, Stadejek T, Opriessnig T:** Circulation of porcine parvovirus types 1 through 6 in serum samples obtained from six commercial polish pig farms. *Transbound Emerg Dis*, 64, 1945-1952, 2017. DOI: 10.1111/tbed.12593
- Oh WT, Kim RY, Nguyen VG, Chung HC, Park BK:** Perspectives on the evolution of porcine parvovirus. *Viruses*, 9, E196, 2017. DOI: 10.3390/v9080196
- Reuter G, Boros Á, Delwart E, Pankovics P:** Novel circular single-stranded DNA virus from turkey faeces. *Arch Virol*, 159, 2161-2164, 2014. DOI: 10.1007/s00705-014-2025-3
- Baker KS, Leggett RM, Bexfield NH, Alston M, Daly G, Todd S, Tachedjian M, Holmes CEG, Cramer S, Wang LF, Heeney JL, Suu-Ire R, Kellam P, Cunningham AA, Wood JLN, Caccamo M, Murcia PR:** Metagenomic study of the viruses of African straw-coloured fruit bats: Detection of a chiropteran poxvirus and isolation of a novel adenovirus. *Virology*, 441, 95-106, 2013. DOI: 10.1016/j.virol.2013.03.014
- Yang S, Liu Z, Wang Y, Li W, Fu X, Lin Y, Shen Q, Wang X, Wang H, Zhang W:** A novel rodent Chapparovirus in feces of wild rats. *Virology*, 13, 133, 2016. DOI: 10.1186/s12985-016-0589-0
- Palinski RM, Mitra N, Hause BM:** Discovery of a novel Parvovirinae virus, porcine parvovirus 7, by metagenomic sequencing of porcine rectal swabs. *Virus Genes*, 52, 564-567, 2016. DOI: 10.1007/s11262-016-1322-1
- Xing X, Zhou H, Tong L, Chen Y, Sun Y, Wang H, Zhang G:** First identification of porcine parvovirus 7 in China. *Arch Virol*, 163, 209-213, 2018. DOI: 10.1007/s00705-017-3585-9
- Burland TG:** DNASTAR's Lasergene Sequence Analysis Software. In, Misener S, Krawetz SA (Eds): Bioinformatics Methods and Protocols. Methods in Molecular Biology, 71-91, *Methods Mol Biol*, Humana Press, Totowa, 2000.
- Tamura K, Stecher G, Peterson D, Filipksi A, Kumar S:** MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol*, 30, 2725-2729, 2013. DOI: 10.1093/molbev/mst197
- Zádori Z, Szelei J, Lacoste MC, Li Y, Gariépy S, Raymond P, Allaire M, Nabi IR, Tijssen P:** A viral phospholipase A₂ is required for parvovirus infectivity. *Dev Cell*, 1, 291-302, 2001. DOI: 10.1016/S1534-5807(01)00031-4
- Belyi VA, Levine AJ, Skalka AM:** Sequences from ancestral single-stranded DNA viruses in vertebrate genomes: The parvoviridae and circoviridae are more than 40 to 50 million years old. *J Virol*, 84, 12458-12462, 2010. DOI: 10.1128/JVI.01789-10
- Xiao CT, Halbur PG, Opriessnig T:** Molecular evolutionary genetic analysis of emerging parvoviruses identified in pigs. *Infect Genet Evol*, 16, 369-376, 2013. DOI: 10.1016/j.meegid.2013.03.017
- Kailasan S, Agbandje-McKenna M, Parrish CR:** Parvovirus family conundrum: What makes a killer? *Annu Rev Virol*, 2, 425-450, 2015. DOI: 10.1146/annurev-virology-100114-055150
- Martynova EU, Schal C, Mukha DV:** Effects of recombination on densovirus phylogeny. *Arch Virol*, 161, 63-75, 2016. DOI: 10.1007/s00705-015-2642-5
- Vannamahaxay S, Chuammitri P:** Update on canine parvovirus: Molecular and genomic aspects, with emphasis on genetic variants affecting the canine host. *Kafkas Univ Vet Fak Derg*, 23, 847-856, 2017. DOI: 10.9775/kvfd.2017.17673
- Cadar D, Csághola A, Kiss T, Tuboly T:** Capsid protein evolution and comparative phylogeny of novel porcine parvoviruses. *Mol Phylogenet Evol*, 66, 243-253, 2013. DOI: 10.1016/j.ympev.2012.09.030