

Genomic Characterization of Goose Parvovirus and Muscovy Duck Parvovirus Co-infection in Fujian, China

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Article Code: KVFD-2015-13848 Received: 10.06.2015 Accepted: 02.08.2015 Published Online: 02.08.2015

Abstract

In the present study, we sequenced and analyzed the complete genomes of the goose parvovirus (GPV) and the duck parvovirus (MDPV) isolates derived from a co-infected dead Muscovy duckling without any symptoms of other emerging Muscovy duck diseases. The genomic organization and sequences analysis indicates that the size of the full-length inverted terminal repeats regions determines the genomic length of GPV and MDPV, with which the lengths of the non-structural protein and capsid proteins coding region were highly conserved. According to the phylogenetic analysis, there are two genotypes of duck parvoviruses co-circulating in Muscovy ducks in China

Keywords: *Goose parvovirus; Muscovy duck parvovirus; Genome; Analysis*

Çin'in Fujian Eyaletinde Kaz Parvovirus ve Muscovy Ördek Parvovirus Koenfeksiyonunun Genomik Karakterizasyonu

Özet

Bu çalışmada, herhangi bir semptom göstermeyen bir Muscovy ördek palazından izole edilen kaz parvovirus (GPV) ve ördek parvovirus (MDPV) koenfeksiyon izolatlarının tüm genom sekansı ve analizi gerçekleştirilmiştir. Tüm uzunluk ters terminal tekrar bölgelerinin büyüklükleri GPV ve MDPV genomik boyutu belirlemektedir, ve yapısal olmayan protein ve kapsid proteinleri kodlayan bölgelerin uzunluklarının oldukça yüksek düzeyde korunduğu genomik organizasyon ve sekans analizleri ile gösterilmiştir. Filogenetik analizlere göre ördek parvoviruslarının iki genotipi Çin'de Muscovy ördeklerinde birlikte bulunmaktadır.

Anahtar sözcükler: *Kaz parvovirus, Muscovy ördek parvovirus, Genom, Analiz*

INTRODUCTION

Anseriform dependoparvovirus 1 belonging to the family *Parvoviridae* that classified into two subfamilies (*Densovirinae* and *Parvovirinae*), which are further classified in thirteen genera (5 within *Densovirinae* and 8 within *Parvovirinae*). Anseriform dependoparvovirus 1 now have two groups that include goose parvovirus (GPV) and the duck parvovirus (MDPV). The MDPV is closely related to the GPV based on the southern hybridization assays and shared more than 80.0% sequence identity with GPV. GPV can cause highly contagious and fatal disease in goslings and Muscovy ducklings; whereas MDPV only cause disease with Muscovy ducklings^[1,2].

The MDPV and GPV contain single-stranded DNA genome of approximately 5.1-kb in length, sharing closely related in genome and neutralization. The genome contains two major open reading frames (ORFs). The left ORF1 encodes the non-structural protein (NS) involved in viral replication, and the right ORF2 encodes the viral capsid proteins VP1, VP2 and VP3. The VP2 and VP3 shared the same C-terminal of the VP1, which were produced by alternate splicing^[3,4].

The MDPV and GPV were reported once epidemic in many Muscovy duck breeding regions, such as Hungary, China, Japan, Thailand, France, and the USA. Though the attenuated vaccine against MDPV and GPV were used



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for more than twenty years in China, the MDPV and GPV are still mainly diseases in China waterfowl (especially for Muscovy ducks) industry. Many small Muscovy ducks farms, with poor breeding conditions, often sporadic outbreak with MDPV or GPV infection.

In this study, we demonstrated the occurrence of MDPV and GPV co-infection in a Muscovy duckling of age 15-day without any other waterfowl common diseases by sequencing the complete genomes of the two viruses. Derivation of the genomic sequences of the MDPV and GPV provides useful tools for studying viral infections in Muscovy ducklings as well as facilitates a better understanding of the phylogenetic relationship between MDPV and GPV.

MATERIAL and METHODS

Case History

In January 2013, a dead Muscovy duckling at the age of 15-day was collected from a farm that the breed regions located in Fujian, Southeast China. The Muscovy ducks shown mass symptoms including watery diarrhea, wheezing and locomotory dysfunction, with typical GPV-related and MDPV-related infection clinical signs. Twenty-five out of forty (62.5%) were dead at the age of 15-day. All the Muscovy duckling were purchased from commercial Muscovy duck farms, which had no previous history of MDPV or GPV exposure, and no attenuated vaccines against MDPV or GPV were used.

To determine the pathogens which were responsible for the disease, tissues were sampled from the dead ducklings for GPV or MDPV tests. The PCR results of the sample testing revealed that the succumbed duckling were co-infected with GPV and MDPV. All classical endemic and emerging viruses outbreaks in Muscovy duck flocks, such as avian influenza virus, Tembusu virus, duck hepatitis virus, Muscovy duck reovirus and novel duck reovirus, could be excluded as the causative agent by PCR (RT-PCR).

Sample Collection and DNA Extraction

The liver, spleen and intestinal cavity from the dead Muscovy duck aged 15 days were collected and homogenized in sterile phosphate-buffered saline (PBS, pH7.2) and centrifuged at 8,000 rpm for 30 min at 4°C. Supernatants were filtered through 0.45 µm and 0.22 µm filters (Merck KGaA, Darmstadt, Germany) and stored at -80°C prior for DNA extraction. DNA was extracted using the Viral DNA Kit (Omega Bio-Tek, GA, USA) according to the manufacturer's instructions.

Genome Sequencing

The GPV and MDPV genomes were amplified by polymerase chain reaction (PCR) method using the corresponding primers according to the similar methodology by Shien^[5]

(Table 1), which overlapped fragments encompassing the entire GPV and MDPV genome, designated as GPV strain G7 and MDPV strain FJM5, respectively. All PCR products were purified using the Gel Extraction Kit (Omega Bio-Tek, GA, USA) and ligated into the pBackZero8-T vector cloning kit (Takara, Dalian, China). In each case, five positive clones were selected at random and sequenced (Sangon Biotech, Shanghai, China) in both directions using an ABI model 3730 automatic DNA sequencer (ABI, CA, USA). The obtained sequences were used to compile the complete genome sequences assembly with Lasergene package (DNASar, v7.1, Madison, WI, USA).

Genomic Characterization and Phylogenetic Analysis

Sequence comparison and percent identity was calculated using the Megalign program in the same package by CLUSTAL-W method. Phylogenetic analysis was performed with MEGA 6.0 using the neighbor-joining method with the maximum-likelihood model. Bootstrap scores were generated from 1,000 replicates.

Considering the VP1 coding region contains the immuno-dominant region that can induce neutralizing antibodies and the VP3 is the most abundant region with high genetic stability, we aligned the full-genome sequences, the VP1 genes and the VP3 genes in this study. Two Muscovy duck-origin GPV virus (PT strain, GenBank No: JF926695 and DY strain, GenBank No: EF515837) and the attenuated vaccine MDPV virus (P1 strain, GenBank No: JF926698) were added when constructing the VP1 gene and VP3 gene phylogenetic tree for evolution analysis.

RESULTS

Genomic Organization and Sequence Analysis

The genome of G7 was found to be 5106 nucleotides in length. The genome contained two major ORFs. The left ORF encodes the non-structural protein (NS) with 627 amino acids (aa) (nt 537-2420), and the right ORF encodes the viral capsid proteins VP1 with 732 aa (nt2439-4637) with the VP3 began at position nt 3008, respectively. However, the G7 genome shared the highest identity (98.9%) with the SYG61v strain, which was licensed as a vaccine virus used in goslings for preventing Dersy's disease in China over three decades^[6]. When compared with other reported GPV genomes, the G7 genome shared 94.1% to 98.8% nucleotides sequence identities, respectively. Compared with other reported MDPV genomes, the G7 genome shared 79.4%-83.2% nucleotides sequence identities, respectively.

The genome of FJM5 was found to be 5017 nucleotides in length. The non-structural protein (NS) encodes 627 aa (nt 490-2373), the VP1 encodes 732 aa (nt2392-4590), the VP3 encodes 534 aa (nt 2986-4590), respectively. The inverted terminal repeats (ITRs) was found to be 359 bp

Table 1. Primers used to amplify the complete genome of GPV and MDPV
Table 1. GPV ve MDPV tam genomlarını amplifiye etmekte kullanılan primerler

Type	Primers	Position	Sequences	Product Length
GPV	G-F1	1-22	CTCATTGGAGGGTTCGTTTCGT	241
	G-R1	218-241	GCATGCGCGTGGTCAACCTAACAGCCGAAAA	
	G-F2	166-185	TGACGTGTTTCCGGCTGTTA	617
	G-R2	762-782	TTCGTCCTGGTTGAACTGATT	
	G-F3	528-548	GTCGGAGAGATGGCACTTTCT	1028
	G-R3	1535-1555	GTGGTGGCAGGTCCGTAGAGC	
	G-F4	1309-1331	CTTCTCAAATAATAGACAAGTG	1137
	G-R4	2425-2445	TAGACATATCTGCTTTGAGTC	
	G-F5	2317-2337	ATGAACATGGGTGGTATGATT	1299
	G-R5	3595-3615	ACTAGAATGCACTCCGGTCAT	
	G-F6	3330-3349	AACCATTGGGAATCAGACC	1410
	G-R6	4687-4709	TCCAATGAGACTCAAGGACAAGA	
MDPV	M-F1	1-21	CTCATTGCAGGGTTCGTTTCGT	238
	M-R1	214-238	ACGCACTTCCTTTGATGACGTATTT	
	M-F2	165-188	CTTTGATGACGTATTTCCGGTTGT	669
	M-R2	812-833	TGTACTCACTGCCTTCTTCCAA	
	M-F3	500-521	TTCATTGCTGCTCTGCTCTCA	1228
	M-R3	1705-1727	CTTTTGCGGATTCCACTACTTTA	
	M-F4	1542-1562	TATGGCTCTACGGACCTGCGA	1182
	M-R4	2701-2723	TGAGCTGCTGGTCTGACGCTTTA	
	M-F5	2573-2596	AGAAAACCCCAACGAAAAGACAAT	1173
	M-R5	3721-3745	TAGCCTGTCTAAATCCTGTGAATGA	
	M-F6	3595-3616	AGCGAGATTCAATGACAGAAGT	1323
	M-R6	4898-4917	TCAAGGCTGATCGGGCGCAT	

GPV and MDPV amplification primers according the position in the GPV B strain genome (GenBank No. U25479, 5106 bp) and MDPV FM strain genome (GenBank No. U22967, 5132 bp), respectively. F and R denote the forward and reverse primers, respectively

in length, which was present at the 5' and 3' terminal ends of the genome. The FJM5 genome shared the highest identity (98.6%) with the FM strain, which was isolated in Hungary. When compared with other reported MDPV isolates SAAS-SHNN and MDPV-GX5 genome, the FJM5 genome shared 90.3% and 92.7% nucleotide sequence identities, respectively. Compared with other reported GPV genomes, the FJM5 genome shared 77.7%-79.6% nucleotides sequence identities, respectively. Nucleotide identities of the G7 VP1 gene with other GPV and MDPV isolates varied between 88.7%-98.6% and 80.0%-88.4%, respectively. Whereas, nucleotide identities of the FJM5 VP1 gene with other MDPV and GPV isolates varied between 85.2%-99.1% and 80.0%-85.4%, respectively.

The inverted terminal repeats (ITRs) of G7 and FJM5 was found to be 444 bp and 359 bp in length, respectively, which was presented at the 5' and 3' terminal ends of the genome structure. The ITRs region sequences of the GPV and MDPV isolates download from the GenBank were summarized in Table 2, which can conclude that the size of the full-length ITRs determine the genomic length of GPV and MDPV.

The complete genome of GPV strain G7 and MDPV strain FJM5 had been submitted to GenBank under accession No. KR029617 and KR075689, respectively.

Phylogenetic Analysis

Phylogenetic tree based on the GPV and MDPV isolates revealed that the GPV strain G7 clustered together with other GPV strains, and also the MDPV strain FJM5 clustered together with other MDPV strains (Fig. 1-1).

The phylogenetic tree based on the VP1 (Fig. 1-2) and VP3 (Fig. 1-3), we can found that the G7 belonged to the GPV clusters; also the FJM5 belonged to the MDPV clusters. However, four Muscovy duck-origin parvovirus (GPV PT strain, GPV DY strain, MDPV SAAS-SHNN strain and MDPV MDPV-GX5 strain) shared more closely than other GPV and MDPV isolates, the four isolates clustered in the subgroup, with specific lineage. From the phylogenetic tree based on the VP1, the four Muscovy duck-origin parvovirus VP1 genes belonged to the MDPV clusters (Fig. 1-2). Whereas, from the phylogenetic tree based on the VP3, the four Muscovy duck-origin parvovirus VP3 genes belonged to

Table 2. Comparison the ITRs sizes of the genome from GPV and MDPV**Table 2.** GPV ve MDPV genomlarında ITR boyutlarının karşılaştırılması

Type	GenBank No.	Strain	Genome	ITR	Host
GPV	U25749	B	5106	444	goose
	EU583392	VG32/1	5104	443	vaccine
	EU583391	06-0329	5054	418	goose
	EU583390	82-0231	5050	416	goose
	EU583389	82-0231V	4980	381	vaccine
	HQ891825	GDaGPV	5106	444	goose
	JF333590	SH	5106	444	goose
	KC178571	Y	5106	444	Muscovy duck
	KC184133	E	5125	443	goose
	KC478066	SHFX1201	5050	416	swan
	KC996729	SYG61V	5102	442	vaccine
	KC996730	YZ99-6	5046	414	goose
	KM272560	LH	5047	414	goose
	KR029617	G7	5106	444	Muscovy duck
	MDPV	U22967	FM	5132	457
KC171936		SAAS-SHNN	5061	381	Muscovy duck
KM093740		MDPV-GX5	5132	384	Muscovy duck
KR075689		FJM5	5017	359	Muscovy duck

the GPV clusters (Fig. 1-3). The results indicated that more genotype duck parvovirus circulating in Muscovy ducks, leading more diverse evolutionary directions of MDPV.

DISCUSSION

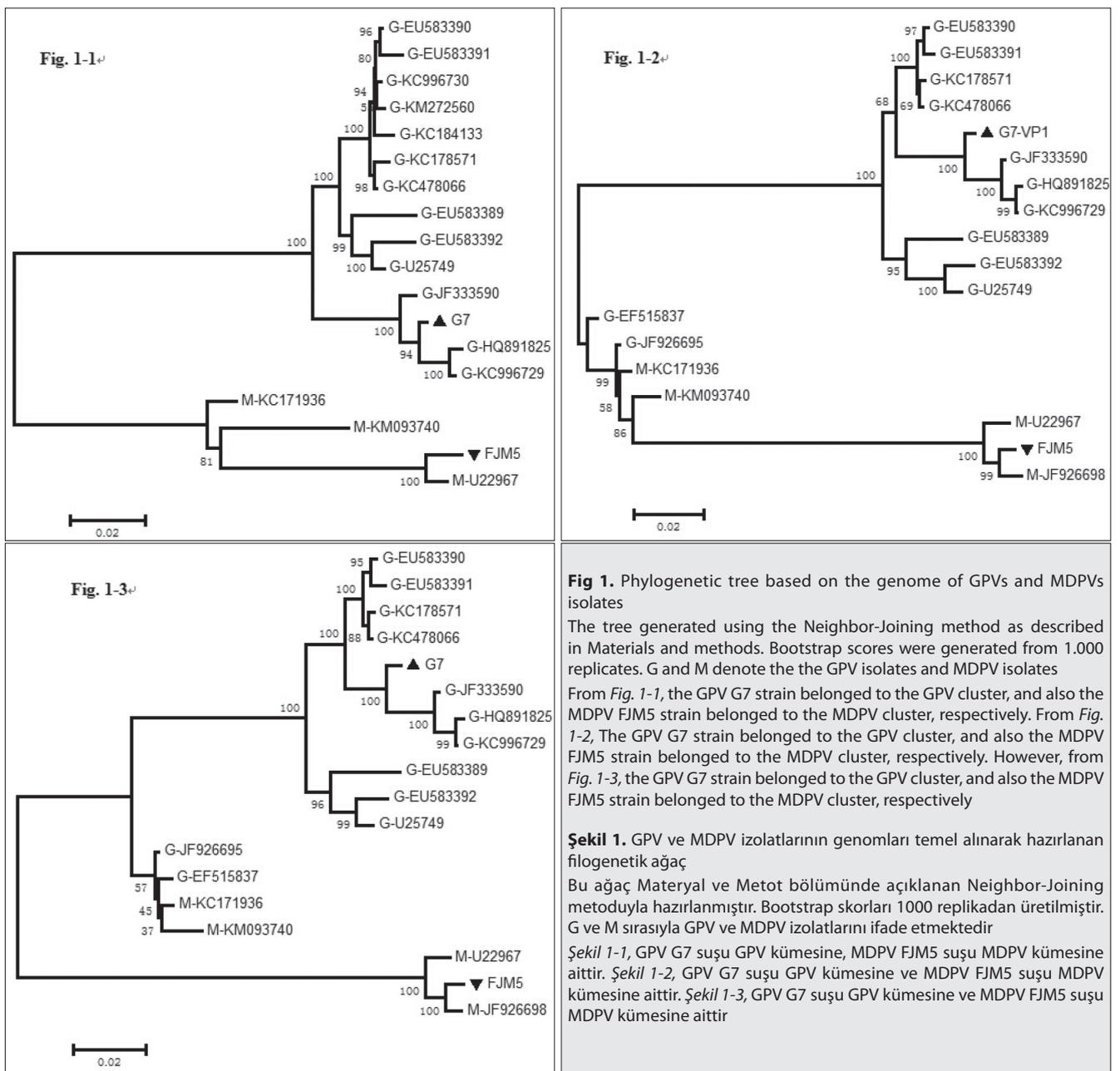
When compared with the GPV reference isolates, the GPV attenuation strains and their parental strains or field viruses showed that only 2.9% different between 82-0321 and 82-0321V (exclude the ITRs region sequences), the MDPV attenuation strains and their parental strains or field viruses showed that only 1.0 % different between P (GenBank No. JF926697) and P1 (GenBank No. JF926698). The results indicated that the genomic sequences of the GPV and MDPV had highly stable in the field for more than three decades, which also verified the GPV G7 strain and MDPV FJM5 strain may the pathogen for the dead Muscovy duck.

Comparing the GPV and MDPV genome structure retrieved from GenBank, the lengths of the NS and VP1 coding region were highly conserved; all references isolates had a NS coding region of 1884 nucleotides and a VP1 coding region of 2199 nucleotides. The length of the ITRs region determines the genomic length of GPV and MDPV, with different nucleotides deletions. Though deletions can be observed compared with the virulent B and FM strain, but no deletion affected the formation of the hairpin structure, no matter the deletions occurs

in the "stem" or "bubble" region of ITRs, because of all substitutions occurred in pairs, and the sequences in each pair were complementary to each other so that these substitutions did not affect the formation of the hairpin structure.

Genetic natural recombination has been reported among parvoviruses, especially with the persistent immunological pressure, which was thought to play an important role for parvoviruses evolution [7]. Poonia B reported new duck parvovirus from Muscovy duck, only shared 84.6% sequence identity with GPV and 84.5% identity with MDPV [8]. Wang reported a part of VP gene sequence of the GPV PT strain isolated from Muscovy ducks had the characteristics of MDPV sequence [9]. Zhu recently reported a recombinant Muscovy duck parvovirus with two putative genetic recombination regions between GPVs and MDPVs [10]. The phylogenetic tree showed that the GPV DY strain and MDPV MDPV-GX5 strain also had genetic natural recombination between GPVs and MDPVs. Meanwhile, the GPV DY strain and PT strain (though no ITRs regions were sequenced) may belong to the new-genotype MDPV.

In summary, although the co-infection with GPV and MDPV shared similar symptoms with GPV or MDPV infection as previously reported, the co-infection of GPV and MDPV in the pathogenicity and virulence of certain isolates still needs further investigation.



ACKNOWLEDGMENTS

This work was supported by the China Agriculture Research System (CARS-43), Special Fund for Agro-scientific Research in the Public Interest (201003012), the Chinese Natural Sciences Foundation (U1305212), the Hundred-Talent Program (Fujian Academy of Agriculture Sciences) (YC2015-12), and the Natural Sciences Foundation of Fujian Province (2015J1114). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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