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Short Communication

Complete Genome Sequence of a Novel Duck Parvovirus Isolated in Fujian, China

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Abstract

In the present study, we sequenced and analysed the complete genomes of a novel duck parvovirus (NM100) isolates derived from Muscovy ducks in Fujian, Southeast China. According to the phylogenetic analysis, based on the complete genome and VP1 gene showed that novel duck parvovirus strain NM100 belong to the MDPV clusters, whereas the VP3 gene showed that strain NM100 belong to the GPV clusters. Two putative genetic recombination events were detected using similarity plots analysis. These findings suggest that a novel duck parvovirus circulating in Muscovy duck flocks with recombination in nature, which enable us to understand the molecular characteristics and evolutionary diversity of waterfowl parvoviruses.

Keywords: *Duck parvovirus, Recombination, Phylogenetic analysis*

Çin'in Fujian Bölgesinde İzole Edilen Yeni Bir Ördek Parvovirus'unun Tüm Genom Sekansı

Özet

Bu çalışmada, Fujian, Güneydoğu Çin'de Muscovy ördeklerinden türetilmiş yeni bir ördek parvovirus izolatlarını (NM100) sıralayıp tam genomlarını analiz ettik. Tam genom ve VP1 genine dayalı filogenetik analize göre, yeni ördek parvovirus suşu NM100'ün MDPV kümelerine ait olduğunu, oysa VP3 geni, NM100 suşunun GPV kümelerine ait olduğunu gösterdi. Benzerlik alanları analizi kullanılarak, iki olası genetik rekombinasyon olayları tespit edildi. Bu bulguların, doğada Muscovy ördek sürülerinde rekombinasyon yoluyla dolaşan yeni ördek parvovirusu olduğu değerlendirilmektedir ki, bunlar da su kuşu parvovirusunun moleküler özelliklerini ve evrimsel çeşitliliğini anlamamızı sağlar.

Anahtar sözcükler: *Ördek parvovirus, Rekombinasyon, Filogenetik analiz*

INTRODUCTION

Waterfowl parvoviruses can cause diseases with high mortality and morbidity to goslings and Muscovy ducklings. Genomic analysis and antibodies neutralization test revealed that the waterfowl parvoviruses could be divided into two groups: the goose parvovirus (GPV) group and Muscovy duck parvovirus (MDPV) group. GPV can cause highly contagious and fatal disease in goslings and Muscovy ducklings; whereas MDPV only cause disease with Muscovy ducklings. The MDPV is highly related to GPV, exhibiting more than 80.0% nucleotide sequence identity [1-4]. Recently, GPV were detected in swan, Cherry Valley ducks and *Anser cygnoides* in China [5-7].

The genome of GPV and MDPV are about 5.1 kb in length, single-stranded DNA and contain two major open reading frames (ORFs). The left-hand side of the genome encodes the non-structural protein, while the right-hand of the genome encodes the capsid proteins (VP1, VP2 and VP3). The VP2 and VP3 are contained within the carboxyl terminal portion of VP1, deriving from the same gene with differential splicing [1].

In this study, we isolated sequenced and analysed the complete genome of a novel duck parvovirus strain NM100. Derivation of the genomic sequences of novel duck parvovirus strain NM100 implied that the virus had two putative genetic recombination events with

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recombination in nature between MDPV and GPV, which provides insights with the genome characterization and aetiology for waterfowl parvoviruses circulating in China.

MATERIAL and METHODS

Case History

A commercial Muscovy duck flock was experienced elevated mortality associated with typical MDPV syndromes, such as locomotory dysfunctions, weight loss, buccal respiration, and watery ocular discharges, at the spring of 2012 in Fujian, China. Most of the sick Muscovy ducklings were younger than 21-day-old and the mortality was nearly 45%.

Virus Isolation and Nucleic Acid Extraction

The virus (designated NM100) was isolated described previously [7], using 10-day-old Muscovy duck embryos by suspension into the allantoic cavities. All Muscovy duck embryos were collected from commercial Muscovy duck farms, which had no previous history with MDPV or GPV infections, and no MDPV or GPV vaccines used before. The virus was harvested after three passages of infected Muscovy duck embryos. Five Muscovy duck embryos were used as control in order to make sure that no vertical transmission waterfowl parvoviruses were detected by the waterfowl parvoviruses universal primers described by us before [8]. Genomic nucleic acids were extracted using the Total DNA/RNA Isolation Kit (Omega Bio-Tek, GA, USA) according to the manufacture's instructions. Duck-origin viral pathogens were tested by using PCR (RT-PCR) technology, only waterfowl parvoviruses universal primers were detected positive for the isolated virus.

Genome Sequencing

The strain NM100 genome were amplified by polymerase chain reaction (PCR) according to the similar strategy described previously [4], with overlapped fragments encompassed the completely GPV and MDPV genome. The PCR products were purified and then cloned. In each case, five positive clones were randomly selected and sequenced (Sangon Biotech, Shanghai, China) to both directions using an ABI model 3730 automatic DNA sequencer (ABI, CA, USA). We connected the overlapped gene fragments into the NM100 full-length genome with software Lasergene (DNAStar, v7.1, Madison, WI, USA), and submitted to GenBank.

Genomic Characterization, Homologous Recombination Analysis and Phylogenetic Analysis

For comparative studies, the complete genome sequences of waterfowl parvoviruses strains were retrieved from GenBank *(Table 1)*. Four strains of the virus (P [10], P1 [11], PT [12,13] and D [13]), which only had the NS and VP1 gene coding region sequences isolated from Muscovy ducks in Fujian, were subjected to phylogenetic analysis. Sequence comparison and genomic homology was determined using the ClustalW method. Phylogenetic analysis was performed by MEGA 6.0 using the neighbour-joining method with the maximum-likelihood model. Bootstrap scores were generated from 1000 replicates.

Table 1. *Virus description and Genbank accession numbers for waterfowl parvoviruses sequences used in this study*
Tablo 1. *Bu çalışmada kullanılan su kuşu parvovirus dizileri için virus açıklamaları ve Genbank erişim numaraları*

Accession Number	Strain	Host	Date	Region	Reference
U25749	B	goose	1960s	Hungary	[1]
EU583390	82-0231	goose	1982	TW, China	[2]
KC178571	Y	Muscovy duck	2011	AH, China	[3]
KC478066	SHFX1201	swan	2012	SH, China	[5]
KT343253	SDLC01	cherry valley duck	2015	SD, China	[6]
KC996729	SYG61V	a	a	JS, China	[9]
KT232256	FJ01	*Anser cygnoides*	2013	FJ, China	[7]
U22967	FM	Muscovy duck	1994	Hungary	[1]
JF926697	P	Muscovy duck	1988	FJ, China	[10]
JF926698	P1	a	a	FJ, China	[11]
KM093740	MDPV-GX5	Muscovy duck	2011	GX, China	[15]
KC171936	SAAS-SHNH	Muscovy duck	2012	SH, China	[14]
JF926695	PT	Muscovy duck	1997s	FJ, China	[12,13]
JF926696	D	a	a	FJ, China	[13]
KU641556	NM100	Muscovy duck	2012	FJ, China	TS

*Anhui, **AH;** Fujian, **FJ;** Jiangsu, **JS;** Shanghai, **SH;** Taiwan, **TW**. a means the vaccine candidates not writing the host and date. **TS;** this study*

The genome recombination events were detected using the Simplot 3.5.1, the GPV vaccine strains SYG61v [9] and MDPV virulent strain FM [1] were used for detection the similarity plots analysis. A multiple comparison-corrected P-value cut-off of 0.01 was used throughout.

RESULTS

Genomic Organization

The genome of NM100 was found to be 5073 nucleotides in length. The non-structural protein (NS) encodes 627 aa (nt 518-2401), the VP1 encodes 732 aa (nt 2420-4618), the VP3 encodes 534 aa (nt 3014-4618), respectively. The inverted terminal repeats (ITRs) was found to be 387 nt in length, which was present at the 5′ and 3′ terminal ends of the genome. The complete genome sequences has been submitted to GenBank under the accession No.KU641556.

Phylogenetic Analysis

The phylogenetic tree based on the NS and VP1 gene coding region (NM100, position nt 518-4618) sequences *(Fig. 1-1)* and VP1 gene coding region (NM100, position nt 2420-4618) sequences *(Fig. 1-2)* indicate that NM100 was at the same genetic evolution clades with the Muscovy parvovirus recombinant strains (SAAS-SHNH) [14], which belonged to the MDPV and N-MDPV cluster. GPV isolates (except for GPV-PT strain and its deviated vaccine strain D) were all at the GPV genetic evolution clades.

The phylogenetic tree based on the VP3 gene coding region (NM100, position nt 3014-4618) sequences *(Fig. 1-3)* indicate that NM100 shared different genetic evolution clades with typical MDPV isolates (FM, P and P1), which belonged to the GPV and N-MDPV cluster, near to the GPV clusters rather than typical MDPV cluster. However, GPV isolates (except for GPV-PT strain and its deviated vaccine strain D) were all at the GPV genetic evolution clades and typical MDPV isolates (FM, P and P1) were at the MDPV cluster.

Sequence Comparison

The NM100 genome shared 93.7% nucleotide sequence identities with MDPV strain FM, compared with other reported MDPV isolates SAAS-SHNN and MDPV-GX5 [15], the NM100 genome shared 99.5% and 95.0% nucleotide sequence identities, respectively. Compared with GPV isolates, the NM100 genome shared 84.8%-85.9% nucleotides sequence identities, respectively.

For VP1 coding region sequences nucleotides homology analysis, the NM100 strain shared 89.1% to 89.5 0% nucleotide sequence identities with typical MDPV isolates (FM, P and P1), respectively. The NM100 strain shared 99.9% nucleotide sequence identities with SAAS-SHNN, 99.5% and 99.3% with GPV-PT and its deviated vaccine, 98.9% with MDPV-GX5. Nucleotide identities of the VP1 of GPV isolates varied between 87.9%-89.6%, respectively.

For VP3 coding region sequences nucleotides homology analysis, the NM100 strain shared 85.5% to 85.9% nucleotide sequence identities with typical MDPV isolates (FM, P and P1), respectively. The NM100 strain shared 99.8% nucleotide sequence identities with SAAS-SHNN, 99.4% and 99.3% with GPV-PT and its deviated vaccine, 98.6% with MDPV-GX5. Nucleotide identities of the VP1 of GPV isolates varied between 91.2%-93.1%, respectively.

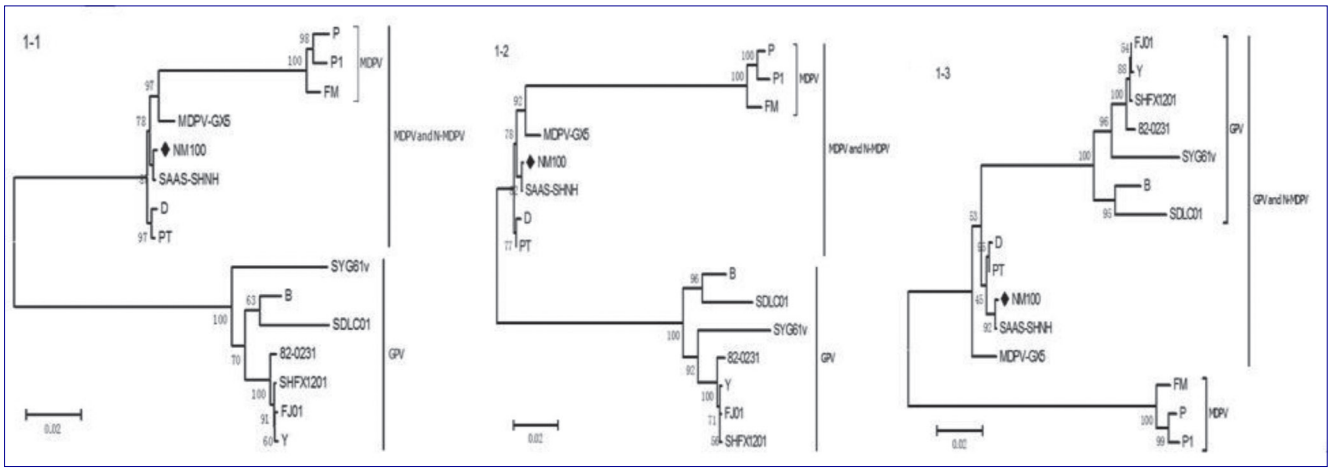

Fig 1. Phylogenetic analysis based on NS and VP1 coding region *(1-1)*, VP1 coding region *(1-2)* and VP3 coding region *(1-3)*

The phylogenetic tree shows that the novel duck parvovirus NM100 NS and VP 1coding region *(1-1)*, VP1 coding region *(1-2)* closely with MDPV clusters, whereas the VP3 coding region belonged to the GPV clusters *(1-3)*.

Şekil 1. NS ve VP1 kodlama bölgesine *(1-1)* dayalı filogenetik analiz, VP1 kodlama bölgesi *(1-2)* ve VP3 kodlama bölgesi *(1-3)*

Materyal ve Metot kısmında tarif edildiği üzere, komşu-birleştirme yöntemiyle oluşturulan ağaç. Öz yükleme puanları 1.000 tekrarla elde edildi. Filogenetik ağaç, yeni ördek parvovirusu NM100 NS ve VP kodlama bölgesini *(1-1)* ve MDPV kümelerine yakın VP1 kodlama bölgesini *(1-2)* gösterirken, VP3 kodlama bölgesi ise GPV kümelerine ait idi *(1-3)*.

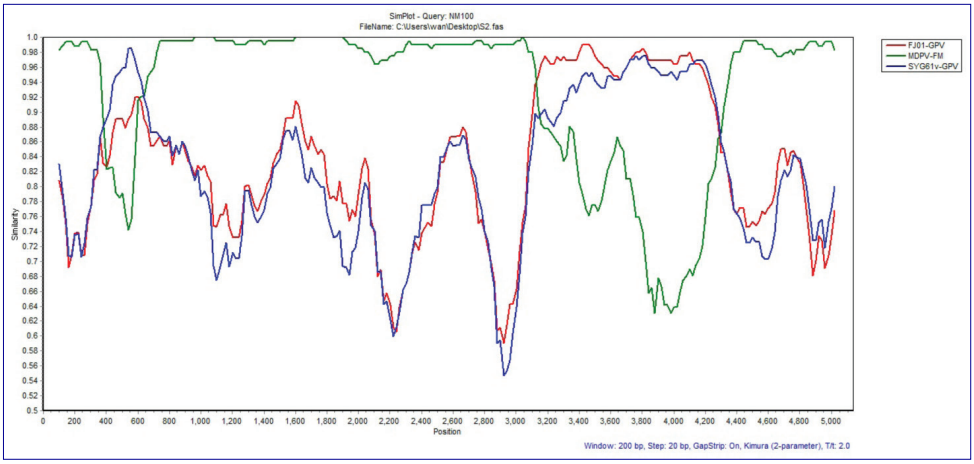

Fig 2. Similarity plots of the full-length sequence of novel duck parvovirus NM100

The schematic drawing demonstrated to denote the recombination position

The GPV virulent strain FJP01 and vaccine strain SYG61v were shown in red and blue, and MDPV virulent strain FM was shown in green. Two recombination breakpoints were observed, one is about 200 base-pair between 3′ ends of the inverted terminal repeat region and parts of 5′ ends of the NS region (position 419 to 610), and the other is nearly 1.150 base-pair (position 3.116 to 4.241) at the VP3 coding region.

Şekil 2. Yeni ördek parvovirusu NM100'ün tam-uzunluk dizisinin benzerlik çizimleri

Şematik çizim rekombinasyon konumunu belirtmek için gösterildi. GPV virulent FJP01 suşu ve aşı suşu SYG61v kırmızı ve mavi olarak, MDPV virulent FM suşu ise yeşil olarak gösterildi. İki adet rekombinasyon kesme noktası gözlendi: birisi, ters çevrilmiş terminal tekrar bölgesinin 3′ uçları ile NS bölgesinin (pozisyon 419-610) 5′ uçlarının parçaları arasındaki yaklaşık 200 baz-çifti, diğeri ise VP3 kodlama bölgesindeki yaklaşık 1.150 baz-çifti (pozisyon 4.241 ile 3.116)

Homologous Recombination Analysis

Using Simplot 3.5.1 software, two putative recombination breakpoints in the nucleotide sequences extending from nt 419 to 610 and from nt 3116 to 4241 *(Fig. 2)*. The first recombination event occurred between strain FM and the GPV strain SYG61v in the 426-611 nt region. The second recombination event occurred between strains FM and SYG61v in the 3127-4249 nt region. Strain FJ01 (GPV) used as GPV virulent control, which was isolated in Fujian, the same as strain NM100.

DISCUSSION

Previous studies had found that DNA viruses have a wide range of genome recombination, especially the parvoviruses under Family *Parvoviridae* [16]. Genus Bocaviruses has a large number of natural recombination phenomena [17]. Regarding waterfowl parvovirus genome recombination, which can change the pathogenic types, U.S. researchers have found new parvovirus strains in Muscovy ducks. The whole genome of this strain (PSU-31010) and its main coding region have not been completely established, but the homology rate between the known gene fragments and classic MDPV and GPV in this region was 84.5% and 84.6%, respectively [18]. Further, its genetic evolution tree belongs to the MDPV subset, and relates to clades different than the classic strain of the MDPV virus. Wang et al.[12] reported Muscovy duck origin GPV (GPV PT strain), which had the VP1 unique region the same features as MDPV. Recombinant waterfowl parvovirus (SAAS-SHNH) was subsequently found among Muscovy Ducks in the Shanghai area, with a genome structure the same as the MDPV genomic structure [14]. Recently, Cheery Valley duckling-origin GPV, which cause beak atrophy and dwarfism syndrome (BADS), genomic characteristics, showed that the virus is close to European GPV isolates, but separated from Asian GPV isolates, which had not reported in China before [6].

In our study, we isolated the NM100 with 10-day-old Muscovy duck embryos, the phylogenetic tree created from NS and VP1 region, the VP1 region and the VP3 region showed more evolution diversity between waterfowl parvoviruses. From the NS and VP1 region, the VP1 region, the NM100 shared closer with MDPV. Whereas, phylogenetic tree based on the VP3 region, the NM100 shared closer with GPV. Also, MDPV isolates (designated by the sequences submitter) (MDPV-GX5, GVP-PT and its deviated vaccine D) shared the same evolution phylogenetic with NM100. The two putative recombination breakpoints in the nucleotide sequences extending from nt 419 to 610 and from nt 3116 to 4241, especially the right recombination regions in the novel MDPV isolates NM100's VP3 coding region.

In summary, a novel duck parvovirus (N-MDPV), designated NM100 was obtained, sequenced, and characterized. It is clear from the multiple sequence alignments and phylogenetic analysis that NM100 represents

a distinct member of the MDPV related parvoviruses. These results suggest that recombination between GPVs and MDPVs may play significant roles in viral infectivity, host range, and pathogenicity. Further investigation of the pathogenicity of this virus on other commercial waterfowl species and the recombinant routes of this virus remain pressing questions for future research.

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Conflict of Interest

The authors declare that they have no any competing interests.

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