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Short Communication

Complete Genome Sequence of Goose Parvovirus Isolated from *Anser cygnoides* in China

Chun-he WAN [1,2] Yu HUANG [2] Shao-hua SHI [2] Guang-hua FU [2]
Long-fei CHENG [2] Hong-mei CHEN [2] Qiu-ling FU [2] Cui-teng CHEN [2] Kai-hui HU [1]

[1] College of Life Sciences, Fujian Agriculture and Forestry University, Fuzhou 350002, CHINA
[2] Institute of Animal Husbandry and Veterinary Medicine, Fujian Academy of Agricultural Science/Fujian Animal Disease Control Technology Development Center, Fuzhou 350013, CHINA

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Abstract

In the present study, we sequenced and analyzed the complete genome of goose parvovirus isolates derived from *Anser cygnoides*. Comparing with other GPV isolates, the results indicated that the event may cause by GPV direct infection to *Anser cygnoides*. These findings suggest that the *Anser cygnoides* could serve as a potential host for GPV and enable us to understand the molecular characteristics and evolutionary diversity of GPV.

Keywords: *Goose parvovirus, Anser cygnoides, Genome, Analysis*

Çin'de *Anser cygnoides*'ten Izole Edilen Kaz Parvovirusu'nun Tam Genomik Dizilimi

Özet

Bu çalışmada, *Anser cygnoides*'ten elde edilen kaz parvovirusu genomunun dizilimi yapıldı ve analiz edildi. Diğer GPV izolatlarıyla kıyaslandığında, sonuçlar olayın doğrudan *Anser cygnoides*'e bağlı bir GPV enfeksiyonuna bağlı olabileceğini gösterdi. Bu bulgulara göre, *Anser cygnoides*'in GPV için potansiyel bir konakçı (ev sahibi) olarak hizmet edebildiği ve GPV'nin moleküler özelliklerinin ve evrimsel çeşitliliğinin anlaşılmasını sağlayabildiği kanısına varıldı.

Anahtar sözcükler: *Kaz parvovirusu, Anser cygnoides, Genom, Analiz*

INTRODUTION

Goose parvovirus (GPV), also named Derzsy's disease virus, is the causative pathogen that results in high mortality and morbidity in domestic ducklings and goslings under the age of three weeks, however not cause fatal diseases with adult birds [1,2]. Goose parvovirus described as 20-22 nm in diameter with an icosahedral outer appearance, which belonging to the Anseriform dependoparvovirus 1 species of the dependoparvovirus genus, under the *Parvoviridae* family [3].

The goose parvovirus genome is approximately 5100 nucleotides long with single-stranded DNA and no helper viruses are required for virus replication in host cells. The GPV genome contains two major open reading frames (ORFs). The left ORF encodes the non-structural protein (NS), while the right ORF encodes for three capsid proteins VP1, VP2 and VP3, derives from the same gene by alternate splicing. The VP2 and VP3 genes shared the same carboxyl terminal portion of VP1 gene [4].

In this study, we sequenced and analyzed the complete genome of goose parvovirus strain FJ01 that was isolated from *Anser cyanide's*. Derivation of the genomic sequences of goose parvovirus from *Anser cygnoides* implied that *Anser cygnoides* might serve as a potential host for goose parvovirus, which provides insights with the genome characterization and etiology for goose parvovirus.

MATERIAL and METHODS

Case History

A commercial *Anser cygnoides* flock was experienced elevated mortality associated with lethargy, weight loss,

İletişim (Correspondence)
+86 591 87572396 (Yu Huang); +86 591 83789492 (Kai-hui Hu)
huangyu_815@163.com (Yu Huang); wanchuntian@126.com (Kai-hui Hu)

dysphagia, ataxia, and watery ocular discharges at October 2012 in Fujian Province, China. Most of the sick *Anser cygnoides* goslings were younger than 21-day-old and the mortality was nearly 40%. To determine the pathogens which were responsible for the disease, we collected the liver, spleen and intestinal cavity from the succumbed *Anser cygnoides* goslings and subjected to PCR (RT-PCR) method to test all possible classical endemic and emerging viruses outbreaks in goose flocks such as goose parvovirus, goose herpesvirus, goose adenovirus, avian paramyxovims type l, avian influenza virus, avian Tembusu virus, goose circovirus and goose reovirus.

Virus Isolation and DNA Extration

The tissues were 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 to virus isolation. The goose parvovirus isolated from *Anser cygnoides*, designated FJ01, was isolated by suspension into the allantoic cavities of 10-day-old goose embryos. The embryos died 84-120 h post inoculation and dead embryos were observed. All goose embryos were purchased from commercial goose farms, which had with no history of GPV exposure or vaccination with attenuated GPV vaccine. The viruses were harvested after three passages of infected goose embryos. DNA was extracted using the Viral DNA Kit (Omega Bio-Tek, GA, USA) according to the manufacture's instructions.

Genome Sequencing

The GPV strain FJ01 genome were amplified by polymerase chain reaction (PCR) according with the similar strategy described previously [3], with overlapped fragments encompassed the completely GPV genome. The PCR products were purified using the Gel Extraction Kit (Omega Bio-Tek, GA, USA) and then ligated into the pBackZero8-T vector with cloning kit (Takara, Dalian, China). 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 FJ01 full-length genome with software Lasergene (DNAStar, v7.1, Madison, WI, USA).

Genomic Sequence Alignment and Phylogenetic Analysis

For comparative studies, the complete genome sequences of GPV virulent strains and attenuated vaccine strains were retrieved from GenBank *(Table 1)*. Sequence comparison and genomic homology was determined using the ClustalW method. Phylogenetic analysis was performed by MEGA 6.0 using the neighbor-joining method

Table 1. *Virus descriptions and GenBank accession numbers for sequences used in this study*
Tablo 1. *Çalışmada kullanılan dizilimler için virus tanımlamaları ve GenBank girişi*

Accession Number	Strains	Genome Size (nt)	ITR	Origin[a] (Province, Country)	Host	Date	Reference
EU583389	82-0321V	4980	381	TW, China	Goose	**A**	[5]
EU583390	82-0321	5050	416	TW, China	Goose	1982	[5]
EU583391	06-0329	5054	418	TW, China	Goose	2006	[5]
EU583392	VG32/1	5104	443	Germany	Goose	**B**	[5]
HQ891825	GDaGPV	5106	444	GD, China	Goose	1978	[6]
JF333590	SH	5106	444	SH, China	Goose	2009	NA
KC178571	Y	5106	444	AH, China	Muscovy duck	2011	[7]
KC184133	E	5125	443	AH, China	Goose	2012	NA
KC478066	SHFX1201	5050	416	SH, China	Swan	2012	[9]
KC996729	SYG61v	5102	442	JS, China	Goose	**C**	[8]
KC996730	YZ99-6	5046	414	JS, China	Goose	1999	NA
KM272560	LH	5047	414	JS, China	Goose	2012	[10]
KR029617	G7	5106	444	FJ, China	Muscovy duck	2013	[3]
KT232256	FJ01	5104	443	FJ, China	*Anser cygnoides*	2012	TS
U25749	B	5106	444	Hungary	*Anser anser*	-	[4]
U22967	FM	5132	457	Hungary	*Cairina moschata*	-	[4]

[a] Origin abbreviations: Anhui, AH; Fujian, FJ; Guangdong, GD; Jiangsu, JS; Shanghai, SH; Taiwan, TW; ***A:*** *82-0321V live vaccine strains, which was derived from 82-0231 after 64 passages in Muscovy duck eggs, two in geese fibroblasts, and four in duck embryos;* ***B:*** *means VG32/1 live vaccine strains, which can purchase from Impfstoffwerk Dessau-Tornau GmbH (Rodleben, Germany);* ***C:*** *means SYG61v live vaccine strains, which were used in mainland China;* ***TS,*** *this study;* ***NA,*** *not available;* ***-,*** *unknown*

with the maximum-likelihood model. Bootstrap scores were generated from 1.000 replicates.

RESULTS

Genomic Organization

The GPV strain FJ01 genome is 5104 nucleotides (nt) in length and has a basic structure similar to previously reported GPV genomes, belonging to a complete replication component virus with 29.12% A, 24.08% G, 23.55% T and 23.24% C. The genome is flanked on the 5′ and 3′ terminal ends by 443 nt inverted terminal repeats (ITRs) regions. The distal 405 nt of each repeat form a U-shaped hairpin structure consisting of a 181 base-pair double-stranded "stem" region and a 43 nt bubble region, which serve as the origin of GPV replication. The sequenced genome has a NS coding region of 1884 nucleotides and a VP1 coding region of 2199 nucleotides *(Fig. 1)*.

The complete genome of GPV strain FJ01 isolated from *Anser cygnoides* had been submitted to GenBank under accession number KT232256.

Comparison of the Genomic Sequences and Phylogenetic Analysis

To identify the nucleotide sequences of goose parvovirus isolated from *Anser cygnoides*, we compared strain FJ01 with the GPV virulent and attenuated strains which were retrieved from GenBank *(Table 1)*. The nucleotide homology of FJ01 to virulent strains varies from 94.2% to 99.9%. Compared with the attenuated strains SYG61v, 82-

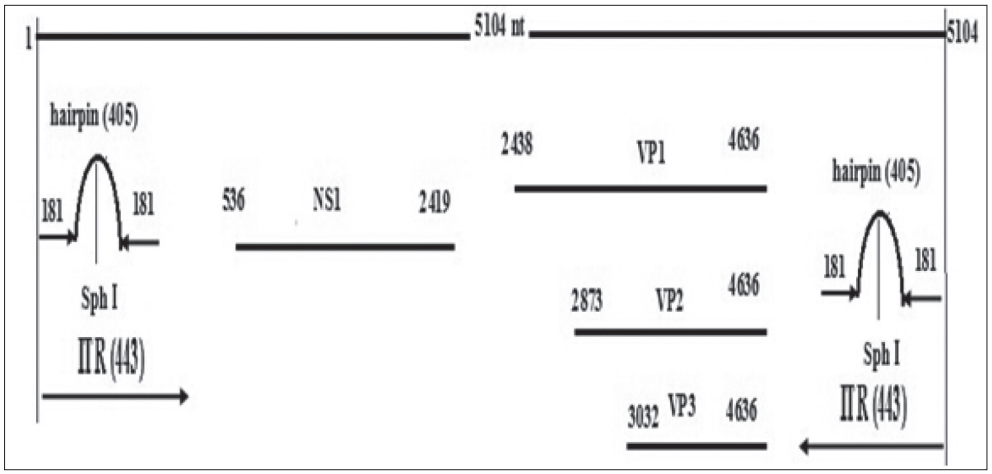

Fig 1. A graphical representation of the genome organization of FJ01 strain. ITR means inverted terminal repeat. Sph□means there has a Sph□ enzyme cutting site (GCATGC) in the bubble. Numbers in the graph mean the position in the genome

Şekil 1. FJ01 suşunun genom organizasyonunun grafik sunumu. ITR invert terminal tekrarını gösterir. Sph□, baloncukta Sph□ enzimi keskin tarafı (GCATGC)'ye sahip demektir. Grafikteki sayılar genomdaki pozisyonu tanımlamaktadır

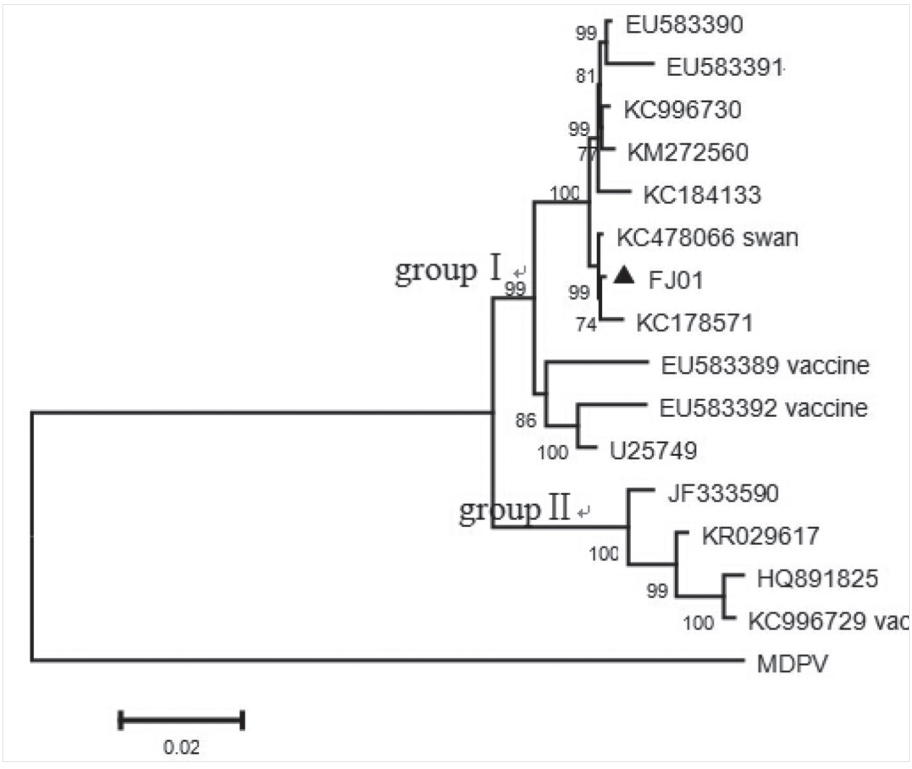

Fig 2. Phylogenetic tree based on the genome of GPVs isolates
The tree generated using the Neighbor-Joining method as described in Materials and Methods. Bootstrap scores were generated from 1000 replicates. MDPV denote the MDPV isolates FM

Şekil 2. GPV izolatları genomu esaslı filogenetik ağaç
Ağaç, Materyal ve Metot'ta tanımlanan Komşu-katılımı yöntemi kullanılarak oluşturuldu. Bootstrap skorları 1000 kez tekrarla oluşturuldu. MDPV, MDPV FM izolatları demektir

0321V and VG32/1, the FJ01 shares nucleotide identity of 94.3%, 96.9% and 96.9%, respectively.

A plylogenetic tree was generated based on the complete GPV genome nucleotide sequences retrieved from GenBank. The Muscovy duck parvovirus (MDPV) strain FM was used as MDPV respective strain (GenBank accession number U22967). The plylogenetic tree contains two groups, which indicates that at least two types of GPV virulent viruses are circulating in China. The GPV strain FJ01 appears closer to the GPV isolates SHFX1201, which was isolated from a swan in Shanghai, China *(Fig. 2)*.

DISCUSSION

The swan goose *Anser cygnoides* is confined to the Eastern Palearctic, which was bred in China, Mongolia and parts of Russia. There were very few researches about the virus disease outbreaks to the swan goose *Anser cygnoides* species reported previously. Previous observation and research indicated that the goose parvovirus can infect both goslings and ducklings. Then, goose parvovirus was isolated from swans in China in 2012 [9]. Earlier studies have confirmed that goose parvovirus genome remained genetically stable in the field. However, based on the phylogenetic analysis in the present study, the GPV isolates can be divided into two major groups (Group I and Group II), which means more genetic diversity between GPVs. In our study, the GPV strain FJ01 showed highest similarity to those of GPV strains, which suggested the emergence of GPV strain FJ01 was more likely resulted from a direct GPV infection.

Wang recently reported GPV strain MDGPV/PT shared genetic recombination with Muscovy duck parvovirus in the NSP gene [11], and their research indicated that inter-genotype recombination within the VP2 gene cluster contributes to the genetic diversity of the VP2 genes of Taiwanese GPV field strains [12]. Simplot program was used to analyze the recombinant event of the GPV strain FJ01 with the vaccine strain SYG61v and virulent MDPV strain FJM5 in China [3], with no recombination observed (data not shown).

In summary, this report presents the first evidence that goose parvovirus can infect *Anser cygnoides* directly. Bivalent attenuated vaccine against GPV had been used to prevent GPV infections in geese and duck flocks in China for decades, however whether attenuated vaccine against GPV can be used for *Anser cygnoides* flocks to prevent GPV infection needs further investigation.

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