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

INTRODUCTION

Waterfowl paroviruses can cause diseases with high mortality and morbidity to goslings and Muscovy ducklings. Genomic analysis and antibodies neutralization test revealed that the waterfowl paroviruses could be divided into two groups: the goose parovirus (GPV) group and Muscovy duck parovirus (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 parovirus strain NM100. Derivation of the genomic sequences of novel duck parovirus strain NM100 implied that the virus had two putative genetic recombination events with
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 paroviruses were detected by the waterfowl paroviruses 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 manufacturer's instructions. Duck-origin viral pathogens were tested by using PCR (RT-PCR) technology, only waterfowl paroviruses universal primers were detected positive for the isolated virus.

**Genomic Characterization, Homologous Recombination Analysis and Phylogenetic Analysis**

For comparative studies, the complete genome sequences of waterfowl paroviruses 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>
<thead>
<tr>
<th>Accession Number</th>
<th>Strain</th>
<th>Host</th>
<th>Date</th>
<th>Region</th>
<th>Reference</th>
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<tbody>
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<td>B</td>
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<td>Hungary</td>
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<tr>
<td>KC478066</td>
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<td>swan</td>
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<td>SH, China</td>
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<td>KT343253</td>
<td>SDLC01</td>
<td>cherry valley</td>
<td>2015</td>
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<td>KT232256</td>
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<tr>
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<td>a</td>
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<td>Muscovy duck</td>
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<td>JF926695</td>
<td>PT</td>
<td>Muscovy duck</td>
<td>1997s</td>
<td>FJ, China</td>
<td>[12,13]</td>
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<tr>
<td>JF926696</td>
<td>D</td>
<td>a</td>
<td>a</td>
<td>FJ, China</td>
<td>[13]</td>
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<tr>
<td>KU641556</td>
<td>NM100</td>
<td>Muscovy duck</td>
<td>2012</td>
<td>FJ, China</td>
<td>TS</td>
</tr>
</tbody>
</table>

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 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 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-SHN 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% nucleotide sequence identities with typical MDPV isolates (FM, P and P1), respectively. The NM100 strain shared 99.9% nucleotide sequence identities with SAAS-SHN, 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 varies 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-SHN, 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.
Complete Genome Sequence ... 

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 strains 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 paroviruses under Family Parvoviridae [16]. Genus Bocaviruses has a large number of natural recombination phenomena [17]. Regarding waterfowl parovirus genome recombination, which can change the pathogenic types, U.S. researchers have found new parovirus 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 parovirus (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 paroviruses. 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.
a distinct member of the MDPV related paroviruses. 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.

Acknowledgments

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Conflict of Interest

The authors declare that they have no any competing interests.

REFERENCES