Genetic characterization and phylogenetic analysis of Novel Duck-Origin Goose Parvovirus in Anhui Province, Eastern China

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Brief Report

Keywords: N-GPV, C-GPV, MDPV, parvovirus, VP1, VP2, ITRs, ORFs

DOI: https://doi.org/10.21203/rs.3.rs-202531/v1

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**Abstract**

Recently, a novel duck-origin goose parvovirus (N-GPV) was reported to cause short beak and dwarfism syndrome in ducks. In this study, we performed complete genome sequencing and analyzed three different duck-derived paroviruses that infected different breeds of ducks. Phylogenetic trees based on gene sequences indicated that they were classical goose parvovirus (C-GPV), Muscovy duck parvovirus (MDPV), and N-GPV, respectively. Furthermore, potential recombination events were found. These results improve our understanding of the diversity of duck-derived paroviruses in the Anhui province, eastern China, and provide a reference for the prevention of associated diseases.

**Main Text**

The *Parvoviridae* is an ancient virus family that is divided into three subfamilies based on common characteristics and phylogenetic relationships: the *Densovirinae*, the *Hamaparvovirinae*, and the *Parvovirinae* [1, 2]. Waterfowl paroviruses, which belong to the *Parvovirinae* subfamily, are small non-enveloped single-stranded DNA viruses with genomes of 5.0–5.1 kb [3]. Their genome contains two major open reading frames (ORFs): the left ORF encodes the non-structural proteins NS1 and NS2, and the right ORF encodes the structural proteins VP1, VP2, and VP3 [4, 5]. There are two inverted terminal repeats (ITRs) at both ends of the sequence, including two stem regions and a bubble with *Sphl* restriction sites that can form a hairpin structure [6-8]. Waterfowl paroviruses, such as goose parvovirus (GPV) and Muscovy duck parvovirus (MDPV), are transmitted within and across waterfowl species [9, 10]. Derzsy’s disease, also known as “goose plague,” is mainly caused by GPVs that infect goslings around 1 month old, and the associated mortality rate is as high as 90% [11, 12]. MDPVs usually infect Muscovy ducklings under 3 weeks of age and cause movement disorders [13], stunting, watery diarrhea, and abnormal feather development; the associated mortality rate is 10–80% [12, 14, 15]. GPVs and MDPVs have caused huge economic losses with respect to the waterfowl breeding industry [16]. Previous studies reported a novel duck-origin goose parvovirus (N-GPV) that could cause short beaks and dwarfism syndrome (SBDS) in Cherry Valley ducks (Pekin duck) and mule ducks in certain areas of China [12, 17]. Recently, an N-GPV was also found in the Sheldrake duck in Linwu, Fujian [19]. Of note, compared with classical GPV (C-GPV) and MDPV, N-GPV has lower mortality and morbidity (less than 3% and 30%, respectively). However, the ducks infected with N-GPV show beak atrophy, paralysis, diarrhea, and slow growth, leading to losses in the duck industry [18-21]. According to phylogenetic comparison analysis, N-GPV is related to GPV, with a genome identity higher than 91.3%. However, there is still a need for further genetic characterization of N-GPV. Understanding the genetic relationships between N-GPV strains in different regions would be very useful for further the prevention of this disease. Thus, in this study, tissue samples from different breeds of ducks were tested for duck paroviruses. Furthermore, phylogenetic and recombination analyses were conducted to better understand the prevalence of waterfowl paroviruses in the Anhui province, eastern China.

In 2019–2020, a total of 52 samples were collected from Cherry Valley ducks, mule ducks, and Sheldrakes showing lethargy, loss of appetite, and diarrhea in the Anhui and Jiangsu provinces, eastern
China. Liver, kidney, and spleen samples were collected and homogenized in 1 mL of sterile phosphate-buffered saline (PBS; pH 7.2). After centrifugation at 10,000 g for 10 min, freeze-thawing was repeated three times and the supernatant was stored at −80°C until use.

Viral DNA was extracted using the TIANamp Virus DNA/RNA Kit (Tiangen, Beijing, China) according to the manufacturer’s protocol. A pair of specific detection primers for conventional polymerase chain reaction (cPCR) was designed using Primer Premier 5 software (DNASTAR, Madison, WI, USA) according to the reference strain in the GenBank (Accession Number: MH444513.1). The cPCR reaction system was composed of 12.5 μL high-fidelity enzyme 2× Premix Taq®2.0 (TaKaRa, Dalian, China), 2 μL DNA template, 0.4 μM each of forward and reverse primers, and RNase-free H₂O up to 25 μL. The amplified product was cloned into the pMD19-T vector (TaKaRa), and the recombinant plasmid was sent to General Biological System Co., Ltd. (Chuzhou, Anhui, China), for sequencing. After BLAST analysis of the sequencing results, we designed primers to allow the amplification of the complete N-GPV genome sequence. The information regarding the primers and PCR conditions are shown in Table 1. The complete genome sequences of C-GPV and MDPV were amplified using the primers previously reported [22, 23].

The previously reported complete genome sequences of C-GPVs, N-GPVs, and MDPVs were downloaded from GenBank and used to analyze the variation and phylogenetic relationships between the three detected strains in this study and other duck and goose-related paroviruses. Sequences of the three detected strains and all referenced strains were aligned using the MAFFT software (http://mafft.cbrc.jp/alignment/software/) and analyzed using the MegAlign 6.0 program (DNASTAR) [24]. A phylogenetic tree based on the complete sequences, NS1, and VP1 was established in 1,000 bootstrap replications using the maximum-likelihood method in the MEGA vX software. Recombination events were identified using RDP (R), GENECONV (G), MAXCHI (M), CHIMAERA (C), SISCAN (S), and 3SEQ (Q) packages in the Recombination Detection Program v.4.50 (RDP 4.50) and the SimPlot software.

Overall, the samples were collected from mule ducks (13 samples), Cherry Valley ducks (5 samples) and Sheldrakes (34 samples). Two samples (3.85%) from Cherry Valley ducks and Sheldrakes were identified as positive for GPV, and one sample (1.92%) from a mule duck was identified as positive for MDPV by cPCR.

The two strains of GPV and one strain of MDPV were successfully amplified by PCR; the three virus strains were named AHAU30, AHAU41, and AHAU25, respectively. The sequences were submitted to GenBank under the accession numbers MT646164 (AHAU30), MT646163 (AHAU41), and MT646165 (AHAU25). The nucleotide identities between these three strains and all GPV and MDPV reference strains were 78.1–98.9%. With regard to the Rep and Cap proteins, the AHAU41 strain had higher similarity with C-GPV (97.9–99.1% and 98.0–99.2%), the AHAU30 strain had higher similarity with N-GPV, except with the M15 strain (96.4–99.0% and 95.6–99.7%), and the AHAU25 strain had higher similarity with MDPV (95.4–99.2% and 96.4–99.0%), which indicated that these three strains may belong to C-GPV, N-GPV, and MDPV groups, respectively.
The phylogenetic tree of the complete sequences showed that the waterfowl parvoviruses were divided into three branches, namely C-GPV, N-GPV, and MDPV. The three detected strains belonged to these three branches, respectively, which was consistent with the above sequence alignment results. Of note, the phylogenetic trees based on \( NS1 \) and \( VP1 \) and that based on the whole genome sequences showed different topological structures; however, they all established that the three strains belonged to the three different Anseriform dependoparvovirus 1 (Figure 1). The phylogenetic trees also indicated that the AHAU30 strain found in the Anhui province was most similar to the Sheldrake-origin N-GPV strain recently reported in the Linwu area, forming an independent sub-cluster with other N-GPVs. Next, to investigate whether there was recombination events, the waterfowl parvoviruses were analyzed using the RDP 4.50 software. The results showed that potential recombination events occurred in two strains, AHAU41 and AHAU30. Particularly, two recombination events were found in the strain AHAU41; the major and minor parents of the two events were C-GPV and N-GPV. Additionally, only one recombination event was found in the AHAU30 strain and the major and minor parents of the recombination event were also C-GPV and N-GPV. Simplot analysis showed that the recombinant region in the AHAU30 strain was located in the \( VP1 \) (2,681 bp to 3,293 bp) locus, and those in the AHAU41 strain were located in the \( NS1 \) (204 bp to 957 bp) and \( VP1 \) (3,938 bp to 4,327 bp) loci (Figure 2). Importantly, the recombination events in the AHAU30 and AHAU41 strains were verified using the R, G, M, C, S, and Q methods, and the obtained p values were between \( 4.860 \times 10^{-5} \) and \( 1.420 \times 10^{-13} \).

Waterfowl parvoviruses include GPV and MDPV, causing higher morbidity and mortality rates in geese and ducks, respectively [12]. In recent years, N-GPV has been found in ducks in some provinces of China, leading to duck SBDS, and causing losses in the duck breeding industry [8, 26]. Until now, little is known about the prevalence and evolution of N-GPV in the Anhui province in eastern China. In this study, three types of waterfowl parvoviruses, namely MDPV, C-GPV, and N-GPV were detected in samples collected from three breeds of ducks in different areas of the Anhui province. Our study highlights the diversity of parvoviruses that can infect ducks and provides evidence of the spread of goose parvovirus among waterfowl species. Notably, N-GPV was only widely detected in Cherry Valley and mule ducks [9, 26]. However, in the last year, N-GPV was also detected in Sheldrakes. In the current study, N-GPV was again detected in Sheldrakes, demonstrating again that this virus can infect this species [27].

In our study, to explore the phylogenetic relationships between the three detected waterfowl strains and other reference strains, phylogenetic analyses were performed based on the complete genome sequences and on the sequences of \( NS1 \) and \( VP1 \). The phylogenetic trees exhibited different topological structures, further verifying the results of the sequence alignment. In addition, we found that the N-GPV detected in Sheldrakes formed a separate branch; of note, the diversity of transmission in waterfowl needs further study.

Remarkably, two recombinant strains (AHAU30 and AHAU41) were found in this study with a total of three recombinant events; the parents were N-GPV and C-GPV in all of the events. Previous studies have also reported recombination events between N-GPV and C-GPV, suggesting that such recombination may be widespread. Of note, recombination requires co-infection of the same cell [28]. Therefore, the
recombination events identified in this study suggested co-infection with C-GPV and N-GPV, which further supports the notion of cross-species transmission of GPV, as previously reported. Importantly, co-infection and cross-species transmission of different GPVs is a challenge to prevent parvovirus infection in ducks and is likely to pose a major threat to the duck industry. Nevertheless, the number of samples in this study and the sample collection location was limited. Therefore, further epidemiological investigation is needed to investigate the prevalence of waterfowl parvovirus in eastern China.

In summary, this study contributes to the better understanding of the diversity of duck-originated parvovirus in the Anhui and Jiangsu provinces and highlights the need for the implementation of parvovirus infections control measures to avoid losses in the duck industry.

**Declarations**

**Conflict of interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Author contributions**

All authors participated in this study. Wang Yong and Sun Jianfei participated in the conception of the experiment, Da Zhang, Xu Guo and Wenhao Shen performed data analysis, and Yongdong Li performed the experiment. The manuscript was completed by Wang Yong and Sun Jianfei.

**Acknowledgments**

We would like to thank Editage (www.editage.cn) for English language editing.

**Funding**

This study was supported by the Ningbo Health Branding Subject Fund (No. ppxk2018-10).

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Tables

Table 1 The primers used for novel duck-origin goose parvovirus (N-GPV) detection and complete genome amplification in the study.
| Name                | Sequences (5’-3’) | Position(bp) | Size(bp) | Annealing temperature (°C) |
|---------------------|-------------------|--------------|----------|---------------------------|
| **Detection**       |                   |              |          |                           |
| Forward-D           | ATGCTGTACCTTCTATGGCTG | 1564-1585    | 203      | 52                        |
| Reverse-D           | TACAGGAGTAGGTTCAATACAAACA | 1742-1766    |          |                           |
| **Complete genome amplification** |                   |              |          |                           |
| Forward-1           | CTCATTGGAGGGTTCGGTTG | 1-20         | 183      | 51                        |
| Reverse-1           | CATGCGCGTGGTCAACCTAACAGCCG | 165-190     |          |                           |
| Forward-2           | GCATGCCGCGCGGTAGCCCAATA | 185-208     | 1293     | 54                        |
| Reverse-2           | TCACCCAGCCGCAGGAT | 1459-1477    |          |                           |
| Forward-3           | GCTGGGTGAAGAGAGGTCAAC | 1469-1491   | 1170     | 52                        |
| Reverse-3           | CTTTGTAGGCTGGGCTCTC | 2619-2638    |          |                           |
| Forward-4           | GCCTCCTAAAATCTGGGCTCCT | 2037-2058   | 1406     | 53                        |
| Reverse-4           | GCTCATCATCCGTAAAAACTTG | 3421-3442   |          |                           |
| Forward-5           | TTTCAATCGCTTCCTCAGG | 3249-3268    | 840      | 56                        |
| Reverse-5           | TCGTCCGTGACCATAATAC | 4069-4088    |          |                           |
| Forward-6           | TATTATGGTCACGGCAGG | 4073-4092    | 817      | 51                        |
| Reverse-6           | CGGCTGTAGGTTGACCACGGCATG | 4864-4889  |          |                           |