Short Communication

Complete genome sequence of a novel recombinant Muscovy duck parvovirus isolated in the Heilongjiang province of China

Jing Yang[1] Ming Li[1,2*] Tianfei Yu[3*]

Abstract

Muscovy duck parvovirus (MDPV) is fatal for young ducklings of less than 3 weeks of age and has caused severe economic loss in duck production in many countries and regions. We report here on a complete genome sequence of a recombinant MDPV strain YL08 isolated from an ill Muscovy duck in 2008 at Qiqihar, Heilongjiang province, China. This genome, MDPV YL08, was 5061 nucleotides long and retained the two ORFs, which are typical in the Parvovirinae family. The genome of YL08 strain has a putative recombination breakpoint on its VP1 gene. This study has helped our understanding of the genetic diversity and virus evolution of MDPV isolates.

Keywords: Muscovy duck parvovirus, Genome sequence, Phylogenetic analysis, Recombination

[1]*College of Computer Science and Technology, Harbin Engineering University, Harbin, China*
[2]*College of Computer and Control Engineering, Qiqihar University, Qiqihar, China*
[3]*College of Life Science and Agriculture Forestry, Qiqihar University, Qiqihar, China*
****Correspondence**: fionalee629@163.com, yutianfei2001@163.com* (M. Li, T. Yu)
Received February 26, 2021
Accepted November 21, 2021
doi: https://doi.org/10.14456/tjvm.2022.21

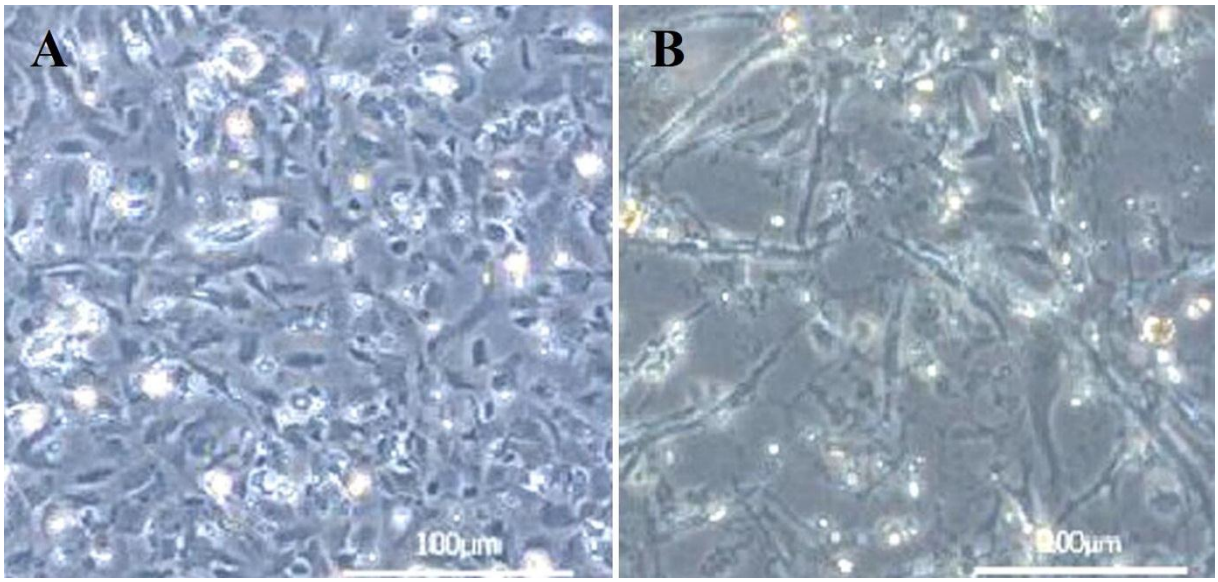
Introduction

Muscovy duck parvovirus (MDPV) is a single-stranded DNA virus that belongs to *Anseriform dependoparvovirus 1* within the *Parvoviridae* family (Soliman *et al.,* 2020; Wan *et al.,* 2019). MDPV is fatal for young ducklings of less than 3 weeks of age and can cause wheezing, locomotor dysfunction and diarrhea (Dong *et al.,* 2019). The whole genome of MDPV is about 5,100 nucleotides (nt) long and contains two major open reading frames (ORFs) and inverted terminal repeats (ITRs) at both ends of its genome (Shen *et al.,* 2020). The left ORF encodes non-structural proteins (NSP) including two proteins, NS1 and NS2, which are derived from the same gene and required for both replication of the viral genome and regulation of structural genes expression (Yu *et al.,* 2016b). The right ORF encodes three structural proteins, VP1, VP2 and VP3, which share a common C-terminal region and play important roles in viral pathogenicity and virulence (Wang *et al.,* 2016).

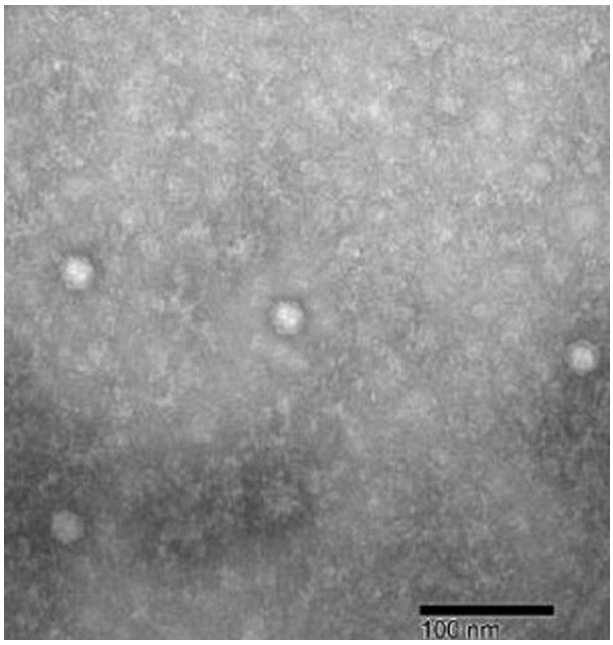
Materials and Methods

The present study aims to determine the complete genome sequence of a Chinese MDPV isolate from a group of Muscovy ducklings in a flock at Qiqihar, Heilongjiang province. Ten overlapped DNA fragments of the MDPV strain YL08 were amplified and compared with the available full-length genomes of the other MDPV isolates to determine their genetic diversity, evolutionary relationship and recombination events. The information about the genome sequence of the MDPV strain YL08 will help to reveal molecular variations of MDPV and facilitate prevention of infection with this virus.

In 2008, more than 60 % of a group of Muscovy ducklings aged approximately 3 weeks in a flock at Qiqihar, Heilongjiang province of the Mainland of China showed characteristic symptoms of MDPV infection with a 36.2 % mortality rate. Liver and spleen samples were collected from an ill Muscovy duckling, placed in sterile containers and then immediately transported to the laboratory on dry ice. Tissue homogenates were prepared in 10 vols of PBS (0.01 M, pH 7.2) and centrifuged at 2,000×g for 20 mins at 4℃. Supernatants were filtered through 0.45 μm and 0.22 μm filter (Ultrafree MC, Millipore) in turn at 2,000×g. A 1: 10 dilution of the supernatants was inoculated into the chorioallantoic sac of 10-day-old embryonated Muscovy duck eggs. It was serially passaged six generation times in embryonated duck eggs, with a final 68% mortality rate. The median embryos lethal dose (ELD50) of the virus in 10-day-old embryonated Muscovy duck eggs was $5 \times 10^{4.1}$/mL. Moreover, it was serially passaged six generation times on primary Muscovy duck embryo fibrolast (DEF) cells, which showed a cytopathic effect (CPE) (supplemental Figure 1). The virus particle existence in the medium of cell culture was confirmed using a transmission electron microscope (TEM) (H-7650; Hitachi, Japan) (supplemental Figure 2). The virus isolate was named as YL08. Furthermore, ten pairs of specific primers (Table 1) based on the genomic DNA sequence of MDPV strain P (GenBank accession no. JF926697) were designed to amplify ten overlapped DNA fragments of YL08 strain with Premix Taq™ (TaKaRa, China). The DNA fragments were sequenced and then the full genome was assembled by SeqMan.



Supplemental Figure 1 Duck embryo fibroblasts under optical microscope (×200). (A) Uninfected cells. (B) MDPV-infected cells at 144 h post inoculation. Cells appeared rounding, shrinking, increasing refractive index and, finally, detachment, increasing the gap between cells, etc.



Supplemental Figure 2 Observation of Muscovy duck parvovirus-like particles by electron microscopy. The virus particles were spherical, about 22 nm in diameter, and had no envelope.

Table 1 Oligonucleotide primers used for amplification of MDPV YL08 isolate genomic fragments by PCR

Primers	Sequence (5'→3')	Size (bp)	Position in genome[a]
P1F	CTCATTGGAGGGTTCGTT	194	1-18
P1R	GCATGCGCCCGATCAG		194-179
P2F	GTATTTCCGGTTGTCAAG	423	160-177
P2R	TCCGAGGGTAACCTGATG		582-565
P3F	GCCTCTTCAGATTTCTTCT	570	526-544
P3R	TCCAGTAACTCTTGCCTC		1095-1078
P4F	ATATGCCCCTTTTCACTG	512	1038-1055
P4R	GGCTATGTTGGTCTTTCC		1549-1532
P5F	ATCTTATGCGGATGGGTG	570	1460-1477
P5R	CTCATTCGCTCGTTCAGG		2029-2012
P6F	AAAGTCCCTACGAATGAA	554	1970-1987
P6R	GACTGAGATTGCTGGTTT		2523-2506
P7F	CGCATCTTGGCGGCATTT	579	2461-2478
P7R	TCGCCCATAGCTCCGCTTC		3039-3021
P8F	AGGCTCCGAACCTGTGGCA	542	2980-2998
P8R	GCGGCAGGGCATAGACAT		3521-3504
P9F	TCAACTCCCGTATGTCCT	552	3445-3462
P9R	CTGGAAGCCTGATCTTTT		3996-3979
P10F	AACAGATAACTATGCGAACT	881	3874-3893
P10R	GCACGTGACCGGAACTTAC		4754-4736

[a] Positions correspond to MDPV YL08 (GenBank accession No. MG932366). F and R denote the forward and reverse primers, respectively.

Results and Discussion

Genome sequences of MDPV strains from different countries were retrieved from Genbank. The related MDPV strains are listed in Table 2. These complete genomic sequences were analyzed using ClustalW (http://www.clustal.org) and MEGA 6.0 (Tamura *et al.,* 2013). Phylogenetic trees of viral genome sequences were constructed using the Maximum Likelihood method in MEGA 6.0 with the substitution model of Tamura and Nei with 1,000 bootstrap replications to estimate the reliability of the tree topology.

The genome of strain YL08 is 5,061 nt in length. The inverted terminal repeats (ITRs) at the 5' and 3' ends of the genome were 421 nt and 420 nt, respectively. The NS1 protein is encoded by 1,884 nt and composed of

627 amino acids (aa) with a predicted molecular weight (MW) of 71.769 kDa. NS1 gene starts at an ATG initiation codon (nt positions 512-514) and terminates with a TAA termination codon (nt positions 2,393-2,395). The NS2 protein is encoded by 1,356 nt and composed of 451 aa with a predicted MW of 51.218 kDa. NS2 gene starts at an ATG initiation codon (nt positions 1,040-1,042) and terminates with the same termination codon as NS1 gene. The VP1 protein is encoded by 2,199 nt and composed of 732 aa. The VP2 and VP3 proteins, which are derived from VP1, are encoded by 1,764 nt and 1,605 nt, respectively, and are predicted to encode 587 aa and 534 aa, respectively. The predicted MW of the VP1, VP2 and VP3 proteins are 81.097 kDa, 65.029 kDa and 59.779 kDa, respectively. VP1, VP2 and VP3 genes start at ATG initiation codon (nt positions 2,414-2,416), ACG initiation codon (nt positions 2,849-2,851) and ATG initiation codon (nt positions 3,008-3,010), respectively, and share the same TAA termination codon (nt positions 4,610-4,612). The genome characteristic of the strain YL08 is shown in Figure 1. The complete genome sequence of MDPV strain YL08 was deposited in GenBank under accession no. MG932366.

Table 2 The genome sequences of MDPV strains cited in this study

Isolate	Accession number	Origin	Collection date	Pathogenicity
GDNX	MH204100	Guangdong, China	2016	Virulent
AH-AQ-2015	KT898977	Anhui, China	2015	Virulent
NM4	KU641557	Fujian, China	2015	Virulent
JS-CZ-2013	KT898978	Jianghina	2013	Virulent
ZJ-JH-2013	KT898979	Zhejiang, China	2013	Virulent
M8	KR029614	Fujian, China	2013	Virulent
NMZJD110	KR075691	Fujian, China	2013	Virulent
FJM2	KR075688	Fujian, China	2013	Virulent
FJM5	KR075689	Fujian, China	2013	Virulent
JX	KR029615	Jiangxi, China	2013	Virulent
FJM3	KR075690	Fujian, China	2013	Virulent
NM100	KU641556	Fujian, China	2012	Virulent
FJ-PT-2012	KT898980	Fujian, China	2012	Virulent
SAAS-SHNH	KC171936	Shanghai, China	2012	Virulent
FJV1	KR029616	Fujian, China	2011	Virulent
MDPV-GX5	KM093740	Guangxi, China	2011	Virulent
JH10	MH807698	Zhejiang, China	2010	Virulent
YL08	MG932366	Heilongjiang, China	2008	Virulent
LH	KY069274	Fujian, China	2008	Virulent
JH06	MH807697	Zhejiang, China	2006	Virulent
ZW	KY744743	Zhejiang, China	2006	Virulent
YY	KX000918	Zhejiang, China	2000	Virulent
FM	NC_006147	France	-	Virulent
FZ91-30	KT865605	Fujian, China	1991	Virulent
P	JF926697	Fujian, China	1988	Virulent
P1	JF926698	Fujian, China	-	Attenuated

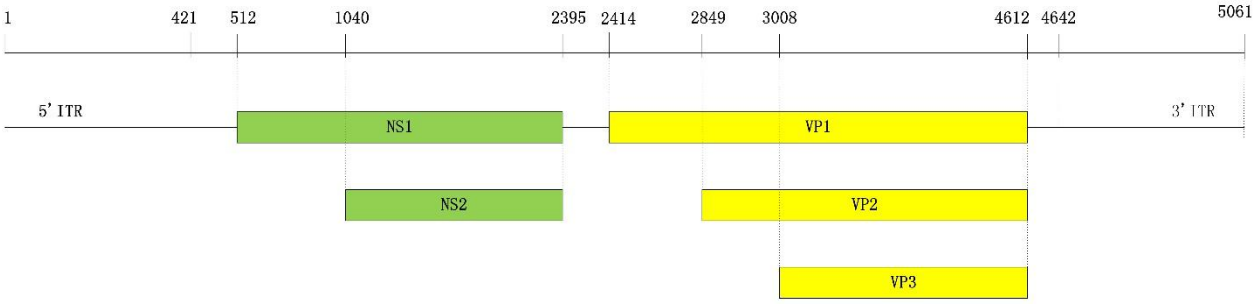


Figure 1 The genome characteristic of MDPV strain YL08

Analysis of the complete genomic sequences using ClustalW (http://www.clustal.org) and MEGA 6.0 software indicated that YL08 shares the highest identity (95.6 %) with YY and 88.6%–94.8 % identity with other MDPV strains. Phylogenetic analysis of the complete YL08 genome sequence revealed two major clusters, classical MDPV and recombinant MDPV. YL08 strain segregated into the classical MDPV group (Figure 2).

Recombinant analysis with SimPlot version 3.5.1 showed that there were two putative recombination breakpoints on the nucleotide sequences nt 602 and nt 1,241 in VP1 gene between the classical MDPV P isolate and recombinant MDPV SAAS-SHNH isolate, leading to the new recombinant strain YL08 (Yu *et al.*, 2016a). Similarity plots analysis of the whole genome of YL08 isolate using the SimPlot program showed the same recombination breakpoints in the nucleotide sequences nt 3,017 (nt 602 on VP1 gene) nt 3,656 (nt 1,241 on VP1 gene) (supplemental Figure 3).

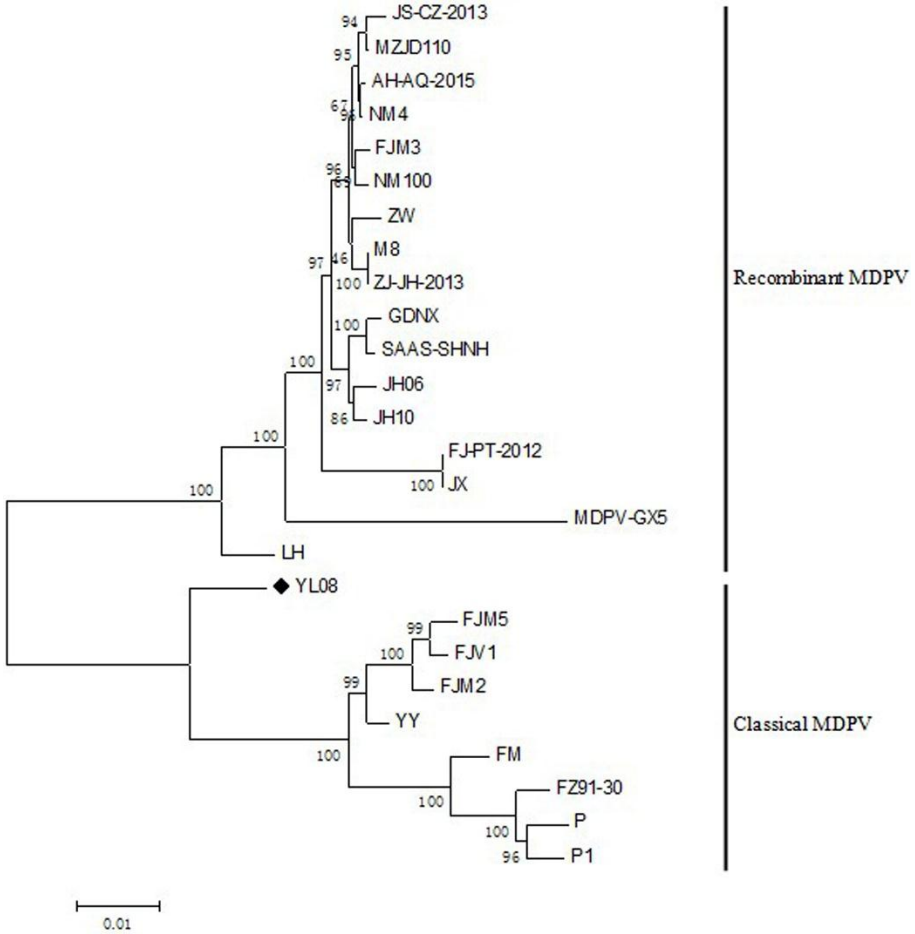
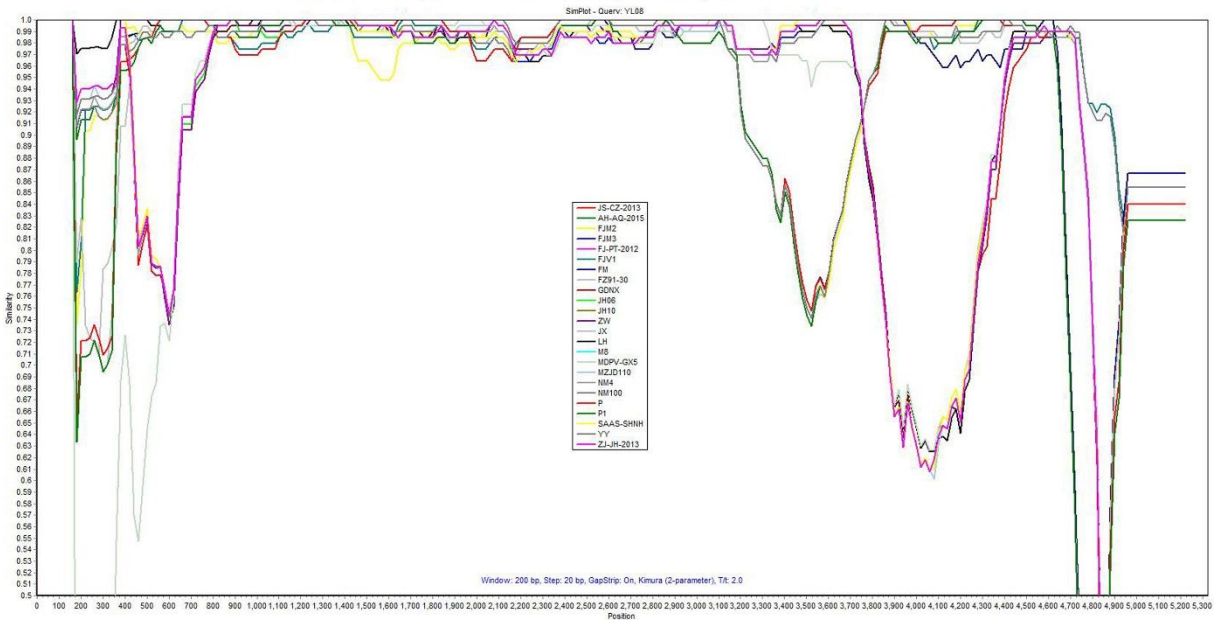


Figure 2 A phylogenetic analysis based on complete genomic sequences of YL08 and 25 other MDPV isolates with the maximum-likelihood method with 1,000 bootstrap replicates using MEGA. Bootstrap value ≥50 % is indicated at the branches. "◆" represents YL08 strain



Supplemental Figure 3 Similarity plots analysis of the whole genome of YL08 using the SimPlot program.

Here, the recombination event was confirmed by incongruent phylogenetic trees constructed on the basis of both the non-recombinant and recombinant regions. The incongruent phylogenetic trees were constructed by the same method described above. We compared the three phylogenetic trees and found that YL08 fell into the classical group with regard to the region (1 - 3016 nt) before the first putative recombination breakpoint (Figure 3A) and the region (3656 - end nt) after the second putative recombination breakpoint (Figure 3C). But, YL08 fell into the recombinant group with regard to the region (3017 - 3655 nt) between the two putative recombination breakpoints (Figure 3B). The dissimilarity between incongruent phylogenetic trees also suggested that the MDPV YL08 strain should undergo a recombination event. Nucleotide sequence comparisons of the divergence and identity of the region (1 - 3016 nt) (supplemental Figure 4), the region (3017 - 3655 nt) (supplemental Figure 5) and the region (3656 - end nt) (supplemental Figure 6) were performed using the MegAlign program. The nucleotide identity between the region (1 - 3016 nt) of YL08 and other reference strains from 92.2% to 99.0%, between the region (3017 - 3655 nt) from 86.4% to 99.1% and between the region (3656 - end nt) from 82.0% to 97.5%.

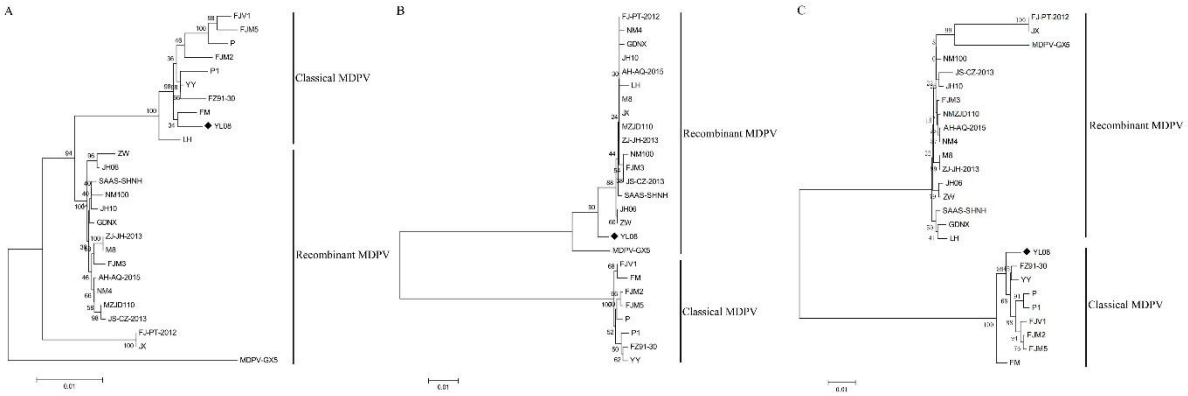


Figure 3 Identification of the gene recombination event by incongruent phylogenetic trees with the neighbor-joining method with 1000 bootstrap replicates using MEGA. Bootstrap value ≥50 % is indicated at the branches. "◆" represents YL08 strain. (A) Incongruent phylogenetic tree based on the region (1 - 3016 nt). (B) Incongruent phylogenetic tree based on the region (3017 - 3655 nt). (C) Incongruent phylogenetic tree based on the region (3656 - end nt).

Percent Identity / Divergence

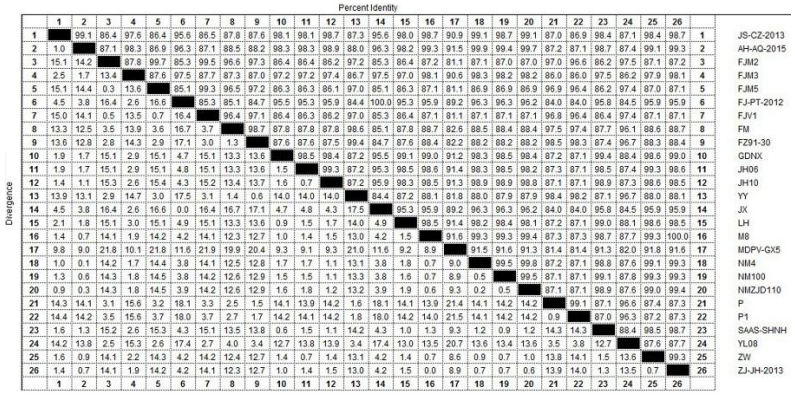
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	
1		98.7	95.7	97.6	95.3	94.6	95.4	95.8	95.5	98.2	98.4	98.6	95.1	94.6	96.7	98.5	92.6	98.8	98.5	99.8	95.3	95.5	98.2	96.4	98.2	98.5	1 JS-CZ-2013
2	0.4		96.9	98.7	96.4	93.9	96.6	96.9	96.4	99.3	99.6	99.7	96.3	93.9	97.7	99.7	93.2	99.9	99.6	98.9	96.2	96.4	99.2	97.3	99.4	99.7	2 AH-AQ-2015
3	2.7	2.3		97.4	98.9	91.7	98.9	97.2	96.7	96.3	96.7	96.7	96.5	91.7	97.5	96.8	91.9	96.9	96.7	95.8	96.6	96.7	96.2	97.5	96.6	96.8	3 FJM2
4	0.6	0.4	2.4		97.0	93.6	97.1	96.0	95.4	98.2	98.6	98.7	95.3	93.6	96.8	98.7	92.9	98.8	98.7	97.8	95.2	95.4	98.2	96.3	98.3	98.7	4 FJM3
5	3.0	2.8	1.1	2.9		91.3	99.4	96.8	96.4	95.9	96.3	96.2	96.1	91.3	97.1	96.4	91.6	96.4	96.4	95.4	97.1	96.3	95.9	97.4	96.3	96.4	5 FJM5
6	3.1	2.9	4.7	2.6	5.1		91.4	91.0	91.0	94.1	93.8	93.9	90.4	100.0	92.6	93.8	90.4	93.9	93.8	94.7	90.7	91.2	94.2	92.2	93.5	93.8	6 FJ-PT-2012
7	2.9	2.6	1.1	2.8	0.6	5.0		96.9	96.5	96.1	96.4	96.4	96.3	91.4	97.2	96.5	91.7	96.6	96.6	95.6	97.1	96.5	96.0	97.6	96.5	96.5	7 FJV1
8	2.4	2.2	1.0	2.2	1.4	5.1	1.2		98.6	96.4	96.8	96.8	98.5	91.0	97.7	96.9	91.6	97.0	96.8	95.9	98.4	98.6	96.4	97.8	96.7	96.9	8 FM
9	2.4	2.4	1.1	2.5	1.5	5.0	1.3	1.0		96.3	96.4	96.3	98.3	91.0	97.6	96.4	91.2	96.5	96.3	95.4	98.6	98.9	96.3	97.5	96.4	96.4	9 FZ91-30
10	0.7	0.5	2.7	0.7	3.1	2.9	2.9	2.5	2.5		99.2	99.2	95.7	94.1	98.2	99.2	93.1	99.3	99.2	98.3	95.1	96.3	99.8	97.9	99.0	99.2	10 GDNX
11	0.6	0.4	2.5	0.5	2.9	3.0	2.8	2.3	2.4	0.6		99.6	96.2	93.8	97.7	99.6	93.2	99.6	99.6	98.6	96.1	96.3	99.2	97.3	99.7	99.6	11 JH06
12	0.5	0.2	2.5	0.4	3.0	2.8	2.8	2.3	2.5	0.6	0.4		96.2	93.9	97.7	99.5	93.2	99.8	99.7	98.8	96.0	96.2	99.3	97.3	99.4	99.5	12 JH10
13	2.1	1.9	0.7	2.0	1.1	4.7	0.9	0.6	0.5	2.3	2.0	2.0		90.4	97.0	96.2	91.1	96.3	96.1	95.3	97.9	98.2	95.7	97.0	96.2	96.2	13 YY
14	3.1	2.9	4.7	2.6	5.1	0.0	5.0	5.1	5.0	2.9	3.0	2.8	4.7		92.6	93.8	90.4	93.9	93.8	94.7	90.7	91.2	94.2	92.2	93.5	93.8	14 JX
15	2.3	2.2	1.4	2.2	1.9	4.6	1.7	1.2	1.2	1.9	2.2	2.2	0.9	4.6		97.7	92.4	97.7	97.7	96.7	97.3	97.6	98.2	99.0	97.5	97.7	15 LH
16	0.6	0.3	2.4	0.4	2.9	3.1	2.7	2.2	2.4	0.6	0.4	0.4	1.9	3.1	2.2		93.2	99.7	99.5	98.8	96.2	96.4	99.2	97.3	99.4	100.0	16 M8
17	6.7	6.4	7.6	6.5	8.0	6.6	7.9	7.3	7.4	6.3	6.4	6.4	6.8	6.6	7.1	6.5		93.3	93.2	92.6	90.9	91.1	93.1	92.3	93.0	93.2	17 MDPV-GX5
18	0.3	0.1	2.3	0.3	2.8	2.9	2.6	2.1	2.3	0.5	0.3	0.2	1.8	2.9	2.2	0.3	6.4		99.7	99.0	96.2	96.4	99.3	97.3	99.4	99.7	18 NM4
19	0.6	0.4	2.5	0.4	2.8	3.0	2.7	2.3	2.4	0.6	0.4	0.3	2.0	3.0	2.2	0.5	6.4	0.3		98.6	96.2	96.3	99.3	97.3	99.4	99.5	19 NM100
20	0.2	0.2	2.5	0.4	2.9	3.0	2.8	2.3	2.4	0.6	0.5	0.3	2.0	3.0	2.3	0.3	6.7	0.1	0.5		95.2	95.4	98.3	96.4	98.4	98.8	20 MZJD110
21	2.8	2.7	1.4	2.9	0.9	5.5	0.9	1.5	1.2	2.9	2.9	2.9	1.0	5.5	1.7	2.8	7.9	2.7	2.8	2.9		98.7	96.0	97.8	96.1	96.2	21 P
22	2.5	2.5	1.3	2.6	1.6	5.0	1.5	1.2	0.9	2.7	2.5	2.6	0.7	5.0	1.3	2.5	7.6	2.5	2.6	2.6	1.2		96.2	97.5	96.4	96.4	22 P1
23	0.7	0.6	2.8	0.7	3.2	2.8	3.0	2.6	2.6	0.2	0.6	0.5	2.3	2.8	1.9	0.6	6.3	0.6	0.6	0.6	3.0	2.7		97.9	98.9	99.2	23 SAAS-SHNH
24	2.6	2.6	1.5	2.7	1.6	5.0	1.4	1.0	1.2	2.2	2.6	2.5	1.0	5.0	1.0	2.6	7.2	2.6	2.6	2.6	1.2	1.4	2.2		97.3	97.3	24 YL08
25	0.9	0.6	2.6	0.7	2.9	3.3	2.7	2.4	2.4	0.8	0.3	0.6	2.0	3.3	2.4	0.6	6.6	0.6	0.6	0.7	2.8	2.4	0.9	2.5		99.4	25 ZW
26	0.6	0.3	2.4	0.4	2.9	3.1	2.7	2.2	2.4	0.6	0.4	0.4	1.9	3.1	2.2	0.0	6.5	0.3	0.5	0.3	2.8	2.5	0.6	2.6	0.6		26 ZJ-JH-2013
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	

Supplemental Figure 4 Divergence (lower left) and percent identity (upper right) among the nucleotide sequences of the region (1 - 3016 nt) of twenty-six MDPV strains.

Percent Identity / Divergence

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	
1		99.8	86.7	100.0	86.7	99.8	86.9	86.5	86.5	99.7	99.7	99.8	86.5	99.8	99.5	99.8	97.0	99.7	99.8	99.8	86.7	86.5	99.7	98.7	99.7	99.8	1 JS-CZ-2013
2	0.2		86.7	99.8	86.7	100.0	86.9	86.5	86.5	99.8	99.8	100.0	86.5	100.0	99.7	100.0	97.2	99.8	99.7	100.0	86.7	86.5	99.8	98.9	99.8	100.0	2 AH-AQ-2015
3	14.7	14.7		86.7	99.8	86.7	99.7	99.4	99.5	86.5	86.5	86.7	99.5	86.7	86.4	86.7	86.9	86.5	86.9	86.7	99.5	99.4	86.5	86.5	86.5	86.7	3 FJM2
4	0.0	0.2	14.7		86.7	99.8	86.9	86.5	86.5	99.7	99.7	99.8	86.5	99.8	99.5	99.8	97.0	99.7	99.8	99.8	86.7	86.5	99.7	98.7	99.7	99.8	4 FJM3
5	14.7	14.7	0.2	14.7		86.7	99.5	99.2	99.4	86.5	86.5	86.7	99.4	86.7	86.4	86.7	86.9	86.5	86.9	86.7	99.7	99.2	86.5	86.5	86.5	86.7	5 FJM5
6	0.2	0.0	14.7	0.2	14.7		86.9	86.5	86.5	99.8	99.8	100.0	86.5	100.0	99.7	100.0	97.2	99.8	99.7	100.0	86.7	86.5	99.8	98.9	99.8	100.0	6 FJ-PT-2012
7	14.5	14.5	0.3	14.5	0.5	14.5		99.7	99.5	86.7	86.7	86.9	99.5	86.9	86.5	86.9	87.0	86.7	87.0	86.9	99.5	99.4	86.7	86.7	86.7	86.9	7 FJV1
8	14.9	14.9	0.6	14.9	0.8	14.9	0.3		99.2	86.4	86.4	86.5	99.2	86.5	86.2	86.5	87.0	86.4	86.7	86.5	99.2	99.1	86.4	86.7	86.4	86.5	8 FM
9	14.9	14.9	0.5	14.9	0.6	14.9	0.5	0.8		86.4	86.4	86.5	99.7	86.5	86.2	86.5	86.7	86.4	86.7	86.5	99.4	99.5	86.4	86.4	86.4	86.5	9 FZ91-30
10	0.3	0.2	14.9	0.3	14.9	0.2	14.7	15.1	15.1		99.7	99.8	86.4	99.8	99.5	99.8	97.0	99.7	99.5	99.8	86.5	86.4	99.7	98.7	99.7	99.8	10 GDNX
11	0.3	0.2	14.9	0.3	14.9	0.2	14.7	15.1	15.1	0.3		99.8	86.4	99.8	99.5	99.8	97.3	99.7	99.5	99.8	86.5	86.4	99.7	99.1	100.0	99.8	11 JH06
12	0.2	0.0	14.7	0.2	14.7	0.0	14.5	14.9	14.9	0.2	0.2		86.5	100.0	99.7	100.0	97.2	99.8	99.7	100.0	86.7	86.5	99.8	98.9	99.8	100.0	12 JH10
13	14.9	14.9	0.5	14.9	0.6	14.9	0.5	0.8	0.3	15.1	15.1	14.9		86.5	86.2	86.5	86.7	86.4	86.7	86.5	99.4	99.5	86.4	86.4	86.4	86.5	13 YY
14	0.2	0.0	14.7	0.2	14.7	0.0	14.5	14.9	14.9	0.2	0.2	0.0	14.9		99.7	100.0	97.2	99.8	99.7	100.0	86.7	86.5	99.8	98.9	99.8	100.0	14 JX
15	0.5	0.3	15.1	0.5	15.1	0.3	14.9	15.3	15.3	0.5	0.5	0.3	15.3	0.3		99.7	96.9	99.5	99.4	99.7	86.4	86.2	99.5	98.6	99.5	99.7	15 LH
16	0.2	0.0	14.7	0.2	14.7	0.0	14.5	14.9	14.9	0.2	0.2	0.0	14.9	0.0	0.3		97.2	99.8	99.7	100.0	86.7	86.5	99.8	98.9	99.8	100.0	16 M8
17	3.0	2.9	14.5	3.0	14.5	2.9	14.3	14.3	14.7	3.0	2.7	2.9	14.7	2.9	3.2	2.9		97.0	97.2	97.2	86.9	86.7	97.0	98.0	97.3	97.2	17 MDPV-GX5
18	0.3	0.2	14.9	0.3	14.9	0.2	14.7	15.1	15.1	0.3	0.3	0.2	15.1	0.2	0.5	0.2	3.0		99.5	99.8	86.5	86.4	99.7	98.7	99.7	99.8	18 NM4
19	0.2	0.3	14.5	0.2	14.5	0.3	14.3	14.7	14.7	0.5	0.5	0.3	14.7	0.3	0.6	0.3	2.9	0.5		99.7	86.9	86.7	99.5	98.6	99.5	99.7	19 NM100
20	0.2	0.0	14.7	0.2	14.7	0.0	14.5	14.9	14.9	0.2	0.2	0.0	14.9	0.0	0.3	0.0	2.9	0.2	0.3		86.7	86.5	99.8	98.9	99.8	100.0	20 MZJD110
21	14.7	14.7	0.5	14.7	0.3	14.7	0.5	0.8	0.6	14.9	14.9	14.7	0.6	14.7	15.1	14.7	14.5	14.9	14.5	14.7		99.2	86.5	86.5	86.5	86.7	21 P
22	14.9	14.9	0.6	14.9	0.8	14.9	0.6	0.9	0.5	15.1	15.1	14.9	0.5	14.9	15.3	14.9	14.7	15.1	14.7	14.9	0.8		86.4	86.4	86.4	86.5	22 P1
23	0.3	0.2	14.9	0.3	14.9	0.2	14.7	15.1	15.1	0.3	0.3	0.2	15.1	0.2	0.5	0.2	3.0	0.3	0.5	0.2	14.9	15.1		99.1	99.7	99.8	23 SAAS-SHNH
24	1.3	1.1	14.9	1.3	14.9	1.1	14.7	14.7	15.1	1.3	0.9	1.1	15.1	1.1	1.4	1.1	2.1	1.3	1.4	1.1	14.9	15.1	0.9		99.1	98.9	24 YL08
25	0.3	0.2	14.9	0.3	14.9	0.2	14.7	15.1	15.1	0.3	0.0	0.2	15.1	0.2	0.5	0.2	2.7	0.3	0.5	0.2	14.9	15.1	0.3	0.9		99.8	25 ZW
26	0.2	0.0	14.7	0.2	14.7	0.0	14.5	14.9	14.9	0.2	0.2	0.0	14.9	0.0	0.3	0.0	2.9	0.2	0.3	0.0	14.7	14.9	0.2	1.1	0.2		26 ZJ-JH-2013
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	

Supplemental Figure 5 Divergence (lower left) and percent identity (upper right) among the nucleotide sequences of the region (3017 - 3655 nt) of twenty-six MDPV strains.



Supplemental Figure 6 Divergence (lower left) and percent identity (upper right) among the nucleotide sequences of the region (3656 - end nt) of twenty-six MDPV strains.

Recently, several reports have shown that natural recombination can occur among the structural protein gene of GPVs (Li *et al.,* 2018; Wang *et al.,* 2015; Wang *et al.,* 2016) or between the structural protein gene of GPVs and MDPVs (Fu *et al.,* 2017; Shen *et al.,* 2015; Wang *et al.,* 2017; Wang *et al.,* 2019; Zhao *et al.,* 2014; Zhu *et al.,* 2014) or between the structural protein gene of MDPVs (Yu *et al.,* 2016). Such natural recombination generates new GPVs or MDPVs genotype and contributes to the genetic variety of waterfowl parvoviruses. Because the MDPVs and MDPV attenuated vaccines have been widely used in China for many years, it is not surprising that attenuated MDPVs and virulent MDPVs have existed on the same farm or even in a single duck. For this reason, in this case, the recombination events could happen in the field conditions. In China, it should be noted that the recombinant MDPVs have become more and more dominant in epidemic events since 2006 (Wang *et al.,* 2019). The frequent appearance of such recombinant MDPVs may pose more challenges in diagnostic testing and the need to develop efficient vaccines against virus variant infection, considering the changes of amino acid in structural proteins can influence the virulence and pathogenicity of MDPV.

In conclusion, this research reports a complete genome sequence of MDPV recombinant strain generated among MDPVs. It suggests that natural recombination may play a very important role in the evolution of waterfowl parvovirus (GPV and MDPV). Meanwhile, the complete genome sequence of strain YL08 also can provide important supplements to MDPV genomics and molecular epidemiological data.

Conflicts of interest: The authors declare no conflict of interest.

Acknowledgements

This work is supported by the Joint Guidance Project of Natural Science Foundation of HeiLongJiang Province of China (Grant No. LH2020C110), the Postdoctoral Science Foundation of Heilongjiang Province of China (Grant No. LBH-Z14071) and Fundamental Research Funds in Heilongjiang Provincial Universities (Grant No. YSTSXK201881).

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