Emergence and genomic analysis of a novel ostrich-origin GPV-related parvovirus in China

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ABSTRACT In recent years, ostrich disease characterized by paralysis and diarrhea has been circulating in some regions of China, causing huge economic losses to the ostrich breeding industry. In our study, clinical samples from diseased ostriches were collected, and only parvovirus was detected. The virus distribution analysis by histopathology and quantitative real-time PCR assays indicated that the virus had a wide range of tissue tropisms. The full-length genome of the ostrich parvovirus (**OsPV**) was sequenced and comprehensively analyzed. Interestingly, the phylogenetic and alignment results indicated that the OsPV and the goose parvovirus (**GPV**) form a separate branch. In contrast to GPV strains, OsPV showed 2 new 14 nucleotide deletions in the inverted terminal repeat (**ITR**) region. Furthermore, recombination analysis indicated that OsPV was a recombination strain between the vaccine strain SYG61v and the virulent strain B strain, with the major parent of OsPV as the SYG61v strain and the minor parent as the B strain. The 14 nucleotide deletions in the ITR region as well as recombination may be some of the reasons for the cross-species transmission of parvovirus from goose to ostrich. The above data will contribute to a better understanding of the molecular biology of the novel OsPV and help to develop the vaccine candidate strain.

Key words: ostrich, parvovirus, paralysis, recombination, cross-species transmission

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INTRODUCTION

Parvoviruses are small (20 nm) non-enveloped icosahedral viruses that belong to the *Parvoviridae* family and that infect and cause diseases in many vertebrate hosts (Boftsi et al., 2020). The International Committee on Taxonomy of Viruses (**ICTV**) classifies the family *Parvoviridae* into 2 subfamilies, *Densovirinae* and *Parvovirinae*, whose hosts are vertebrates and arthropods, respectively (Wan et al., 2018). Recent studies have revealed that the diversity of some parvovirus species has greatly expanded, and some parvoviruses have evolved the capacity to expand their host range by biologically adapting to novel hosts (François et al., 2016). In recent years, parvoviruses have become widespread in birds, especially waterfowl parvoviruses, goose parvovirus (**GPV**), and Muscovy duck parvovirus (**MDPV**). GPVs can infect goslings, Muscovy ducklings, swans, and Anser cygnoides, whereas MDPV has only been discovered in Muscovy ducklings (Liu et al., 2014; Shao et al., 2014; Wan et al., 2015; Wan et al., 2016). Recent studies suggest that a novel duck parvovirus (**NDPV**), identified as a new variant of GPV, can infect ducks and geese (Fan et al., 2017; Ning et al., 2018). Moreover, recombination can also occur between GPV and MDPV, which accelerates the evolution of parvoviruses.

Recently, a disease outbreak characterized by paralysis occurred in farmed ostriches in the Hebei Province, China. The incidence rate was approximately 50%, and most of the diseased ostriches were 1 to 3 months of age. Clinical symptoms mainly include paralysis, inability to stand, and a reduced diet. Ostrich diarrhea can also be present with white feces, gradually resulting in weak and thin ostriches. Because the ostriches are paralyzed for a long time, this can affect intestinal peristalsis and cause intestinal blockages, which can eventually lead to death. Antibacterial and antiviral drugs have no effect on treating this disease, and thus far, no effective

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solution has been reported. More importantly, the agent of this disease is unknown, causing extensive loss within the ostrich farming industry.

Therefore, to determine the pathogen of this disease, sick ostriches were autopsied and examined in this study. Finally, it was determined that the pathogen causing ostrich paralysis was a new ostrich-derived parvovirus (**OsPV**), which was named HB-2019 strain. The whole genome of the virus was also sequenced, and phylogenetic tree analysis showed that the virus was correlated to GPV. Recombination analysis revealed that this OsPV HB-2019 strain is a recombination strain between the vaccine SYG61v strain and the virulent B strain, which may result in cross-species transmission.

MATERIALS AND METHODS

Sample Collection

In 2019, an ostrich farm in the Hebei Province suffered from an outbreak of paralysis and diarrhea. Muscles, sciatic nerve, kidneys, spleen, thymus, heart, pancreas, and gut samples were collected from one diseased ostrich and processed for viral detection and isolation.

Clinical Anatomy and Histopathology

The diseased ostriches were dissected, and tissues from their muscle, kidneys, liver, heart, and spleen were collected and examined. All tissues were stored at −80°C for further study. Parts of the tissues were fixed in 4% neutral formalin at room temperature, embedded in paraffin, and cut into 5-μm-thick sections. After deparaffinization, the sections were stained with hematoxylin and eosin (**H&E**). Microscopic lesions were observed under an Olympus microscope (Olympus, Tokyo, Japan).

Detection of the Potential Pathogen Using Polymerase Chain Reaction

The tissue samples were homogenized in Dulbecco's Modified Eagle Medium (**DMEM**), freeze-thawed three times, and centrifuged at 12,000 × g for 5 min. The supernatants were collected for DNA extraction using a TransGen BiotechEasyPure Viral DNA/RNA Kit according to the manufacturer's instructions. As the causing agent of paralysis in the ostriches is unknown, a variety of suspicious pathogens were detected, including avian influenza virus (**AIV**), avian paramyxovirus-1 (**APMV-1**), avian encephalomyelitis virus (**AEV**), avian orthoreovirus (**ARV**), GPV, and Mycoplasma synoviae (**MS**). The primers for the different agents used in this study are listed in Table 1. Amplification bands were sequenced to verify the results.

Viral Isolation

GPV was found to be the most homologous to OsPV HB-2019, and the virus was further isolated from 10-day-old goose embryos. The tissue samples were ground and homogenized in physiological saline, freeze-thawed 3 times, and centrifuged at 12,000 × g for 15 min at 4°C. The supernatants were filtered using 0.22-μm sterile filters to remove contaminants and inoculated into the chorioallantoic cavity of 10-day-old goose embryonated eggs (300 μL/egg). The inoculated embryonated eggs were checked daily for survival. The allantoic fluid and

Table 1. The primers and probe used in this study.

Primers	Nucleotide sequence(5′→ 3′)	Length of products (bp)
AIV-Forward	CGTAGACGCTTTGTCCAGAATGC	428
AIV-Reverse	GTCCTCATTGCCTGCACCATC	
AEV-Forward	AGAGGGTTTTGTGACGGGTACA	268
AEV-Reverse	CAGTGGCGTGTAGAAAGGGATC	
APMV-1-Forward	TGATGTGGAGGTAGACCCGAAGA	183
APMV-1-Reverse	CTGAGCCCGACAGATAGATTGAG	
DRV-Forward	TCGCACTATTGACGCACTTACT	801
DRV-Reverse	GAGGTGTTGATACCACGAGGAG	
MS-Forward	GTGGGGAGCAAACAGGATTAG	611
MS-Reverse	GTGACGAGCGGTGTGTACAAG	
GPV-Forward	AAACTTACTGAGCCCGTTCCTG	659
GPV-Reverse	GCGACGCTGTCTGCTTTATTGA	
ITR-1-Forward	TCATTGGAGGGTTCGTTCGT	188
ITR-1-Reverse	GCATGCGCGCGGTCAACCTAA	
ITR-2-Forward	GCATGCGCGCGGTCAGCCCAA	795
ITR-2-Reverse	GTATGCTGCAGTCACGGTCTT	
T2-Forward	GGGAGGACAGAATAAGAC	1,437
T2-Reverse	TTTTCAGCATCATCAAGT	
T3-Forward	TTGTTCTCATCAGTCGCTCCA	1,417
T3-Reverse	GCGGCAGGGCATAGACAT	
T4-Forward	CATCACAAAGACCACCAG	1,088
T4-Reverse	AAGCTCCAAGAACATCAA	
ITR-3-Forward	ACACTACTACAGCTCCTACGAGTT	712
ITR-3-Reverse	GCATGCGCGCGGTCAGCCCAA	
OsPV-Forward	CAAATTCCATCCTTCTCCGAATCT	86
OsPV-Reverse	TCTGCAGGCACTGGTGTATTCTTGA	
OsPV-Probe	CTGCACAATCCACCACCGCAGGTGTTC	

embryo bodies were harvested 6 days after inoculation and stored at −80°C for further testing.

Whole Genome Amplification of OsPV HB-2019 Strain and Sequencing

The sequencing results of the detected bands were input into the NCBI Basic Local Alignment Search Tool (BLAST) to screen for the sequences with the highest homology, and 6 pairs of primers were designed (Table 1). The genomes were amplified using PrimeStar HS DNA polymerase (TaKaRa Biotechnology, Dalian, People's Republic of China). Amplified DNA fragments were visualized via electrophoresis on a 1% agarose gel. Polymerase chain reaction (**PCR**) products were purified and cloned into pEasy-Blunt vector and sequenced by Sangon Biotech.

qPCR

To further detect the distribution and content of the virus in different tissues, 3 g of each sample collected from the diseased ostriches were used for homogenization. The supernatants were collected for DNA extraction, according to the manufacturer's instructions. The primers and probes are listed in Table 1 and were synthesized by Sangon Biotech. The reaction was carried out in a LightCycler PCR (Roche), and the optimal reaction was carried out as follows: 95 °C for 30 s, followed by 35 cycles of 95 °C for 5 s and 60 °C for 35 s (Wang et al., 2017b).

Phylogenetic and Recombination Analysis

For the phylogenetic analysis, the whole genome sequences of GPV, MDPV, and novel-goose parvovirus (**N-GPV**) were retrieved from the NCBI nucleotide database as reference sequences. Detailed information and GenBank numbers of these viruses are shown in Table 2. The phylogenetic tree was constructed by MEGA 5.0 software, using the neighbor-joining method with 1,000 bootstrap replicates. The generated tree was annotated by ITOL (https://itol.embl.de/) (Yin et al., 2021). Multiple sequence alignments were analyzed using DNASTAR software to determine sequence homology. To detect probable recombination events, the OsPV HB-2019 strain was analyzed using the Recombination Detection Program v.4.43 (RDP4) and SimPlot version 3.5.1. Different methods implemented in RDP4 were used to detect recombinant events under default settings for different detection programs. The results of the recombination analysis using RDP4 are shown in Supplementary Table 1. If the recombination event was supported by at least 3 methods with a *P*-value of $<10^{-6}$ or a recombination score above 0.6, the recombination event was considered as true and analyzed via Simplot (Wang et al., 2017a). Simplot analysis was conducted by setting the window width and step size to 200 bp and 20 bp, respectively.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism 6 (GraphPad, La Jolla, CA). Statistical significance was assessed using a Student's t test. Differences were considered to be statistically significant at $P < 0.05$.

RESULTS

Clinical Syndrome and Histopathologic Changes

The morbidity of ostrich farms in Hebei Province is approximately 50% (17/30), and all the sick ostriches were 1 to 3 months of age. The classic symptoms exhibited by the diseased ostriches included paralysis and diarrhea (Figure 1A-a). Bleeding spots were also detected in multiple viscera, especially in the intestines, muscle, and thymus. In addition, the knee joint exhibited prominent effusion and swelling, as shown in Figure 1A-b. HE staining results of different tissue sections showed that a massive infiltration of hemocytes was present in the leg muscle, and muscle fibers in the muscular layer exhibited differing degrees of swelling and degeneration. The renal tubules also showed degeneration and necrosis with massive lymphocytic infiltration in the interstitium. Hemorrhagic tissue was also observed in the thymus and bursa (Figure 1B).

Detection of the OsPV and Complete Genome Sequencing

To determine the causative pathogen of the outbreak in the studied ostrich farm in the Hebei Province, PCR was used to detect the potential pathogens causing paralysis and diarrhea. The results showed that the samples were parvovirus-positive, but negative for AIV, APMV-1, AEV, ARV, and MS (Figure 2A). The newly detected viral strain was named OsPV HB-2019. Six PCR products were obtained which covering the full length of the viral genome. The nucleotide sequence of the complete OsPV HB-2019 genome has been deposited in GenBank (accession No. MT010852).

Virus Isolation and Propagation

The goose embryos were inoculated with the supernatant of the tissue homogenate and propagated for five passages, and no obvious changes or deaths occurred in infected goose embryos on the fifth passage until 6 dpi. The allantoic fluids and embryo bodies were harvested for the PCR assay. PCR results showed that viral DNA was present in the allantoic fluid after 5 passages (Supplementary materials 1). However, the viral load was extremely low, which may be related to the presence of maternal antibodies in non-SPF goose embryos.

Table 2. Background information of 64 selected virus strains used in this study.

GenBank No.	Strains	Collection year	Country of origin	Host
MT010852	OsPV HB	2019	China	Ostrich
KM272560	LH	2012	China	Goose
KC996730	YZ99-6	1999	China	Goose
KR091960	YZ	2013	China	Goose
KR091959	WX	2013	China	Goose
KT232256	FJ01	2012	China	Anser cygnoides(eggs)
KC478066	SHFX1201	2012	China	swan
KC178571	Y	2011	China	Muscovy duck
KR136258	Yan-2	2013	China	yan goose
EU583391	06-0329	2008	China	Goose
EU583390	82-0321	2008	China	Goose
KC184133	E	2012	China,	Goose
KY475562	RC16	2016	China	Goose
MH209633	DY16	2016	China	Goose
MF942876	SQ0412	2017	China	Goose
MH717785	RC70	2017	China	Goose
MH717784	RC45	2017	China	Goose
U25749	B	1995	Hungary	Anser
EU583392	VG32/1	2008	China	Goose
KU684472	GPV GER	2015	Poland	ornamental duck
KC996729	SYG61v	2008	China	Goose
HQ891825	GDaGPV	1978	China	Goose
KT598506	GPV-98E	1998	China	Goose
KT598505	GPV-98D15	2012	China	Goose(duck embryo)
KY511292	FJ	2018	China	Goose
JF333590	SH	2009	China	Anser
KR029617	G7	2013	China	Muscovy duck
MF438102	MDE	2015	China	Mule duck
EU583389	82-0321V	2008	China	Goose
KT935536	JS1	2015	China	Pekin duck
KT935531	JS1	2015	China	Pekin duck
KY511124	GPV SD	2015	China	Duck
MF441223	SDHZ1604	2015	China	Cherry Valley ducks
KU641558	CVSD01	2015	China	Anas platyrhynchos
MF441227	AH1605	2016	China	Cherry Valley ducks
MF441222	SDLY1602	2015	China	Cherry Valley ducks
MF441221	SDLY1512	2015	China	Cherry Valley ducks
MF441226	JS1603	2016	China	Cherry Valley ducks
MF441225	AH1606	2016	China	Cherry Valley ducks
KT751090	QH15	2015	China	Peking duck
KX384726	GPV SC16	2016	China	Cherry Valley ducks
MF441224	SDDY1605	2016	China	Cherry Valley ducks
MH444514	GD	2016	China	Mule duck
MH444513	AH	2019	China	Cherry Valley ducks
KY679174	GPV DS15	2015	China	Cherry Valley ducks
KT343253	GPV SDLC01	2015	China	Cherry Valley ducks
MK736656	HuN18	2018	China	Linwu Sheldrake
KU844283	M15	2015	China	Mule duck
KR075689	FJM5	2013	China	Muscovy duck
KR029616	FJV1	2011	China	Muscovy duck
KU844281	P	1988	China	Muscovy duckling
KR075688	FJM2	2013	China	Muscovy duck
KU844282	P1	2016	China	Muscovy duck
KT865605	FZ91-30	1991	China	Muscovy duck
KX000918	YY	2000	China	Muscovy duck
NC006147	FM	1995	China	Cairina moschata
MG932366	YL08	2008	China	Muscovy duck
KY069274	LH	2008	China	Mule duck
KM093740	MDPV-GX5	2011	China	Muscovy duck
KC171936	SAAS-SHNH	2012	China	Muscovy duck
MH807698	JH10	2010	China	Muscovy duck
MH204100	GDNX	2016	China	Muscovy duck
MH807697	JH06	2006	China	Muscovy duck
KY744743	ZW	2006	China	Muscovy duck

Distribution of Viruses in Different Tissues of Disease Ostrich

Different tissues from the sick ostriches were homogenized, and qPCR was used to detect the distribution of the virus. The results showed that OsPV HB-2019 is widely distributed in different organs. As shown in Figure 3A, viral DNA copies were all above 10^6 in the muscles, sciatic nerve, kidneys, spleen, thymus, heart, and pancreas. Moreover, the viral load in the gut was also high, especially in the duodenum, jejunum, ileum, and cecum (Figure 3B).

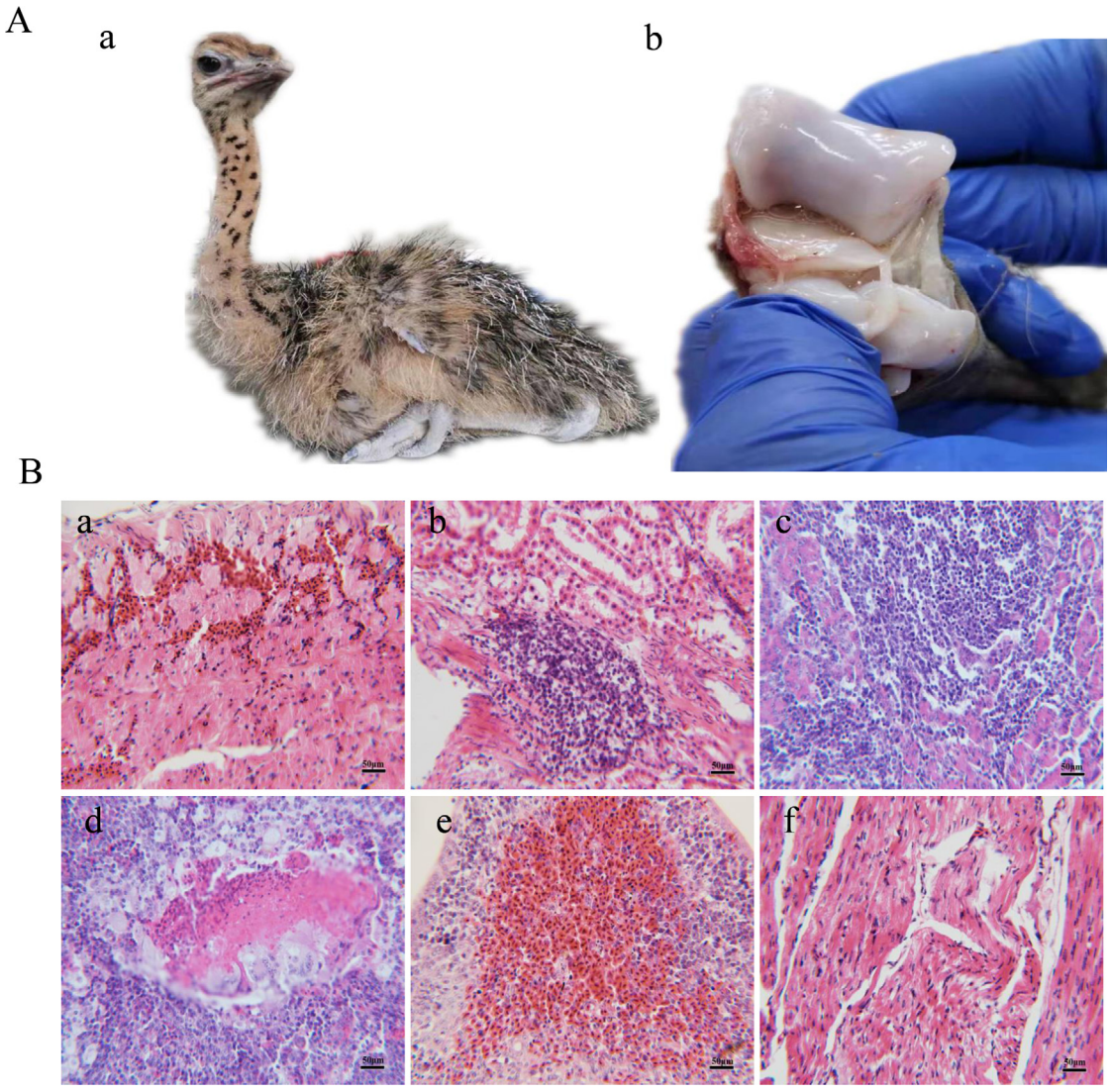

Figure 1. Clinical syndrome and histopathologic changes. The diseased ostrich characterized with paralysis (A-a), and the joint exhibiting prominent effusion and swelling (A-b). Microscopic examination of the histopathologic changes in different tissues collected from diseased ostrich (B-a) muscle, (B-b) kidney, (B-c) liver, (B-d) thymus, (B-e) bursa of fabricius, and (B-f) myocardium. The scale bar equal 50 μm.

Genomic Characterization and Homology Analysis

The complete genomic sequence of OsPV HB-2019 was determined and deposited in the GenBank database under the accession number MT010852. The genomes of OsPV HB-2019 were 5,046 nucleotides (**nt**) in length, including 2 ITRs at the 3′ and 5′ ends. OsPV HB-2019 shared 92.36−99.80%, 92.81−95.2%, and 85.21−86.36 sequence identity with classic GPV, N-GPV, and MDPV respectively. The NS sequence shared 93.63 −99.68%, 96.23−99.36%, and 83.15−83.25% and the VP sequence shared 94.86−99.86%, 94.63−99.41%, and 80.23−90.15% sequence identity with classic GPV, N-GPV, and MDPV, respectively. Besides, the amino acid sequence of NS and VP shared 96.97−99.36%, 96.17

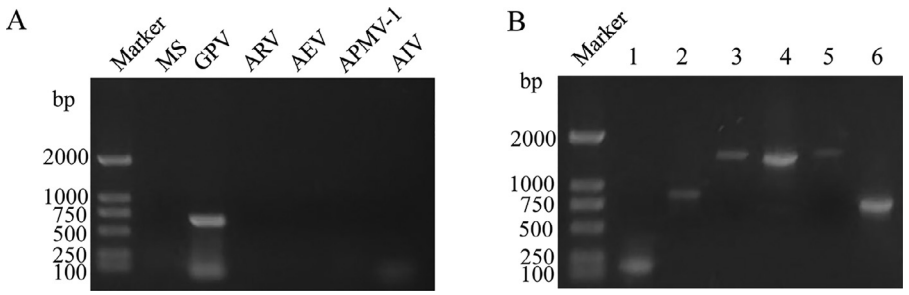

Figure 2. PCR amplification of different agents and complete genome of ostrich parvovirus. Six potential pathogens were detected with corresponding primers, including MS, GPV, ARV, AEV, APMV-1 and AIV (A). The complete genome of OsPV was divided into six fragments for amplification, and every fragment was cloned to pEasy-Blunt vector for further sequenced (B).

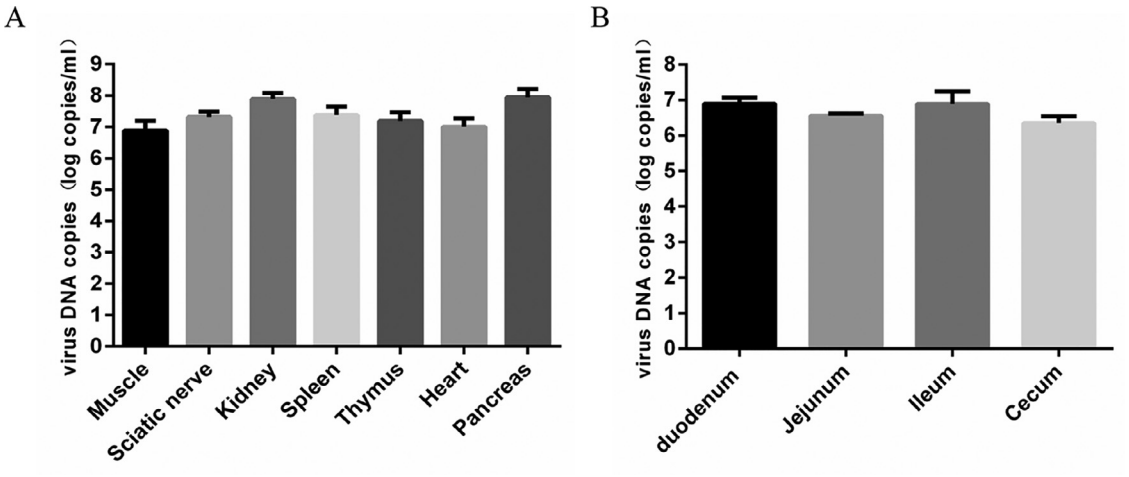

Figure 3. Analysis of virus DNA copies in selected tissues. Virus DNA was isolated from different tissue samples and used in triplicate to determine the virus DNA copies per mL using qPCR (A). The amount of viral DNA in different stages of the intestine were also detected using qPCR (B).

−97.13%, 89.31−90.91% and 95.08−99.59%, 95.90−97.95%, 91.39−92.49% identity with classic GPV, N-GPV, and MDPV, respectively (Table 3). Multiple sequence alignment showed that OsPV is characterized by the same deletion as the GPV DY16 strain at the 3'-ITR and 5′- ITR when compared to the GPV virulent B and the vaccine SYG61v strains.

Phylogenetic Analysis

Phylogenetic analyses were conducted using the whole genome of OsPV and other parvoviruses, including classic GPV, MDPV, and N-GPV strains available in GenBank. As shown in Figure 4, whole genome phylogenetic analysis resulted in 64 parvovirus strains classified into three major clusters, with OsPVs and GPV RC16 strains (goose parvoviruses isolated in Chongqing in 2016) forming a separate branch. Phylogenetic and alignment analyses showed that OsPV is a branch of the GPV (Figure 4A). There was a greater genetic distance between the OsPVs and SYG61, and an even greater distance between OsPV and N-GPV.

Recombination Analysis

Simplot and RDP software were used to analyze the recombination of OsPV HB-2019, and 2 potential recombination events were detected in strain OsPV HB-2019. Recombination analysis performed with RDP4 showed that the breakpoints were located in VP1 (nt 2,494 and 2,724) and VP1 (nt 3,806 and 4,318; Figure 5A). These breakpoints separated the genome into five regions; three being closely related to virulent B strains of GPV from Europe (Figure 5B), and the remaining two being closely related to vaccine strain SYG61v of GPV (Figure 5C). The results, therefore, show that OsPV HB-2019 is the result of recombination between the virulent B strain and the vaccine strain SYG61v of GPV.

Table 3. Comparison of nucleotide and amino acids homology of whole genome, NS sequence, and VP sequence between OsPV HB-2019 and other waterfowl parvovirus.

Sequence name	Classic GPV	N-GPV	MDPV
Whole genome	92.36%−99.80%	92.81%−95.20%	85.21%−86.36%
NS nucleotide sequences	93.63%−99.68%	96.23%−99.36%	83.15%−83.25%
VP nucleotide sequences	94.86%−99.86%	94.63%−99.41%	80.23%−90.15%
NS amino acid sequences	96.97%−99.36%	96.17%−97.13%	89.31%−90.91%
VP amino acid sequences	95.08%−99.59%	95.90%−97.95%	91.39%−92.49%

DISCUSSION

As stated earlier, since 2019 there have been outbreaks of diseases characterized by ostrich paralysis and diarrhea in ostrich farms in many areas of the Hebei Province. In this study, we investigated the pathogen that caused this disease in ostrich farms and detected OsPV in the samples collected from the diseased ostriches. Tests for other viral agents, such as AIV, APMV-1, AEV, ARV, and MS were negative, which is consistent with the findings of a previous study (Yuan et al., 2020). The vaccine generated by inactivating GPV can prevent and treat ostriches to a certain extent, therefore, we speculate that ostrich parvovirus (OsPV) was the pathogen causing ostrich diarrhea and paralysis; however, this needs further investigation. OsPV possesses the highest genome homology to classical GPV; therefore, goose embryos were used for further passaging. Interestingly, OsPV can propagate in goose embryos; however, the viral load was extremely low, which may be related to the non-SPF goose embryos which carrying maternal antibodies. Based on the clinical symptoms, histopathology, and distribution of viruses in various tissues, we know that OsPV has a wide range of tissue tropism. Other waterfowl parvoviruses, such as GPV and MDPV, are mainly distributed in the spleen and intestines after infection. This may be

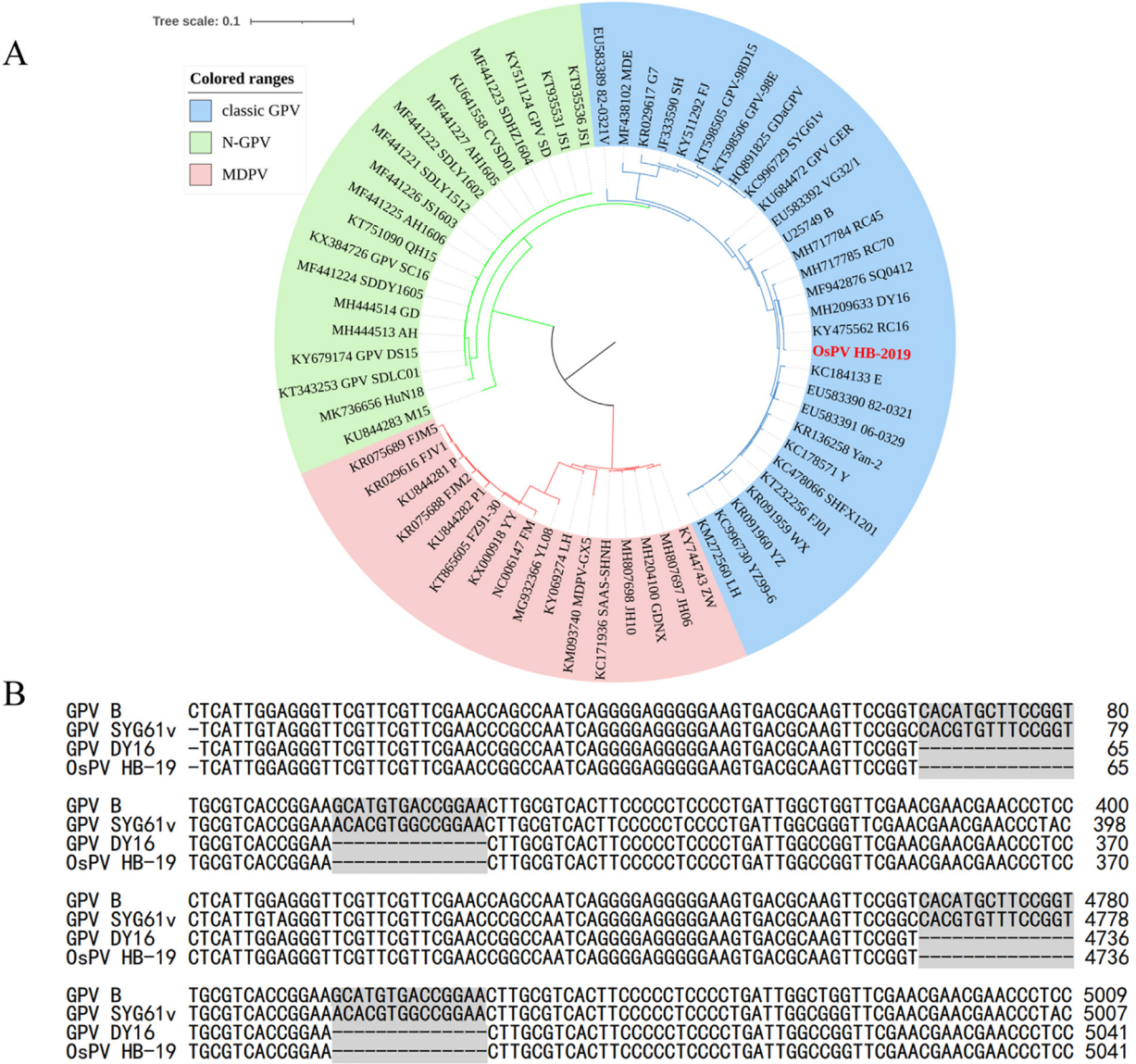

Figure 4. Phylogenetic analysis of OsPV HB-2019 (red font) and other waterfowl parvovirus isolates available in GenBank database based on the complete genomic sequences. MDPV in this study is indicated with a pink background, classic GPV is indicated with a blue background, and N-GPV is indicated with a green background. Reference sequences obtained from GenBank are indicated by their accession numbers and strain names. The neighbor-joining method in MEGA 5.0 was used for the construction of phylogenetic tree (A). Sequence alignments of the ITRs of the OsPV HB-2019 and GPVs (SYG61v, DY16, and B strain). The shaded part indicates the deletion locations (B).

the main reason ostrich parvoviruses cause multiple organ damage in ostriches. In addition, the virus can be detected in ostrich eggs (data not shown), indicating that the virus has the characteristics of vertical transmission.

The whole genome sequence of OsPV HB-2019 consisted of 5,046 nt, and the phylogenetic tree showed that it was closely related to GPV isolates, with 92.36 to 99.80% similarity. The nucleotide sequence similarity was highest with the GPV DY16 strain, a classical virulent GPV strain (Wang et al., 2019). In addition, OsPV HB-2019 had the same deletion as the GPV DY16 strain. According to previous reports, GPV only infects geese and Muscovy ducks, but parvoviruses have a wide host range (Li et al., 2018). However, the factors that cause cross-species transmission of this virus are unknown. Interestingly, the genome of OsPV possesses 2 deletions at the ITR compared to the GPV vaccine SYG61v and the virulent B strains (Figure 4B). The ITR not only functions as an origin of genome replication but also contains several transcription factor binding sites, including E-box, activating transcription factor/cyclic AMP-responsive element binding (ATF/CREB), and an upstream stimulatory factor known as MLTF (Yuan et al., 2020). The deletion sequence in the ITR may be one of the reasons why the GPV infects ostriches. Genetic recombination evidently plays an important role in parvovirus evolution, and several previous studies have provided evidence of natural genetic recombination in waterfowl parvoviruses (Ohshima and Mochizuki, 2009; Zhu et al., 2014; Liu et al., 2020). The resultant recombinant viruses may escape the immunization response elicited by outdated vaccine strains, induce virulence, and eventually lead to cross-species transmission. A previous study confirmed that the recombinant MDPV remains pathogenic to Muscovy

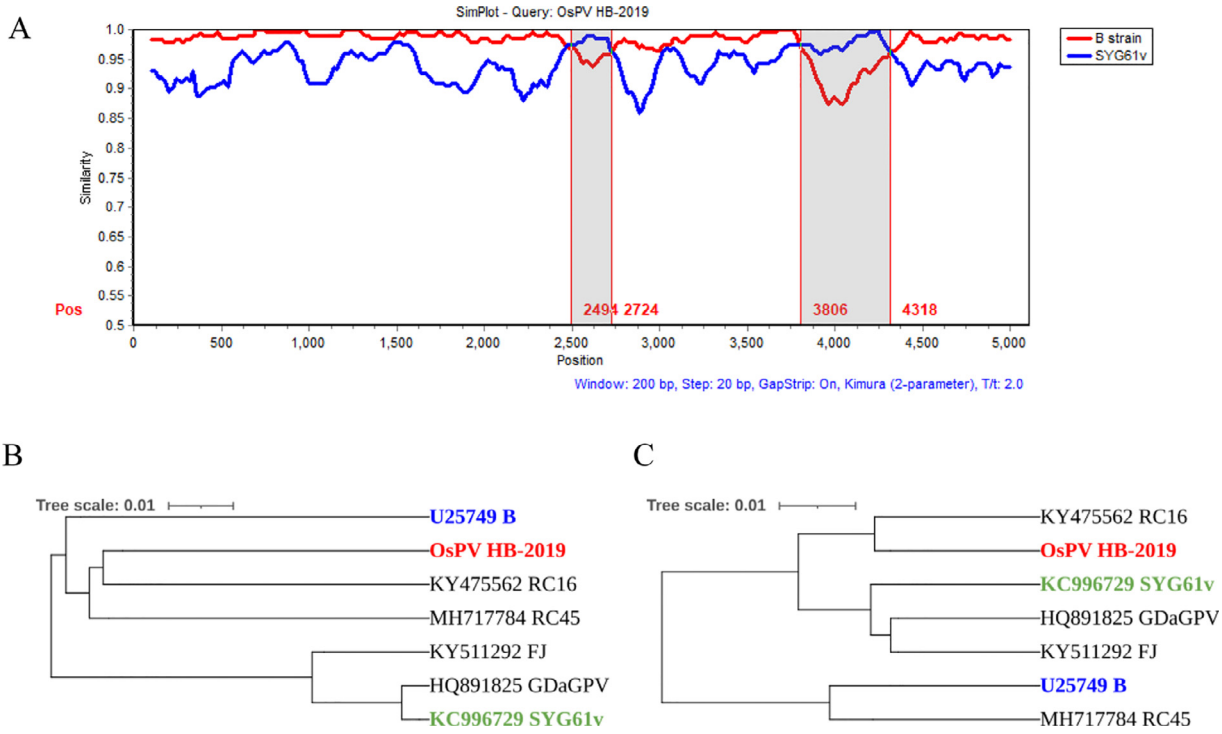

Figure 5. Genome recombination analysis of the OsPV HB-2019 strain. (A) Recombination analysis was calculated using the Simplot 3.5.1 software. The complete genome of OsPV HB-2019 was chosen as the query sequence. Blue line: SYG61v, red line: B strain. Recombination breakpoints are shown with red lines, and the locations are shown at the bottom. The background color of the major parental regions is white, whereas that of the minor parental regions is gray. Y-axis: Similarity score, X-axis: nucleotide position. Phylogenies of the major parental and minor parental regions are shown below the similarity plot (B, C).

ducks that are older than 19 d and is also associated with high morbidity and mortality rates and intestinal embolism (Chen et al., 2002; Zhu et al., 2014). In addition, different GPV strains can recombine, resulting in a new genotype among GPV strains (Wang et al., 2015). In our study, 2 recombination events were detected using RDP4 and Simplot in the OsPV HB-2019 strain. The VP genes of OsPV from 2,470 nt−2,728 nt to 3,839 nt −4,338 nt showed the highest similarity with the corresponding gene in the vaccine strain SYG61v, whereas the other regions of the genome showed the highest similarity with those of virulent B strain GPV. Recombination of multiple viruses can cause cross-species transmission, such as circovirus in pigs and porcine deltacoronavirus (Li et al., 2019; Ye et al., 2020). The GPV is a waterfowl virus. Although it has a wide range of host tropism, it is currently mainly limited to waterfowl and other animals. Therefore, how GPV breaks through the interspecies barrier and infects ostriches still remains unclear. Our data revealed that OsPV HB-2019 was a GPV recombination strain, therefore, we speculate that recombination may be one of the main reasons for its cross-species transmission; however, it needs to be further investigated using a reverse genetic system and animal experiments.

In conclusion, parvoviruses, originating from ostriches, causing symptoms of paralysis and diarrhea were detected. This virus is characterized by a wide range of tissue tropism, and the full-length genome of the original ostrich parvovirus, OsPV HB-2019 strain, was sequenced. Phylogenetic tree and sequence alignment results confirmed that OsPV HB-2019 had the highest nucleotide sequence similarity with the GPV DY16 strain, a classical virulent GPV strain. Compared with other waterfowl parvoviruses, two 14 nucleotide deletions were located in the ITR. In addition, the OsPV was a recombination virus, and it was the first to provide evidence of genetic recombination events between GPV, which may be associated with cross-species transmission from goose to ostrich. Therefore, it is necessary to monitor the recombination of parvoviruses from different species, which will contribute to preventing cross-species transmission.

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Ethical statement: Animal experiments in this study were approved by the Animal Welfare and Ethics Committee at Laboratory animal center of HeBei Agriculture University (Approve Number 2020025).

DISCLOSURES

The authors declare no potential conflicts of interest with respect to the research, authorship and publication of this article.

SUPPLEMENTARY MATERIALS

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