Isolation and characterization of a duck reovirus strain from mature ducks in China

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ABSTRACT In 2018, a disease characterized by splenic hemorrhage and necrosis killed ducks in a duck farm in Guangxi province, China. A duck reovirus strain was isolated from the tissues of the dead ducks by inoculating duck embryos and BHK-21 cells. Electron microscopy of the cultured the isolate showed that the viral particles were nearly round in shape and approximately 70 nm in diameter, and they were designated DRV-GL18. Sequence analysis showed that the GL18 strain viral genome was 23,419 nt in length and had 10 dsRNA segments. Phylogenetic analysis of cDNA amplicons of segments encoding the protein σC which are outer capsid proteins showed that the isolate belongs to the branch of the epidemic strains of duck reovirus. The Recombination Detection Program (**RDP**) and SimPlot program analyses suggested potential genetic recombination events in the M2 segments. Pathogenicity experiments revealed that GL18 produced severe hemorrhaging in livers and necrosis in the spleen of infected SPF ducklings. A death rate of 50% in the experimental ducklings was calculated during the first 7 d, and the rest of the ducklings were observed to undergo spleen necrosis. These data suggested that GL18 is a duck reovirus isolate with severer pathogenicity, and it could be a candidate for development of vaccine. This is the first reported isolation of duck reovirus from mature ducks.

Key words: duck reovirus, phylogenetic analysis, spleen necrosis

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INTRODUCTION

Duck reovirus (**DRV**) is a member of genus *Orthoreovirus* in the family *Reoviridae*, an important poultry pathogen in ducks and causes serious economic loss in the duck breeding industry. DRV, which can infect Cherry Valley duck, Pekin duck, Muscovy duck and other fowls normally (Liu et al., 2011 ; Li et al., 2016; Chen et al., 2019); avian reovirus (**ARV**), which isolated from chicken (Tang et al., 2016); and muscovy duck reovirus (MDRV), which infect Muscovy duck (Yun et al., 2013) , belong to the species *Avian orthoreovirus*. Similar to ARV, DRV also contains 10 double-stranded (ds) RNA genome segments that are divided into 3 size classes by sodium dodecylsulfate-polyacrylamide gel electrophoresis: small segments (S1−S4), medium segments (M1−M3) and large segments (L1−L3) (Benavente and Martínez-Costas, 2007; Liu et al., 2011; Wang et al., 2020) . The viral genomes encode 8 structural proteins (λA, λB, λC, μA, μB, σA, σB, and σC) and 3 or 4 nonstructural proteins (μNS, σNS, p10, and p18). The S1 gene of DRV codes p10, p18, and σC. The remaining genetic segments each contain only one open reading frame (**ORF**) that encodes one protein (Hellal Kort et al., 2013; Du et al., 2020; Wang et al., 2020).

In 1997, the first report indicated that reoviruses were isolated from Muscovy ducks with swollen lesions and hemorrhagic spleen and liver with white necrofic foci in Fujian Province, China. Then, outbreaks of a disease known as "Duck spleen necrosis diseases" (**DSND**) were characterized by spleen necrosis and liver hemorrhage in Pekin ducks that have been reported constantly since 2006 in China, disease agent was confirmed to be orthoreovirus which called duck reovirus (**DRV**), and it resulted in 10% to 15% mortality in 5-day-old ducklings (Liu et al., 2011). According to research of Chen et al. (2012), the DRV-TH11 strain caused 40% mortality in ducks of various age with serious and large necrotic foci in the spleens. In 2016, the DRV-Ych strain caused an outbreak of disease in Shandong Province, and it was characterized by intestinal hemorrhage with

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approximately 5% mortality (Cao et al., 2019). Since then, DRV outbreaks have also been reported in other Chinese provinces, showing that different DRV isolated strains have different pathogenicities (Li et al., 2016a; Wang et al., 2020; Zheng et al., 2016).

In recent years, with the development of the poultry industry in China, outbreaks of ARVs have been reported continually. ARVs spread more widely, and the host of infection is also charging and becoming more multifarious. Based on nucleotide sequences of the σC gene, ARVs can be divided into 3 subgroups: ARV, MDRV and DRV. Different ARV subgroups have differences in pathogenicity. Compared with ARV and MDRV, DRV has more widespread pathogenicity and can infect many kinds of ducks (including Pekin ducks and Muscovy ducks), chickens, geese and birds (Jones, 2000; Palya et al., 2003; Li et al., 2016b; Yan et al., 2021). DSND plays an important role in the current duck industry, and DRV has become one of the most common agents of duck diseases since 2006.

Compared with previous reports, a suspected DRV outbreak that can be fatal to adult ducks, occurred at a duck farm in Guangxi Province, China. Pathological examination showed swelling, hemorrhage and necrosis of the liver and spleen. In this study, a new strain of DRV was isolated from the tissues of dead ducks. We conducted a series of assays including viral isolation, phylogenetic analysis and pathogenicity experiments, to analyze the viral molecular features and biological characteristics.

MATERIALS AND METHODS

Tissues and Cells

Samples (liver, spleen, lung, etc) are gathered from ducks farm commercially for the purpose of eggs production. It takes 80 to 100 ducks per 1,000 square meters of farmland, in Guangxi Province in 2018. The infected ducks displayed depression and reluctant activities. The average mortality and morbidity rates were 0.5% and 5%. Gross lesions of yellowish-white focal necroses were observed in the spleen. BHK-21 cells were purchased from ATCC and maintained in our laboratory.

Animals and Ethics Statement

Zero-day-old SPF duck eggs were purchased from the National Laboratory Poultry Animal Resource Center, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences (**CAAS**) and hatched in our laboratory. The animal experiments in this study were authorized and the protocol was approved by the Animal Ethics and Animals Welfare Committee of Shanghai Veterinary Research Institute, CAAS (project identification code. SVLAC/XM-A-21003).

Viral Nucleic Acid Detection

DNA and RNA of the collected tissues were extracted using Tissue DNA Kit (OMEGA, Georgia) and TRIzol reagent (Invitrogen), respectively. PCR/RT-PCR for the detection of duck adenovirus (**DAdV**), muscovy duck parvovirus (**MDPV**), duck plague virus (**DPV**), duck circovirus (**DuCV**), DRV, newcastle disease virus (**NDV**), avian influenza virus (**AIV**), and duck hepatitis virus-1 (**DHV-1**) was performed using the primers listed in Table 1. M-MLV reverse transcriptase (Promega, Madison) was used to synthesize cDNA with RNA as template. The amplified PCR products were sequenced by Tsingke biotechnology Co, Ltd (Beijing, China).

Table 1. Primer sequence of the viruses for detection.

Name	Sequence of primers (5'→3')	Size (bp)	References
DAdV-F	CACCTCAACAAGGAGCACAA	614	This study
DAdV-R	CATTCCCTTTAGCTCCGACT		
MDPV-F	ACTCTGACTGAGACTCGATAGTG	659	This study
MDPV-R	GATCTTCTGTCCACTTGCTTCT		
DPV-F	TGCAACGAGGAGAGTTATTG	516	This study
DPV-R	GTGCATGAGGCATTTAGAAC		
DuCV-F	TTGAGGAGTCGCTGGGAGGA	410	This study
DuCV-R	GACGACTACGTCATTTCCCG		
NDV-F	CTTGCAGCTGCAGGGATTGT	460	Fan (2016)
NDV-R	GCATCTTCCCAACTGCCACT		
AIV-F	TTCTAACCGAGGTCGAAAC	220	Fan (2016)
AIV-R	AAGCGTCTACGCTGCAGTCC		
DHV-1-F	GGTGATTCTAACCAGTTAGG	715	This study
DHV-1-R	TTCAATTTCCAGATTGAGTTC		
DRV-F	ATGGATCGCAACGAGGTGATAC	320	This study
DRV-R	GAGGATAGCTCGGACCGGACAT		
σC-F	ATGGATCGCAACGAGGTGATACG	966	This study
σC-R	CTAGCCCGTGGCGACGGTGAA		
σC-qPCR-F	GGAGTCGTCTCACTCCAAGC	189	Ding et al. (2016)
σC-qPCR-R	TTTGCGAAGACATGAGCAAC		

Virus Isolation and Characterization

Virus Isolation The collected samples were homogenized with sterile phosphate-buffered saline (**PBS**). After centrifugation at 6,000, 9,000, and 12,000r for 10 min at 4°C, the samples were successively centrifuged. The supernatant was filtered with a 0.22 μm filter. The filtered supernatant was inoculated into 10-day-old duck embryos through the allantoic cavity route and into BHK-21 cells. BHK-21 cells cultured in T25 flasks were inoculated with 1 mL of the virus supernatant for 1.5 h, and then replaced and maintained in DMEM supplemented with 2% fetal bovine serum (**FBS**) at 37°C with 5%CO_2, and cytopathic effects (**CPE**s) were observed daily (Liu et al., 2011; Yun et al., 2013; Wang et al., 2019). Cell cultures were harvested and lysed by freezing-thawing for 3 times when the cells were incubated with virus for 120 h. The frozen and thawed supernatant was blindly passaged 5 times in BHK-21 cells. The virus isolated strain was harvested for subsequent experiments.

Electron Microscopy

The BHK-21-adapted virus supernatant was purified by differential centrifugation to acquire virions. First, the supernatant was clarified by centrifugation at different speeds (3,000, 6,000, 9,000, and 1,2000 r) for 30 min at 4°C to remove the cellular debris. Second, the clarified supernatant was ultra-centrifuged for 6 h at 30,000 r at 4°C and then the precipitated virions were resuspended by cold PBS. Finally, the purified virus particles were negatively stained with phosphotungstic acid for transmission electron microscopy (**TEM**) examination (Yun et al., 2013).

RT-PCR

The cell-virus culture supernatant was harvested for RT-PCR assays to detect viral nucleic acids to identify and exclude pathogens by using the primes of *DRV-F/R* (listed in Table 1). The PCR assay contained 1 μL of each of the forward and reverse primers, 10 μL of 2 × Taq Master Mix (Vazyme, Nanjing, China), 6 μL of ddH_2O, and 2 μL of cDNA.

Indirect Immunofluorescence Assay

To detect viral antigen-DRV σC proteins, BHK-21 cells were cultured in 6-well plates until the cell monolayers reached approximately 90% confluency, the medium was removed and the cells were rinsed thrice with PBS. A well of cells was infected with 0.5 mL virus supernatant and incubated at 37°C for 1.5 h, and another well was incubated with 0.5 mL DMEM without FBS at the same time as the negative control group. At 24 hpi, the cells were fixed with precooled 4% paraformaldehyde at room temperature for 10 min, and then incubated with 0.3% Triton X-100 in PBS at room temperature. After incubation with 5% skim milk at 37°C for 1 h, the cells were subsequently incubated with the primary antibody which was a monoclonal antibody (**mAb**) against DRV σC proteins (Our laboratory kept, 1:1000 v/v) at 37°C for 1 h, followed by incubation with goat anti mouse lgG secondary antibody (Invitrogen, 1:2000 v/v). Finally, cells were incubated with 1 μg/mL DAPI (Beyotime, China). Cells were examined by using a confocal laser microscopy (Zeiss, Germany) (Wu et al., 2018; Shi et al., 2022).

Western Blot Assay

Performing the same operations as Section 2.3.4, cells were inoculated with virus supernatant and medium, respectively. At 24 hpi, cells lysed with RIPA lysis buffer (50 mM Tris, Ph7.4, 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1%SDS, sodium orthovanadate, leupeptin, [beyotime, China]) were collected for Western blot assays to detect DRV σC proteins and β-actin proteins. The supernatant of lysates were subjected to 12% SDS-PAGE gels and transferred to nitrocellulose membrane. The membranes using mAb against DRV σC (described previously, 1:1000 v/v) and mAb against β-actin (Beyotime, China, 1:1000 v/v) as primary antibodies and HRP-conjugated goat anti mouse IgG (CST, Danvers, MA, 1:10000 v/v) as a secondary antibody. Proteins were visualized with ECL (Thermo Fisher, Waltham, MA) on a chemilumniescene image analysis system (Tanon, China) (Sklan et al., 2007; Wu et al., 2018).

Viral Genome Sequencing and Analysis

To acquire the full-length genome of GL18, RT-PCR was amplified with 10 pairs of primers (Table 2) based on the genomic sequence of the TH11 strain which is maintained by our lab. RT-PCR contained 2 μL of each

Table 2. Primers for the amplification of complete genome sequences.

Gene	Name	Sequences of primers (5'→3')	Reference
S1	*S1-F*	GCTTTTTTCTTCTCTGCCCATG	This study
	S1-R	GATGAATAGCTCTTCTCATCG	
S2	*S2-F*	GCTTTTTCTTCCACGATGGCGC	This study
	S2-R	GATGAGTACGTCCACGTGCTG	
S3	*S3-F*	GCTTTTTGAGTCCTCAGCGTG	This study
	S3-R	GATGAATAGGCGAGTCCCGC	
S4	*S4-F*	GCTTTTTGAGTCCTTGTGC	This study
	S4-R	GATGAATAAGAGTCCAAG	
M1	*M1-F*	GCTTTTCTCGACATGGCCTATC	This study
	M1-R	GATGAGTAACCGAGTCCGCCG	
M2	*M2-F*	GCTTTTTGAGTGCTAACCTTTC	This study
	M2-R	GATGAGTAACGTGCTAACCCAG	
M3	*M3-F*	GCTTTTTGAGTCCTAGCGTGG	This study
	M3-R	GATGAGTAACCGAGTCCGCCG	
L1	*L1-F*	GCTTTTTCTCCGAACGCCGAAATG	This study
	L1-R	GATGAGTAACCTCCAACGAGAG	
L2	*L2-F*	GCTTTTTCCTCACCATGCATG	This study
	L2-R	GATGAGTAACACCCTTCTAC	
L3	*L3-F*	GCTTTTCACCCATGGCTCAG	This study
	L3-R	GATGAGTAACACCCTTCTACTGG	

of the forward and reverse primers, 25 μL of 2 × Phanta Max Master Mix (Vazyme, Nanjing, China), 18 μL of ddH_2O, and 3 μL of cDNA. Amplifications were performed at 95°C for 3 min, followed by 33 cycles of 95°C for 30 s, 55°C (according to the Tm value of primers), 72°C for 1.5 min (S segments for 1.5 min, M segments for 2.5 min, L segments for 3.5 min), and final extension at 72°C for 10 min. The PCR products were isolated by agarose gel electrophoresis and purified using a FastPure Gel DNA Extraction Mini Kit (Vazyme, Nanjing, China). Purified products were cloned into pEASY-Blunt Zero Vector (Vazyme, Nanjing, China) and transformed into Tran5α Chemically Competent Cell (TransGen, Beijing, China). Plasmid DNA was sequenced by Tsingke Biotechnology Co, Ltd (Beijing, China).

The nucleic acid sequence identity was analyzed by BLAST in GenBank. A phylogenetic tree was constructed by MEGA 7.0.26 with the maximum-likelihood method by 500 bootstrap replicates. To detect any potential recombination events, the whole genome of strain DRV-GL18 was screened for 7 different algorithms (RDP, GENECONV, Bootscan, MaxChi, Chimera, SiScan and 3Seq) by using the Recombination Detection Program (RDP version 4.46) . Then, the potential recombination events were identified by Bootscan analysis (SimPlot version 3.5.1).

Pathogenicity of GL18 in SPF Ducklings

Twenty 0-day-old SPF duck embryos were placed in incubator at 37°C for 28 d until hatching. Thirteen ducklings hatched eventually and were divided into 2 groups: 5 duckings were raised as negative control, 8 ducklings were orally and subcutaneously infected with 0.5 mL 10^5 EID_{50}/mL allantoic fluid of the GL18 strain. In addition, 1-day-old ducklings were raised on negative pressured isolators that implement weaning automatic water and free food intake. Clinical signs were observed 3 times a day and recorded for 14 d to evaluate the pathogenicity of the isolate strain in ducklings. Dead ducklings were examined for pathogenic lesions when duckings were dying. All surviving ducklings were euthanatized and anatomized. Tissues were collected and stored at −80°C.

Histopathology Assay

Fresh tissues were collected from the infected ducklings and fixed with 4% paraformaldehyde. Histological sections were routinely prepared after paraffin embedding. The paraffin sections were prepared for hematoxylin and eosin (**H&E**) staining. Histopathological changes in the tissues were observed under an optical microscope.

Immunohistochemistry Assay

Immunohistochemistry assays were performed on the sides of the liver, spleen and lung using DRV σC mAb as the primary antibody and HRP-conjugated goat anti-mouse IgG as the secondary antibody (as described previously). Sections were heated at 65°C to melt paraffin and after antigen retrieval and 3% hydrogen peroxide treatment, using 5%BSA to block antigen. The primary antibody (1:500 v/v) was added at 4°C overnight incubation and the secondary antibody (1:5000 v/v) was added at 37°C for 1 h. Followed by DAB staining (Solarbio, China), and Slides were stained by hematoxylin (Marcano et al., 2021).

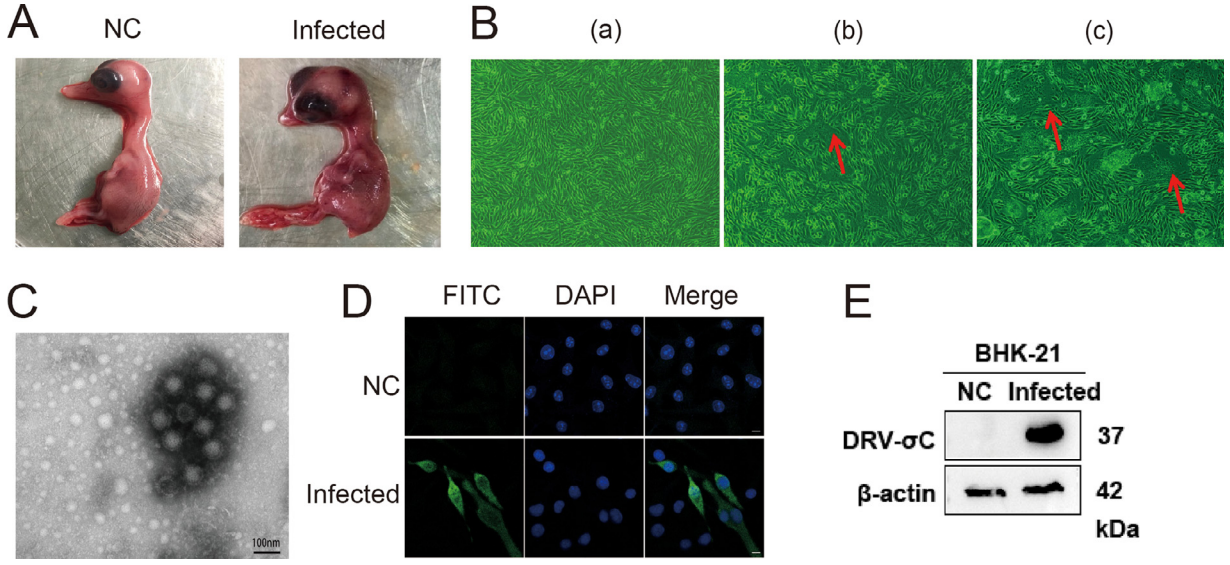

Figure 1. Isolation of DRV from clinical tissue samples of dead ducks. (A) Lesion of duck embryo inoculated with the sample. (B) CPE of infected BHK-21: syncytium as the arrow pointed out. (A) Normal BHK-21; (B) BH8K-21 infected the seventh culture at 24 hpi; (C) BHK-21 infected the seventh culture at 24 hpi. (100 ×) (C) Electron microscopy of the isolate. (D) Detection of DRV-σC expression in BHK-21 by IFA assay. (E) Western blot analysis of BHK-21 infected with the isolate.

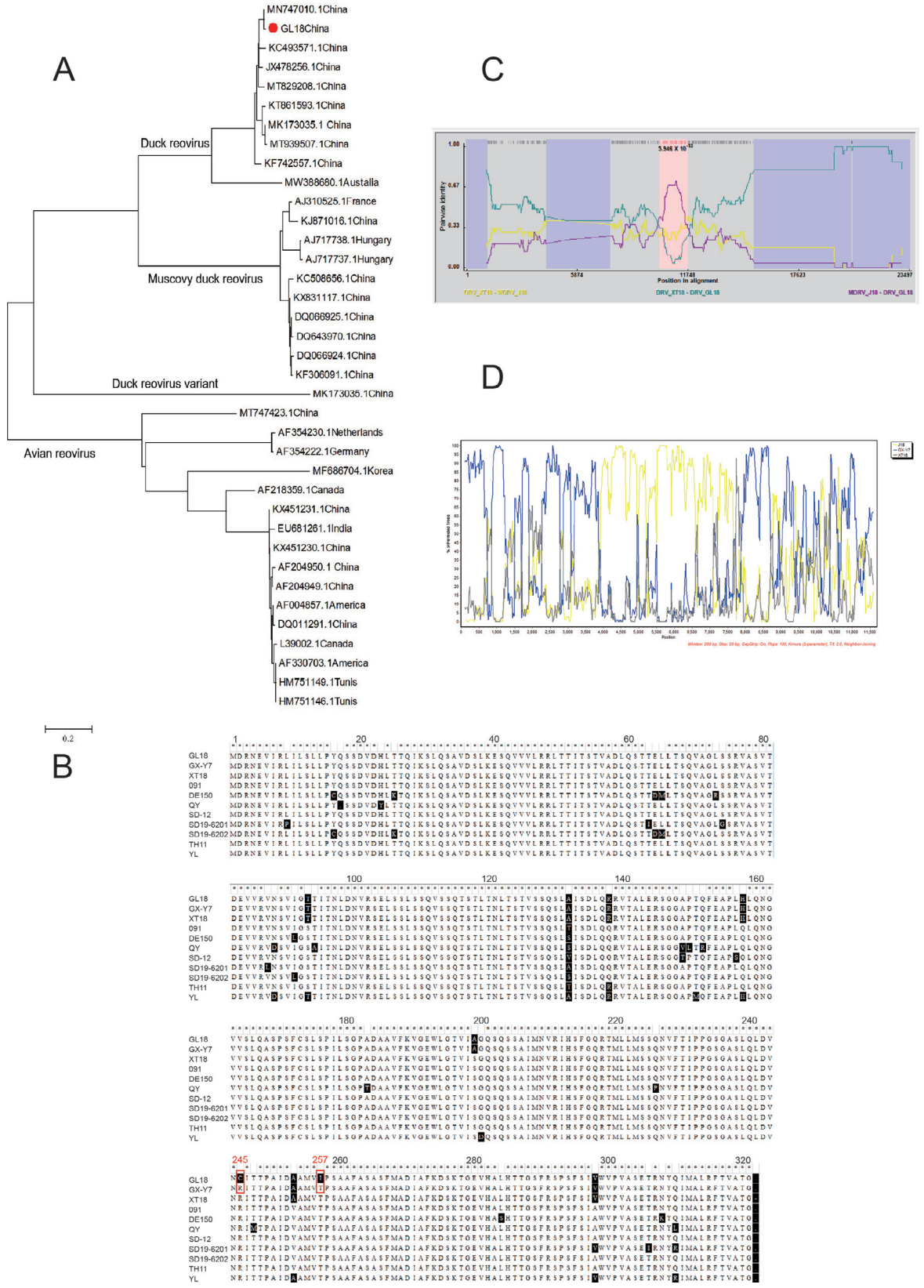

Figure 2. Sequence and analysis of the entire GL18 strain genome. (A) Phylogenetic analysis of σC gene sequences of ARVs isolates with the maximum-likelihood method. (B) Amino acid sequence alignment of the σC protein of GL18 with Chinese DRV isolates. (C) Gene recombination event prediction by RDP. (D) Verification of gene recombination by Simplot.

qPCR Assay

The qPCR assay was preformed to quantify the viral load of tissues and the primers were designed with on DRV σC gene sequence as template, named *σC-qPCR-F* and *σC-qPCR-R* (sequences presented in Table 2). The standard plasmid pMD19T-σC was stored in our lab.

Table 3. Virus strains with the most similarity of nucleotide sequences.

Segments	Segment length (bp)	Virus with most genic similarity	Similarity	Accession numbers
S1	1−1568	DRV-GX-Y7	99%	MN747010.1
S2	1−1324	DRV-GX-Y7	99%	MN747011.1
		DRV-XT18	99%	MK749404.1
S3	1−1202	DRV-GX-Y7	99%	MN747012.1
S4	1−1192	DRV-GX-Y7	99%	MN747013.1
M1	1−2284	DRV-GX-Y7	99%	MN747007.1
		DRV-XT18	99%	MK749401.1
M2	1−2158	DRV-GX-Y7	99%	MN747008.1
		DRV-SY	99%	MK955822.1
M3	1−1996	DRV-TH11	99%	JX440512.1
		DRV-091	99%	JX478255.1
L1	1−3359	DRV-GX-Y7	99%	MN747004.1
		DRV-SH12	99%	MH510245.1
L2	1−3830	DRV-TH11	99%	KC493573.1
		DRV-HN5d	99%	KT861588.1
L3	1−3906	DRV-GX-Y7	99%	MN747006.1

RESULTS

Viral Nucleic Acids Detection

Three dead ducks we obtained and the tissues (liver, spleen, lung, etc) of the same duck were mixed as a sample. PCR/RT-PCR was performed by nucleic acids of extracted the tissues, and we determined that three samples all only contained DRV, without DAdV, MDPV, DPV, DuCV, NDV, AIV, and DHV-1. Then, we selected one of the three samples which showed strong positive sample for follow-up experiments.

Viral Isolation and Characterization

To isolate the virus from the duckling tissues, the mixed tissues homogenate was inoculated into 10-day-old duck embryos and blindly passaged 5 times. Hundred percent mortality was observed for inoculated duck embryos, excluding mechanical death within 24 h. The inoculated embryos were concentrated at 2 to 4 dpi and systemic hemorrhage with scattered hemorrhagic spots were observed (Figure 1A). We infected BHK-21 cells with the supernatant of mixed tissue homogenate and blindly passaged 5 times, but only a few small syncytia were observed. Subsequently, we detected the first to fifth generation of cell cultures for DRV by RT-PCR and all were DRV-positive. This result suggested that this DRV strain could replicate on BHK-21 cells. After seven successive passages, syncytium was observed at 24 hpi. More syncytium, cells aggregation and cells falling from the surface of the flask, were observed at 48 hpi (Figure 1B).

TEM observations showed that the virions were nearly round in shape with a diameter of approximately 70 nm (Figure 1C), similar to virions in DRV-XT18 strain (Wang et al., 2019). We used DRV σC mAb protein of DRV as described previously, to detect infected cells if they contained DRV by indirect immunofluorescent assay and Western blot assay. Both results indicated that the σC protein was expressed in the infected BHK-21 cells (Figure 1D, E). According to the result of IFA assay, we observed that the DRVσC protein was distributed in the cytoplasm of infected BHK-21 cells, it is consistent with previous reference reported (Ding et al., 2016). Therefore the isolate we designed was DRV-GL18 (GL18 hereafter).

Genomic Amplification and Analysis of the GL18 Isolate

Sequencing data showed that the viral RNA was 23,419 bp in full length with 10 segments that were divided into small segments (S1, S2, S3, S4), medium segments (M1, M2, M3) and large segments (L1, L2, L3) from 1191 bp (S1) to 3958 bp (L1). (GenBank accession numbers MW924628−MW924637) (Supplementary Table 1). Therein S1 segment is polycistronic which encodes nonstructural proteins p10, p18 and structural protein σC among 10 segments, the others segments only encode one protein, respectively.

We constructed a phylogenetic tree based on the nucleotide sequences encoding for protein σC and determined the evolutionary relationship of GL18 with other avian reoviruses. GL18 was in the duck reovirus branch which is a branch of avian reoviruses (Figure 2A), and it is close to duck reoviruses which reported recently in China, including MN747010.1 (DRX/GX-Y7) (Wang et al., 2020), KC493571.1 (DRV-TH11) (Chen et al., 2012; Zhu et al.), JX478256.1 (DRV-091) (Ma et al., 2012), and KT861593.1 (DRV-HN5d) (Zheng et al., 2016), the percentage of identity was 99.69%, 96.38%, 97.10%, 95.12%, respectively. GL18 has the most homology with the DRV/GX-Y7 isolate in phylogenetic analysis (Table 3). Amino acid sequence alignment of σC protein showed that 2 amino acids were different between the DRV-GL18 strain (245C, 256I) and DRV/GX-Y7 strain (245R, 256T) (Figure 2B).

Recombination events were detected in the M2 and M3 segments of DRV-GL18 by RDP analysis (Figure 2C), and confirmed the result that M2 and M3 segments had a recombination event by SimPlot software (Figure 2D).

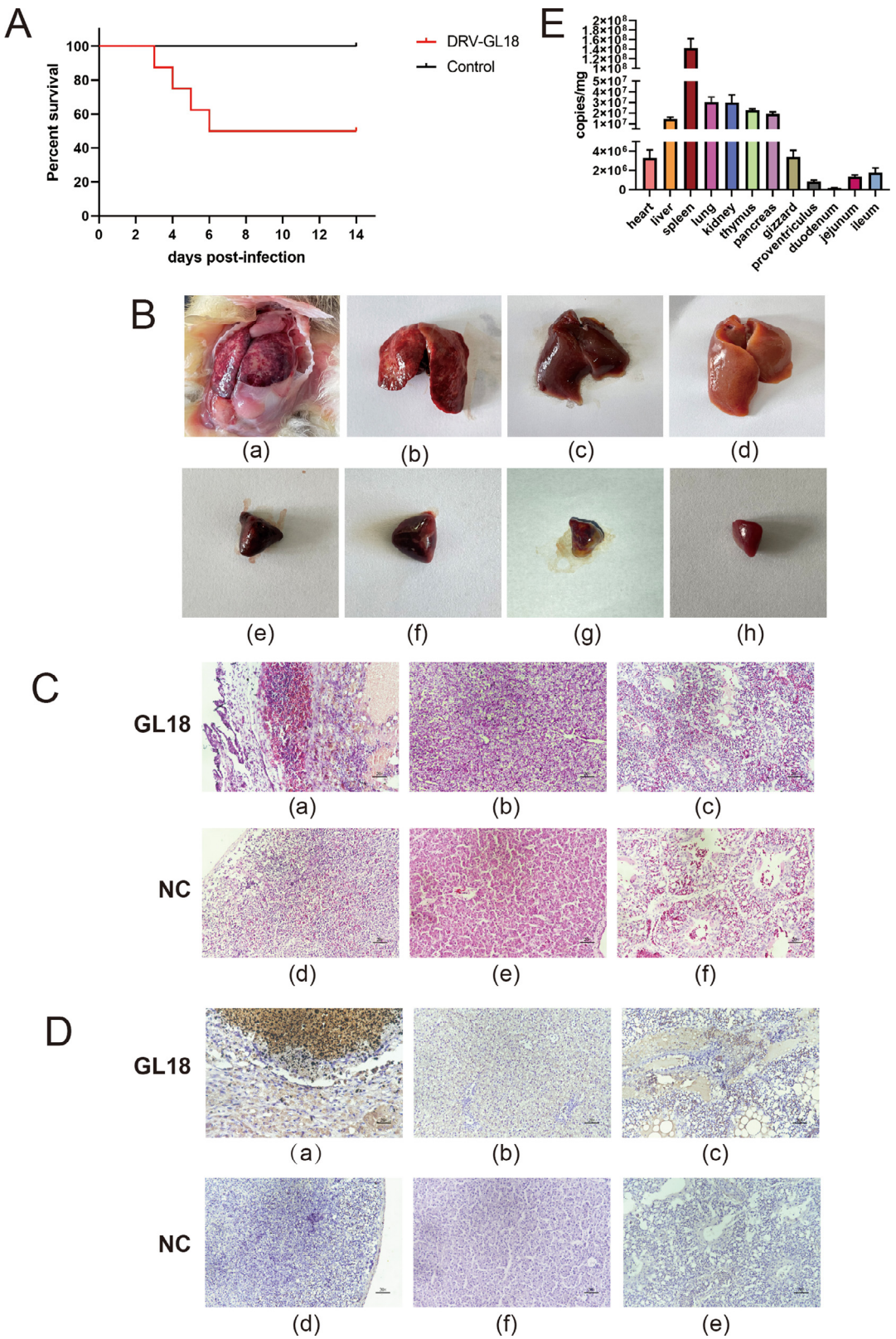

Figure 3. Experimental infection with the GL18 strain. (A) Survival curve of the infected ducklings. (B) Gross lesion of the livers and spleens from infected ducklings. (A)∼(C) Liver lesions of different degrees. (D) Normal liver. (E)−(G) Spleen necrosis with foci. (C) Histopathology examination of the GL18 infected, the spleen, liver and lung performed lesions with varying degrees. (D) IHC assay showed DRV-σC had high expression in the spleen, liver and lung. (E). Viral loads in different tissues from infected ducklings. (scale = 20 μm).

Experimental Infection With the GL18 Strain

Compared with uninfected controls, laboratory infection with GL18 resulted in obvious disease in the infected ducklings. All infected ducklings showed the most apparent clinical signs of depression and anorexia at Day 2 dpi, and death began to appear at Day 3 dpi. At Day 7 dpi, 50% of ducklings were killed, and their

tissues were collected (Figure 3A). Autopsy results of the dead ducklings showed splenomegaly hemorrhagic necrosis and liver with many yellow−white necrotic foci and hemorrhage (Figure 3B). The manifestation of surviving ducklings was slower in growth than the control group. At 14 dpi, the rest of the infected ducklings were killed and pathologically examined; the spleens were necrotic and atrophic, and the livers were hemorrhagic without foci.

The histopathological assays of infected ducklings demonstrated that the lungs had substantial lesion and hemorrhage, the liver cells were structurally disturbed with vacuolar degeneration and filled with red blood cells, and necrosis and hemosiderosis occurred in the red pulp region of the spleen.

Brownish-yellow particles were observed in sections of the infected GL18 group, and a remarkable yellow color was observed in spleen sections. These results suggested that DRV σC was expressed in these tissues of ducklings.

To calculate the viral loads of tissues, collected tissue samples (heart, liver, spleen, lung, kidney, thymus, pancreas, gizzard, proventriculus, duodenum, jejunum, and ileum) were amplified by using σC-qPCR-F/R specific primers, and the results showed that strain GL18 was distributed in all detected tissues, suggesting that strain GL18 could replicate and multiply in these tissues. The highest viral loads were detected in the spleen, followed by the lung and pancreas, thymus, kidney, liver and heart, and the viral loads of digestive and excretory organs.

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DISCUSSION

In our work, the reovirus strain GL18 was isolated from tissue samples of dead adult ducks. GL18 could be fetal to duck embryos, and the formation of syncytia and removal of cells the well flask occurred through further passage in BHK-21 cells. GL18 was confirmed to be a duck reovirus strain by phylogenetic tree analysis based on nucleotide sequences of the ARV σC gene and has high homology with the GX-Y7 isolate. In contrast, the homology of the GL18 M3 segment was more similar to TH11 than to GX-Y7. The other segments were similar to GX-Y7. Autopsy and histopathological analysis suggested that GL18 inoculation in SPF ducklings resulted in spleen necrosis as described in a previous report of DRV. Moreover, the liver had severe hemorrhagic and necrotic lesions in dead ducklings. GL18 was highly pathogenic to 1-day-old ducklings, with a death rate of 50% in our experimental study. Surviving ducklings exhibited slower growth than normal ducklings.

As a DRV outer capsid protein, the σC protein is the attachment protein of DRV. It is worth noting that the amino acid sequence alignment of the σC protein showed that two amino acids were different between the GL18 strain (245C, 256I) and GX-Y7 strain (245R, 256T). Compared with the GL18 strain, the GX-Y7 strain did not cause ducklings to die (Wang et al., 2020). These 2 amino acid sites may be important for the difference in virulence, although this hypothesis needs further research and verification.

Regrettably, our pre-experimental infection with GL18 could not reproduce the mortality in the mature ducks. It is possible that there is improper breeding management or the environment. We used 1-day-old ducklings for experimental infection by referring to the clinical incidence and other scholars' experiments (Liu et al., 2011; Zheng et al., 2016; Luo et al., 2021). GL18 could cause 50% mortality and 100% pathogenicity.

In summary, we isolated a duck reovirus (GL18) from the tissue samples of dead ducks. The isolated strain exhibits severe pathogenicity with classical lesions in 1-day-old ducklings.

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DISCLOSURES

All the authors declared that there are no conflicts of interest with regard to this manuscript.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2022.102345.

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