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Genomic characterization and pathogenicity of a novel fowl adenovirus serotype 11 isolated from chickens with inclusion body hepatitis in China

Qilong Qiao, Minghe Xu, Xiangdong Wang, Jingge Tian, Yihang Zhang, Congcong Song, Junjie Liu, Yan Li, Xingyu Li, Panpan Yang, Mingzhen Song, Yongtao Li, Zeng Wang, Baiyu Wang, and Jun Zhao[1]

College of Veterinary Medicine, Henan Agricultural University, Zhengzhou 450046, China

ABSTRACT Fowl adenovirus serotype 11 (**FAdV-11**) is one of the primary causative agents of inclusion body hepatitis (**IBH**), which causes substantial economic losses in the world poultry industry. In this study, we characterized the genome of the fowl adenovirus serotype 11 (FAdV-11) isolate FJSW/2021. The full genome of FJSW/2021 was 44, 154 base pairs (**bp**) in length and had a similar organization to that of previously reported FAdV-11 isolates. Notably, compared with those of other reported FAdV-11 strains, the preterminal protein (**pTP**) of FAdV-11 FJSW/2021 has six amino acid (aa) insertions (**S-L-R-I-I-C**) between 470 and 475 and one aa mutation of L476F; moreover, the tandem repeat (**TR**) regions of TR1 and TR2 were 33 bp (1 repeat) and 1,080 bp (8 repeats) shorter than those of the Canadian nonpathogenic isolate ON NP2, respectively. The pathogenicity of FJSW/2021 was studied in 10-day-old specific pathogen-free chicken embryos following allantoic cavity inoculation and in 1-day-old, 1-wk-old and 2-wk-old SPF chickens following intramuscular inoculation with 10^7 $TCID_{50}$ of the virus. The results showed that FJSW/2021 can induce typical severe IBH in chicks less than 2 wk old. These findings highlighted the genetic differences between the pathogenic and non-pathogenic FAdV-11 isolates. The data will provide guidance for identifying the virulence factors of FAdV-11 strains. The animal challenge model developed in our study will allow precise evaluation of the efficacy of potential FAdV-11 vaccine candidates.

Key words: fowl adenovirus serotype 11, inclusion body hepatitis, complete genome, pathogenicity

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INTRODUCTION

Fowl adenoviruses (**FAdVs**), which belong to the genus *Aviadenovirus* within the family *Adenoviridae*, are associated with a number of disease conditions, including inclusion body hepatitis (**IBH**), hepatitis-hydropericardium syndrome (**HHS**) and gizzard erosions (**GE**) (Schachner et al., 2018). Fowl adenoviruses are grouped into 5 species, FAdV-A to FAdV-E, based on genomic differences and are further divided into 12 serotypes (FAdV-1-8a and 8b-11) by cross-neutralization tests (Benkő et al., 2022). Inclusion body hepatitis is caused mainly by FAdV-2 and FAdV-11 in FAdV-D and FAdV-8a and FAdV-8b in FAdV-E (Helmboldt and Frazier, 1963; Morshed et al., 2017; Mohamed et al., 2018). Since the first report of IBH in 1963, the disease has spread worldwide. In recent years, outbreaks of IBH have exhibited an increasing trend worldwide and have caused substantial economic losses in many countries (Helmboldt and Frazier, 1963; Gomis et al., 2006; Wilson et al., 2010; Nakamura et al., 2011; Steer et al., 2015; Zhao et al., 2015; Oliver-Ferrando et al., 2017; Morshed et al., 2017; Mohamed et al., 2018; Huang et al., 2019; El-Tholoth and Abou El-Azm, 2019; Abghour et al., 2019, 2021; Cizmecigil et al., 2020; Chen et al., 2020; Hosseini et al., 2020; Mirzazadeh et al., 2020; Şahindokuyucu et al., 2020; Bertran et al., 2021; Chitradevi et al., 2021; Mase et al., 2021; Sabarudin et al., 2021; Lebdah et al., 2022; Li et al., 2022; Liu et al., 2022; Niczyporuk and Kozdruń, 2022; Xie et al., 2022). Inclusion body hepatitis is predominantly observed in broilers and is characterized by a sudden onset of mortality approaching 10 to 30% after 3 to 4 d. A higher mortality rate is observed for birds younger than 3 wk of age. The main lesions of IBH are pale, friable, and swollen livers. Small white foci can be observed on the liver, and petechial or ecchymotic hemorrhages may be present. Swollen kidneys frequently coincide with glomerulonephritis (Wilson et al., 2010; Morshed et al., 2017).

Although outbreaks of FAdV-11-related IBH have been increasingly reported in China and many other geographical areas worldwide, complete genome sequences of FAdV-11 isolates are limited. The pathogenicity of the FAdV-11 isolate has not been assessed

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[1]Corresponding author: zhaoj@henau.edu.cn

systematically. In this study, one FAdV-11 strain was isolated from a broiler flock with clinical signs of IBH, and its complete genome was sequenced. The pathogenicity of the FAdV-11 isolate was also evaluated in SPF chicken embryos and SPF chickens of different ages.

MATERIALS AND METHODS

Ethics Statement

All chicken experiments and procedures conducted in the present study were reviewed and approved by the Ethics and Animal Welfare Committee of Henan Agricultural University, China (HNND2023030301).

Sample Collection and Virus Isolation

Twenty one liver samples from dead chickens were collected from a commercial broiler farm experiencing severe IBH in Fujian Province, China. Total DNAs in the liver samples were extracted. The detection of FAdVs in the samples was performed via PCR with primers for the conserved FAdVs hexon gene region in all 5 species of FAdVs (Forward 5′-CAA RTT CAG RCA GAC GGT-3′ and Reverse 5′-TAG TGA TGM CGS GAC ATC AT-3′) according to methods described previously (Meulemans et al., 2001). The PCR products were subsequently sequenced by Sangon Biotech (Shanghai) Co., Ltd., China. Only FAdV-11-positive liver samples were selected for virus isolation. Virus isolation was conducted using chicken leghorn male hepatocellular (**LMH**) cells. The cells were cultured in DMEM/F12 medium (Thermo Fisher Scientific, MA) supplemented with 10% fetal bovine serum (**FBS**) (AusgeneX, Australia, USA), 100 IU/mL penicillin, and 100 μg/mL streptomycin at 37°C in a humidified 5% CO_2 incubator. The FAdV-11 isolate was named FJSW/2021.

Viral DNA Extraction and Sequencing

Viral DNA was extracted from the lysates of LMH cells infected with the FAdV-11 isolate using a QIAamp DNA Blood Mini Kit (Qiagen, CA) following the manufacturer's instructions and was used as a template for PCR amplification. A total of 36 primer sets, designed on the basis of the released sequences of FAdV-11 strains in GenBank, were used to amplify overlapping DNA fragments spanning the entire viral genome. The amplicons were purified and sent to Sangon Biotech (Shanghai) Co., Ltd., for sequencing. The complete genome sequence was assembled using the Seqman program of the Lasergene sequence analysis software package (DNASTAR, Inc., Madison, WI) and deposited in GenBank.

Sequence Analysis

The complete genome sequence of FJSW/2021 was aligned with other available genome sequences of FAdV reference strains (Table 1) in the GenBank database, and the percentage similarity among different FAdV-11 strains was calculated using Geneious Prime software 2023.02 (https://www.geneious.com/). The existence of homologous open reading frames (**ORF**) in other FAdV-11 strains was used to identify potential ORFs. A phylogenetic tree was constructed using MEGA 7.0 via the neighbor-joining method. A schematic of the genome organization of FJSW/2021 was generated using SnapGene software.

Pathogenicity Assessment of FAdV-11 FJSW/2021 in SPF Chicken Embryos and Chickens

Considering that FAdV-11 can be transmitted vertically and horizontally, experiments were conducted to assess the pathogenicity of FAdV-11 FJSW/2021 to 10-day-old SPF chick embryos and chickens of different ages. Ten 10-day-old SPF chick embryos were inoculated with 10^7 $TCID_{50}$ of virus via the allantoic cavity. The embryos were monitored every 24 h postinoculation (**hpi**). Ten embryos were inoculated with sterile cell culture medium as a control. The embryos were monitored

Table 1. Information on reference FAdV strains.

Strain	Serotype	Genotype	Country	GenBank	Year
HBQ12	FAdV-11	D	China	KM096545	2014
BJH13	FAdV-11	D	China	KM096546	2014
ON NP2	FAdV-11	D	Canada	KP231537	2014
ON P2	FAdV-11	D	Canada	KU310942	2014
380	FAdV-11	D	UK	KT862812	2015
LN/1507	FAdV-11	D	China	KU497449	2016
JL/1407	FAdV-11	D	China	KY012057	2016
MX95-11	FAdV-11	D	Mexico	KU746335	2016
MN509168	FAdV-11	D	Australia	MN509168	2019
GB591	FAdV-11	D	Germany	MK572868	2019
PKFAd18	FAdV-11	D	Pakistan	MN428137	2019
Iran/UT-Kiaee/2018	FAdV-11	D	Iran	MK757569	2019
685-CORR	FAdV-D	D	England	MK572874	2019
CELO	FAdV-1	A	Germany	U46933	1996
340	FAdV-5	B	Northern Ireland	KC493646	2013
ON1	FAdV-4	C	Canada	GU188428	2009
CH/HNJZ/2015	FAdV-4	C	China	KU558760	2016
HG	FAdV-8b	E	Canada	GU734104	2010

every day until 7 d postinfection (**dpi**). Ten 1-day-old, 1-wk-old and 2-wk-old White Leghorn chickens were inoculated intramuscularly with 10^7 $TCID_{50}$ of the virus. The corresponding control animals were inoculated with sterile cell culture media via the same route. All the chickens were monitored daily for 7 d. Postmortem examinations were carried out for deceased chickens during the experiments and euthanized chickens at 7 dpi. Liver and kidney tissues from embryos and chickens were collected for histopathological examination. Routinely, the tissues were fixed with 10% neutral phosphate buffered formalin, embedded in paraffin, and cut into 5 μm thick paraffin sections with a microtome. Dewaxed sections were stained with hematoxylin-eosin. The micropathological lesions were examined under the light microscope. Tissue samples from the heart, liver, spleen, lung, kidney, cecal tonsils, bursa of Fabricius, duodenum, proventriculus and pancreas of 1-wk-old and 2-wk-old chickens were collected for viral load measurement.

Viral Load Measurement

To determine the viral loads in the tissue samples, total DNA was extracted from 100 mg of tissue samples with a TIANamp Genomic DNA Extraction Kit (Tiangen, Beijing, China). The viral loads in the tissue samples were determined by SYBR Green quantitative real-time polymerase chain reaction (**qRT−PCR**) as described previously, with modifications (Grgić et al., 2013). Briefly, the ORF14 gene of FAdV-11 was cloned and inserted into the pMD-18T vector to construct a standard plasmid as an indicator for identifying the presence of the virus. The number of viral genomes was expressed as $\log_{10}$ (copy numbers/mg tissue).

Statistical Analysis

All the statistical analyses were performed using the unpaired t test within the GraphPad Prism software package. For analyses of the viral loads in tissues, tests were performed between different FAdV-11-infected and uninfected chickens. P values less than 0.05 and 0.01 were considered to indicate statistical significance and extreme significance, respectively.

RESULTS

FAdV-11 Isolation and Identification

In January 2021, 2-wk-old broilers on a chicken farm in Fujian Province suffered from severe IBH. The typical gross lesions of affected broilers included enlarged livers with pale dispersive necrosis around the edge and swollen kidneys. The mortality rate is about 60% in the affected broiler flocks. Total DNAs in the liver tissues were extracted for FAdV identification by PCR. A 900-bp specific fragment was amplified from the liver samples by using the primers for the conserved FAdVs hexon gene region in all 5 species of FAdVs. Sequencing results indicated that the liver samples were positive for FAdV-11 but negative for other avian pathogens. After inoculation with the supernatant of FAdV-11-positive liver homogenates, the infected LMH cells exhibited typical CPE, characterized by enhanced refractivity and detachment of the cultures from the flask in the form of bunches of grapes at 72 hpi to 96 hpi (Figure 1). The FAdV-11 isolate FJSW/2021 replicated efficiently in LMH cells, and the peak titer reached $10^{5.2}$ $TCID_{50}$/100 μL at 96 hpi.

Genome Organization and Phylogenetic Analysis of the FAdV-11 Isolate FJSW/2021

The complete genomic sequence of the FAdV-11 isolate FJSW/2021 was deposited in GenBank under the accession number OK336458. The whole genome of FJSW/2021 is 44,154 bp in length and has a composition of 23.4% A, 26.4% C, 26.6% G, and 22.6% T, containing a total G+C content of 53%. The genome of FJSW/2021 shares 95.46 to 99.72% nucleotide identity with other published FAdV-11 strains. FJSW/2021 has 99.72% and 99.45% similarity with the Chinese isolates HBQ12 and BJH13, respectively; 99.36% and 98.85% similarity with the Chinese isolates JL/1407 and LN/1507, respectively; 98.98% similarity with the Mexican nonpathogenic strain MX95-S11; 98.54% and 97.39% similarity with the Canadian pathogenic strain ON P2 and the nonpathogenic strain ON NP2, respectively; 97.93% similarity with the Australian pathogenic strain

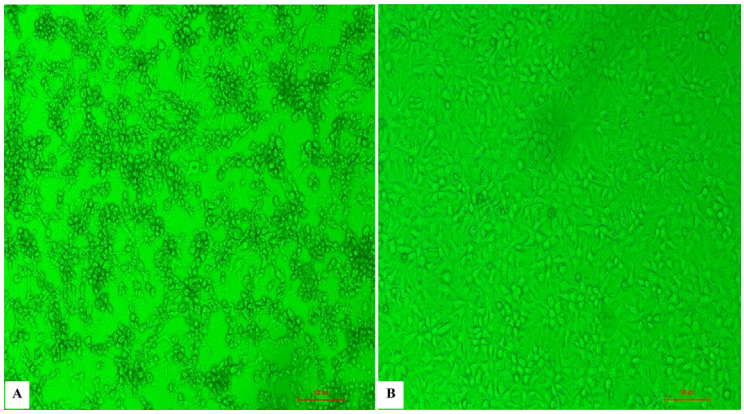

Figure 1. The typical cytopathic effects (CPEs) in LMH cells induced by the FAdV-11 isolate FJSW/2021. (A) FAdV-11 isolate FJSW/2021 infected LMH cells; (B) Uninfected LMH cells.

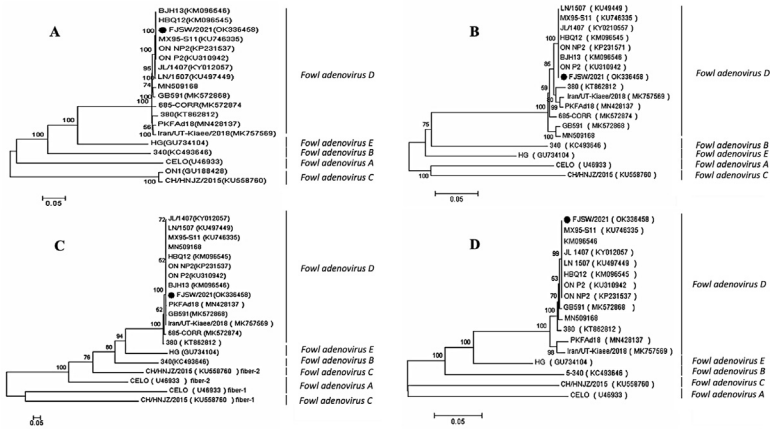

Figure 2. Phylogenetic analysis of FAdV-11 strain FJSW/2021. The phylogenetic trees were based on the nucleotide sequence of the full genome (A), and the amino acid sequences of the fiber (B), hexon (C), and penton base (D) genes.

MN509168; 97.17% similarity with the Pakistani pathogenic strain PKFAd18; 97.07% similarity with the Iranian pathogenic strain Iran/UT-Kiaee/2018; 96.48, 95.46, and 95.99% similarity with the isolate 685-CORR; and 380 from the United Kingdom and the isolate GB591 from Germany, respectively.

Phylogenetic analysis of whole-genome sequences and the amino acid sequences of hexon, fiber and penton bases revealed that FJSW/2021 is clustered together with reported FAdV-11 strains in a small group of Chinese isolates, HBQ12 and BJH13; Mexican isolate, MX95; and Canadian isolates, ON P2 and ON NP2 (Figure 2).

The gene order and putative ORFs in the FAdV-11 isolate FJSW/2021 genome are similar to those of previously reported FAdV-11 strains (Figure 3, Table 2). FJSW/2021 has a 72 bp inverted repeat (ITR) sequence at both ends, which is consistent with the published FAdV-11 genome sequence. The amino acid sequences of major proteins of FAdV-11 strains available in GenBank were aligned using the MegAlign program. The preterminal protein (pTP) of FAdV-11 FJSW/2021 has six amino acid insertions (**S-L-R-I-I-C**) between 470 and 475 and one aa mutation, L476F (Figure 4). Most of the FAdV-11 isolates had identical hexon genes, except for some amino acid mutations in the hexon genes of the Iranian/UT-Kiaee/2018, PKFAd18, MN509168 and 380 strains. The hexon of FJSW/2021 shares 99.6, 99.5, 98.7, and 98.4% amino acid identity with that of Iran/UT-Kiaee/2018, PKFAd18, MN509168 and 380, respectively. This is the same case for the fiber gene. FJSW/2021 has an identical fiber gene to most of the FAdV-11 isolates, except for the isolates of Iran/UT-Kiaee/2018, PKFAd18, MN509168 and 380. The fiber gene of FJSW/2021 shares 99.3, 99.8, 99.6, and 95.1% amino acid identity with that of Iran/UT-Kiaee/2018, PKFAd18, MN509168 and 380, respectively. Notably, there were 17 amino acid deletions (253GVAVADTLTISSNTGTVT269) in the fiber protein of the isolate PKFAd18 and 34 amino acid deletions (295PLTTSSNGLSLKLTPNGSIQSSSTGLSVQTD-PAG328) in the fiber of the isolate 380. The penton base is well conserved in all FAdV-11 isolates.

The tandem repeat regions TR1 and TR2, previously identified in some FAdV-11 isolates, are also present in FJSW/2021. Notably, the pathogenic FAdV-11 isolates had fewer TR1 and TR2 than did the nonpathogenic ON-NP2 strain, as shown in Table 3. The TR1 region of FJSW is 33 bp (1 repeat) shorter than that of the Canadian nonpathogenic isolate ON NP2. There are only 5 repeats of the 135-bp stretch in the TR2 region of FJSW/2021, which is shorter than that in the Canadian nonpathogenic isolate ON NP2 (13 repeats) and the pathogenic isolate ON P2 (8 repeats). The predicted

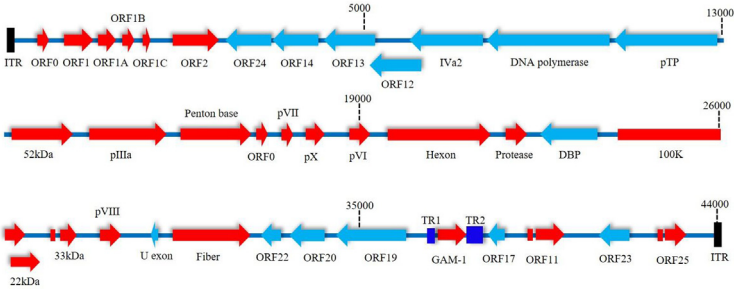

Figure 3. Schematic representatin of genome organization of FAdV-11 strain FJSW/2021.

Table 2. Putative open reading frames in the FAdV-11 isolate FJSW/2021 genome.

Gene	Strand	Location	bp	aa
ORF0	R	575-808	234	76
ORF1	R	847-1338	492	163
ORF1A	R	1339-1497	159	52
ORF1B	R	1500-1730	231	76
ORF1C	R	1735-1878	144	47
ORF2	R	1952-2755	804	267
ORF24	L	2838-3518, 16310-16315	681, 6	228
ORF14	L	3537-4223, 16310-16315	687, 6	230
ORF13	L	4263-5249, 16310-16315	987, 6	330
ORF12	L	5245-6162, 16310-16315	600	307
IVa2	L	6131-7336	1206	401
DNA polymerase	L	7333-11253	3291	1096
pTP	L	11250-13217, 16310-16315	1968, 6	657
52K	R	13275-14483	1209	402
IIIa	R	14470-16425	1956	651
Penton	R	16521-17963	1443	480
VII	R	18004-18240	237	78
X	R	18478-19077	600	199
VI	R	19205-19891	687	228
Hexon	R	20003-22855	2853	950
protease	R	22869-23486	618	205
DBP	L	23602-24915	1314	437
100K	R	25436-28606	3171	1056
22K	R	28278-28832	555	184
33K	R	28278-28617, 28862-29196	340,335	224
pVIII	R	29236-29961	726	241
U exon	R	29980-30213	234	77
Fiber	R	30212-31930	1719	572
ORF22	L	31990-32562	573	190
ORF20	L	33046-33894	849	282
ORF19	L	34279-36501	2223	740
GAM 1	R	38038-38871	834	277
ORF17	L	39693-40163	471	156
ORF11	R	40560-40920,40998-41240,41315-41493	361,243,179	260
ORF23	L	41752-42687	936	311
ORF25	L	43140-43148,43228-43728	9,501	169

22K protein, which is absent in the Chinese isolates HBQ12 and BJH13, was identified in FJSW/2021. Two codon deletions present in a glutamic acid-rich region of the 22K and 33K ORFs, equivalent to the glycine-rich region of 100K, of the nonpathogenic isolate ON NP2 were also found in the isolate 685-CORR from the United Kingdom and Pakistani pathogenic strain PKFAd18 based on the genomic sequences in GenBank. However, this mutation was not identified in the corresponding ORFs of FJSW/2021 or other pathogenic FAdV-11 isolates.

Pathogenicity of the FAdV-11 Isolate FJSW/2021

The pathogenicity of FJSW/2021 in 10-day-old SPF chick embryos and chickens of different ages was assessed. Ten-day-old SPF chick embryos were inoculated with 10^7 $TCID_{50}$ of virus via the allantoic cavity. The onset of mortality in 10-day-old SPF chicken embryos was recorded at 72 hpi. with a mortality of 100% at 168 hpi (Figure 5A). The dead embryos exhibited pale, friable, swollen livers with small white necrotic

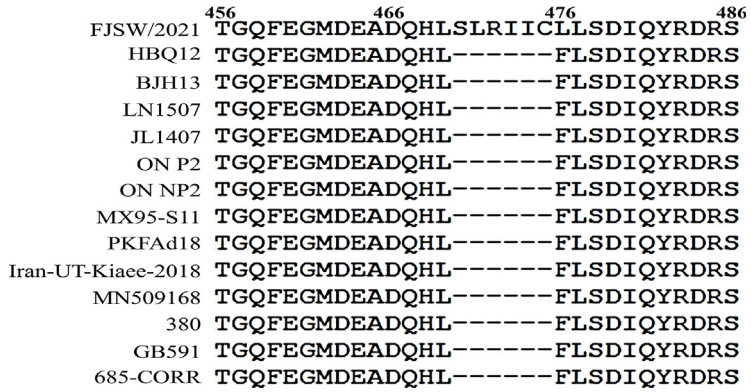

Figure 4. Novel characterization of the preterminal protein of FAdV-11 strain FJSW/2021. The preterminal protein (pTP) of FAdV-11 FJSW/2021 has six amino acid insertions (**S-L-R-I-I-C**) between 470 and 475 and one aa mutation, L476F.

Table 3. Comparison of the tandem repeat region of FAdV-11 isolates.

Isolate	No. of TR		Pathogenicity[1]	Origin
	TR1	TR2		
HBQ12	3	7	+	China
FJSW/2021	3	5	+	China
PKFAd18	3	4	+	Pakistan
MN509168	3	4	+	Australia
MX95-S11	2	7	±	Mexico
Iran/UT-Kiaee/2018	2	7	+	Iran
JL/1407	2	4	+	China
GB591	3	0	??	Germany
380	2	0	??	United Kingdom
685-CORR	1	0	??	United Kingdom
BJH13	0	7	+	China
LN1507	0	6	+	China
ON P2	0	8	+	Canada
ON NP2	**4**	**13**	**-**	**Canada**

[1]+ pathogenic; - nonpathogenic; ± no clinical signs, but cause IBH; ? no pathogenicity data.

foci and petechial hemorrhages. The kidneys were also swollen (Figure 5B).

After intramuscular infection with 10^7 $TCID_{50}$ of the virus, the onset of mortality in 1-day-old SPF chickens was recorded at 48 hpi. with a mortality of 100% at 72 hpi (Figure 5C). Postmortem lesions of pale, swollen livers and swollen kidneys with renal tubules filled with urate were observed (Figure 5D). The infected 1-wk-old chickens were characterized by depression, reduced or no food intake, and crouching with ruffled feathers. Mortality was recorded at 48 hpi and reached 100% at 96 hpi (Figure 5E). Typical gross lesions of IBH, characterized by swollen livers with white dispersive necrosis and enlarged kidneys, were observed in the dead birds (Figure 5F). The infected 2-wk-old chickens exhibited depression and loss of appetite. The onset of mortality was recorded at 96 hpi, and the final mortality reached 50% at the end of the experiment (Figure 5G). Swollen livers with pale dispersive necrosis around the edge and enlarged kidneys were observed in the dead chickens (Figure 5H). Histopathological analysis of the dead chicken embryos and different aged chicks revealed the similar typical IBH lesions characterized by degeneration and necrosis of hepatocytes and basophilic intranuclear inclusion bodies of varying sizes; necrosis; mesenchymal congestion; and vacuolar degeneration of renal tubular epithelial cells (Figure 6).

Viral loads in different tissues were determined by real-time PCR. Viruses were found in all the tested tissues. The number of viral copies in the liver and pancreas was significantly greater than that in other tissues in infected 1-wk-old and 2-wk-old chickens ($p < 0.001$). The difference between the viral loads in the liver and pancreas from infected 1-wk-old and 2-wk-old chickens did not reach statistical significance ($p = 0.4310$ and 0.0963, respectively) (Figure 7).

DISCUSSION

Inclusion body hepatitis, caused by fowl adenoviruses, is an economically important poultry disease that causes substantial economic losses to the world poultry industry. FAdV-11 is one of the primary causative agents for IBH. In recent years, FAdV-11-associated IBH has been increasingly reported in China and other countries around the world (Gomis et al., 2006; Wilson et al., 2010; Nakamura et al., 2011; Steer et al., 2015; Zhao et al., 2015; Morshed et al., 2017; Oliver-Ferrando et al., 2017; Mohamed et al., 2018; Abghour et al., 2019, 2021; El-Tholoth and Abou El-Azm, 2019; Huang et al., 2019; Mirzazadeh et al., 2020; Şahindokuyucu et al., 2020; Cizmecigil et al., 2020; Hosseini et al., 2020; Chen et al., 2020; Sabarudin et al., 2021; Chitradevi et al., 2021; Bertran et al., 2021; Mase et al., 2021; Lebdah et al., 2022; Li et al., 2022; Liu et al., 2022; Niczyporuk and Kozdruń, 2022; Xie et al., 2022). Currently, there are no commercially available vaccines against FAdV-11-related IBH. Considering the coexistence of pathogenic and nonpathogenic strains in avian flocks (Slaine et al., 2016), characterizing the epidemic strain FAdV-11 is critical for identifying genetic markers responsible for the different FAdV-11 pathotypes and developing efficient vaccines matching the corresponding pathotypes.

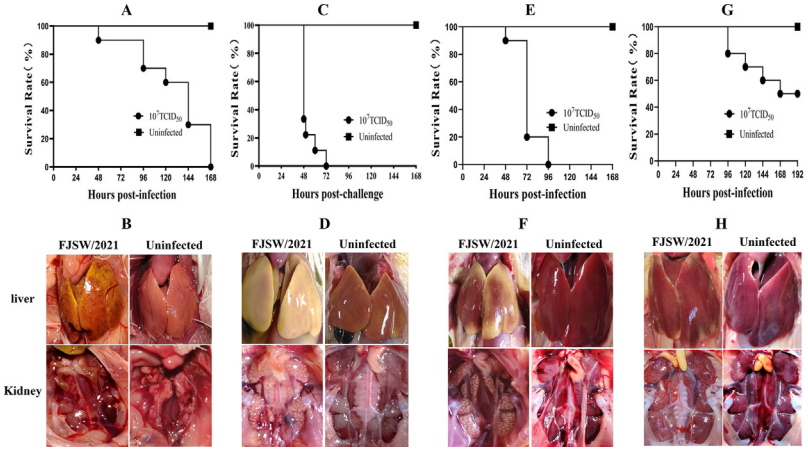

Figure 5. Pathogenicity of the FAdV-11 strain FJSW/2021 to SPF chicken embryos and chickens. Survival rates and postmoterm lesions in 10-day-old chicken embryos inoculated with 10^7 $TCID_{50}$ of FAdV-11 FJSW/2021 via allantoic cavity (A and B); in 1-day-old chicks (C and D), 1-wk-old chickens (E and F), and 2-wk-old chickens (G and H), inoculated with 10^7 $TCID_{50}$ of FAdV-11 FJSW/2021 intramuscularly.

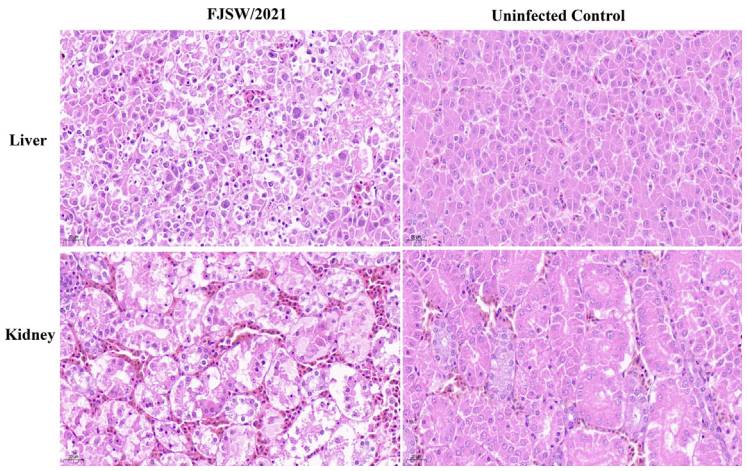

Figure 6. Representative histological lesions in livers and kidneys from chickens infected with the FAdV-11 strain FJSW/2021. Typical IBH lesions characterized by degeneration and necrosis of hepatocytes and basophilic intranuclear inclusion bodies in hepatocytes; necrosis, mesenchymal congestion, and vacuolar degeneration of renal tubular epithelial cells, were observed in chicken embryos and chickens infected with the FAdV-11 strain FJSW/2021.

In this study, we isolated and sequenced one FAdV-11 strain, FJSW/2021, from a broiler flock with clinical signs of IBH in Fujian Province, China. The pathogenicity of FJSW/2021 was assessed in 10-day-old chicken embryos and in 1-day-old, 1-wk-old and 2-wk-old chicks. These collective data will provide guidance for the identification of potential virulence factors of FAdV-11 and the establishment of a challenge model for evaluating the efficacy of vaccines against IBH induced by FAdV-11.

The full-length genome of the FJSW/2021 isolate is 44, 154 bp in length and shares 95.46 to 99.72% nucleotide identity with other published FAdV-11 strains. The genome of FJSW/2021 shares higher nucleotide identity with 4 Chinese FAdV-11 isolates, namely, HBQ12, BJH13, JL/1407 and LN/1507 (98.85−99.72%), the Mexican nonpathogenic strain MX95-S11 (98.98%) and the Canadian pathogenic strain ON P2 (98.54%). Phylogenetic analysis of whole-genome sequences and the amino acid sequences of hexon, fiber and penton bases indicated that FJSW/2021 is clustered together with reported FAdV-11 isolates. Although the gene order and putative ORFs in the FJSW/2021 genome are similar to those of previously reported FAdV-11 strains, FJSW/2021 exhibited some novel characteristics. The preterminal protein (**pTP**) of FJSW/2021 has S-L-R-I-I-C between 470 and 475 and one aa mutation, L476F, compared with other reported FAdV-11 isolates. pTP serves as the protein primer for adenovirus DNA replication and is processed at 2 sites by a virus-encoded protease to yield mature terminal protein (**TP**). Previous studies have demonstrated that either TP or pTP is at least in part responsible for directing adenoviral DNA to appropriate sites in the nucleus during the course of viral infection (Webster et al., 1997). Mutation of the pTP gene may affect the replication process of the virus, resulting in changes in its pathogenicity. The variation of FJSW/2021 in pTP has not been identified in other reported FAdV-11 isolates. However, the actual effects of the six-amino acid insertion on virus replication need to be further investigated. Most of the FAdV-11 isolates have identical hexon, fiber and penton genes, except for some amino acid mutations in the above genes of Iran/UT-Kiaee/2018, PKFAd18, MN509168 and 380. Notably,

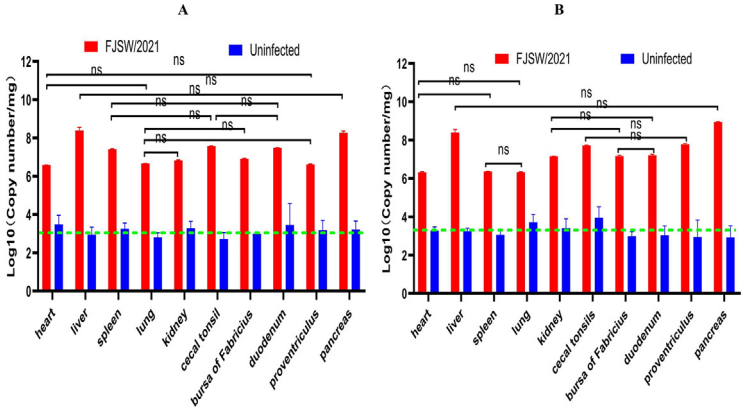

Figure 7. Viral loads in different tissues of chickens infected with the FAdV-11 strain FJSW/2021. The viral loads in different tissues of 1-wk-old chickens (A) and 2-wk-old chickens (B) inoculated intramuscularly with 10^7 $TCID_{50}$ of the FAdV-11 strain FJSW/2021.

there are 17 amino acid deletions (253GVAVADTL-TISSNTGTVT269) and 34 amino acid deletions (295PLTTSSNGLSLKLTPNGSIQSSSTGLSVQTD-PAG328) in the fiber proteins of FAdV-11 isolates PKFAd18 and 380, respectively. However, whether these are the actual characteristics of PKFAd18 and 380 or whether these differences are due to sequencing mistakes remains to be further verified. In addition, 2 previously identified codon deletions present in a glutamic acid-rich region of the 22K and 33K ORFs, equivalent to the glycine-rich region of 100K in the nonpathogenic isolate ON NP2 (Slaine et al., 2016), were also found in the isolate 685-CORR from the United Kingdom and the Pakistani pathogenic strain PKFAd18. However, this deletion was not detected in the corresponding ORFs of FJSW/2021 or other pathogenic FAdV-11 isolates. A total of 100K is crucial for the formation of properly structured hexon trimers. Both the 22K and 33K proteins are important splicing factors for gene transcription, and the 33K protein is critical for virion packaging and maturation (Morris and Leppard, 2009). Whether these mutations result in differences in the pathotypes of these isolates remains to be studied.

The major difference among the FAdV-11 isolates is in the tandem repeat (**TR**) region. TR1 and TR2, previously identified in some FAdV-11 isolates, are also present in FJSW/2021. The TR1 region of FJSW is 33 bp (1 repeat) shorter than that of the Canadian nonpathogenic isolate ON NP2 (Slaine et al., 2016). There are only 5 repeats in the TR2 region of FJSW/2021, which is also much shorter than that in the Canadian nonpathogenic isolate ON NP2 (13 repeats). Notably, there is variation in the length of TR1 and TR2 in the FAdV-11 genome from isolate to isolate (Table 3). Overall, the pathogenic FAdV-11 isolates have fewer TR1 and TR2 comparing to the nonpathogenic ON NP2 isolate. However, whether differences in the lengths of TR1 and TR2 result in differences in the pathotypes of FAdV-11 needs to be identified. We recently developed a reverse genetic platform for FAdV-11 FJSW/2021. The actual effects of the length of TR1 and TR2 on the pathogenicity of FAdV-11 are under investigation.

Since FAdV-11 FJSW/2021 was isolated from a broiler flock with clinical signs of IBH, the pathogenicity of FJSW/2021 in 10-day-old SPF chick embryos and chickens of different ages was assessed in this study. The results indicated that FJSW/2021 was fatal for 10-day-old SPF chick embryos after inoculation with 10^7 $TCID_{50}$ virus via the allantoic cavity and for 1-day-old and 1-wk-old chicken SPF chickens after intramuscular inoculation with 10^7 $TCID_{50}$ of the virus. Typical gross and histopathological lesions of IBH were observed in the dead embryos and chicks. However, the final mortality rate reached only 50% for the infected 2-wk-old chickens. Our results implied the age-dependence of FAdV-11 FJSW/2021 pathogenicity. Actually, the age-dependent pathogenicity has been described for FAdV-4, a major causative of HHS (Yuan et al., 2021). The stand-alone pathogenicity of FAdV-11 isolates has long been contradictory due to the variation between experimental studies (Zhao et al., 2015; Absalón et al., 2017; Wang et al., 2020; Abghour et al., 2021). Based on the existing literature on the pathogenicity of FAdV-11, it seems that most of the FAdV-11 isolates alone does not induce mortality in chickens older than 2 wk of age and co-infections are needed to do so. Notably, there is one report from China Agricultural University showed that oral inoculation of 3-wk-old SPF chickens with $10^{3.5}$ EID_{50} (median embryo infective dose) FAdV-11 isolate HBQ12 could induce 8.6% mortality within 21 d postinfection (Zhao et al., 2015). This indicates that the pathogenicity of different FAdV-11 isolates varies. In this study, we provided the first evidence that intramuscular inoculation of chickens less than 2 wk old with high dose FAdV-11 FJSW/2021 could induce 50 to 100% mortality. There could be several reasons for this phenomenon. The main possible reason could be a high enough challenge dose (i.e., 10^7 $TCID_{50}$) could break through the chick's immune resistance and causing high mortality in younger chicks. Another possible reason is due to the unique genetic properties of FJSW/2021 isolate such as S-L-R-I-I-C in the pTP, and relative shorter TR region of TR1 and TR2.

Given that the age-dependence of of the pathogenicity of FAdV-11 isolates, assessments of the efficacy of vaccine candidates should take selecting chickens of appropriate age into consideration. Considering the age-related severity of FAdV-associated diseases and the vertical transmission properties of FAdVs, there is an increasing need for prevention strategies targeting parental birds that can transfer maternal antibodies to progeny.

In summary, the FAdV-11 strain FJSW/2021 was successfully isolated from broiler flocks with typical clinical signs of IBH in Fujian Province, China. The complete genome sequence of FJSW/2021 is an additional supplement to the FAdV-11 sequence library. Systematic comparison of the genomes of the different FAdV-11 isolates will shed light on the crucial virulence factors of FAdV-11. The appropriate animal challenge model developed in our study will allow precise evaluation of the efficacy of potential FAdV-11 vaccine candidates.

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DISCLOSURES

The authors declare no conflict of interest.

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