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# Analysis of the recombination and evolution of the new type mutant pseudorabies virus XJ5 in China

Luyao Jiang<sup>1,2,3,4†</sup>, Jinlong Cheng<sup>1,2†</sup>, Hao Pan<sup>1,2,3,4</sup>, Fan Yang<sup>1,2,3,4</sup>, Xiemin Zhu<sup>1,2,3,4</sup>, Jiayan Wu<sup>1,2,3,4</sup>, Haochun Pan<sup>1,2,3,4</sup>, Ping Yan<sup>1,2,3,4</sup>, Jinzhu Zhou<sup>1,2,3,4,6</sup>, Qingqing Gao<sup>1,2,3,4</sup>, Changchao Huan<sup>1,2,3,4\*</sup> and Song Gao<sup>1,2,3,4,5\*</sup>

## Abstract

Pseudorabies have caused enormous economic losses in China's pig industry and have recurred on many large pig farms since late 2011. The disease is caused by highly pathogenic, antigenic variant pseudorabies virus (vPRV) strains. Our laboratory isolated a pseudorabies virus in 2015 and named it XJ5. The pathogenic ability of this mutant strain was much stronger than that of the original isolate. After we sequenced its whole genome (GenBank accession number: OP512542), we found that its overall structure was not greatly changed compared with that of the previous strain Ea (KX423960.1). The whole genome alignment showed that XJ5 had a strong genetic relationship with the strains isolated in China after 2012 reported in GenBank. Based on the isolation time of XJ5 and the mutation and recombination analysis of programs, we found that the whole genome homology of XJ5 and other strains with Chinese isolates was greater than 95%, while the homology with strains outside Asia was less than 94%, which indicated that there may be some recombination and mutation patterns. We found that virulent PRV isolates emerged successively in China in 2011 and formed two different evolutionary clades from foreign isolates. At the same time, this may be due to improper immunization and the presence of wild strains in the field, and recent reports have confirmed that Bartha vaccine strains recombine with wild strains to obtain new pathogenic strains. We performed genetic evolution analysis of XJ5 isolated and sequenced in our laboratory to trace its possible mutations and recombination. We found that XJ5 may be the result of natural mutation of a virus in a branch of mutant strains widely existing in China.

**Keywords** Pseudorabies virus, Variant strain, Phylogenetic analysis, Recombination

<sup>†</sup>Luyao Jiang and Jinlong Cheng contributed equally to this work.

\*Correspondence:  
Changchao Huan  
ChangchaoHuan@yzu.edu.cn  
Song Gao  
gsong@yzu.edu.cn

<sup>1</sup>College of Veterinary Medicine, Yangzhou University, Yangzhou 225009, Jiangsu, China

<sup>2</sup>Institutes of Agricultural Science and Technology Development, College of Veterinary Medicine, Yangzhou University, Yangzhou 225009, Jiangsu, China

<sup>3</sup>Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou 225009, Jiangsu, China

<sup>4</sup>Key Laboratory of Avian Bioproduct Development, Ministry of Agriculture and Rural Affairs, Yangzhou 225009, Jiangsu, China

<sup>5</sup>Joint International Research Laboratory of Agriculture and Agri-Product Safety of Ministry of Education of China, Yangzhou University, Yangzhou 225009, Jiangsu, China

<sup>6</sup>Jiangsu Academy of Agricultural Sciences Veterinary Institute, Nanjing 210014, Jiangsu, China



## Introduction

Pseudorabies virus (PRV), also called Aujeszky's disease virus (ADV) or suid herpesvirus type 1 (SuHV-1), is a highly contagious virus that primarily affects swine [1]. Before 2011, porcine pseudorabies occasionally occurred in scattered spots in China, and had high homology with the Ea strain isolated from Hubei in 1990 [2]. However, after 2011, the epidemics of PRV still occurred after immunization with Bartha-K61 vaccines, and the immunized pigs could not be completely protected [3–5]. Further PRV variants such as PRV TJ, PRV HN, PRV HNB, PRV HNX, etc. were isolated, showing higher virulence and higher mortality in pigs. It forms a distant branch from the Ea strain [6–12]. In addition, it was reported that hSD-1/2019, a PRV variant isolated in 2019 from the cerebrospinal fluid of four clinically symptomatic patients who were engaged in work related to pig or pork slaughtering, had similar areas of brain lesion involvement, and two of these patients also had typical retinal detachment. After treatment, they all suffered from severe neurological sequelae, including blindness, memory impairment, minimally conscious state and persistent vegetative state [13]. This evidence suggests that the emergence of some highly virulent PRV strains may pose new challenges to human health and therefore it is important to strengthen preventive, surveillance, diagnostic and treatment measures to prevent further transmission and protect animal and human health [14, 15]. Although swine is the only natural reservoir for PRV, it has been found to infect many other animals, such as dogs, sheep, cattle, rabbits, canines, foxes, wolves, tigers, and bears [16].

Several envelope glycoproteins of PRV (gB, gD, gH, gI, and gL) are essential in the production of virus-neutralizing antibodies and cell-mediated immunity, as well as for virus penetration into neurons. In addition, other glycoproteins, such as gC, gM, and gK, can regulate PRV attachment and fusion [17–22]. During the initial attachment stage, gB and gC bind to the heparan sulfate moiety of the host cell surface proteoglycan and participate in the fusion of the virus and the cell membrane, serving as viral fusion proteins and playing an important role in the entry of the virus into the host cell [23–25]. At the same time, gD binds to the host cell fibronectin 1 receptor, thereby triggering fusion with the host membrane and recruiting the fusion machinery consisting of gB and gH/gL for cell entry [26, 27]. On the other hand, deletion of gE and gI reduced the spread of viral particles between neurons [28]. As an important independent protein gE of PRV, it has been found that only a single amino acid mutation/insertion/deletion of gE can lead to significant changes in its virulence. Therefore, it is particularly important to monitor the above protein mutations. The DNA molecular structure of PRV is double-stranded and linear, approximately 145 kb, and it encodes more than

70 kinds of proteins [29]. The genomic DNA is primarily divided into four parts, namely the unique long segment (95 kb) (unique long; UL), short segment (9 kb) (unique short; US) and terminal repeats (TR) and internal repeats (IR) located on both sides of the short segment. The genome has a high G+C content (more than 73%), and the G+C content is the highest among the discovered herpes viruses. At present, whole-genome sequencing of PRV has become a prevalent practice, and the overall distribution of PRV genes is well-understood. The proteins gB, gC, gD, gE, gI, TK, etc., found at present can be used as the breakthrough point of PRV vaccines (such as the Bartha K61 vaccine) [30–32]. Therefore, it is also very meaningful to monitor the mutation of the above proteins and analyze their evolution.

## Methods

### PRV complete genome sequencing

A case of suspected porcine pseudorabies from an infected pig in Yangzhou city was reported in 2015. The samples were collected from brain tissues of 3-day-old piglets with the onset of the disease, and confirmed PRV positive by PCR and other methods. The samples were ground and treated with antibiotics at 4°C overnight, and the supernatant of grinding solution was filtered through a 0.45 µm sterile filter. The filtered supernatant was seeded on a cell plate filled with a monolayer of Vero cells, and after 1 h of adsorption, the cell supernatant was discarded and spread on 0.8% low-melt AGAR into the cell plate. The plaques were picked and freeze-thawed for three times before further purification, and the virus with high purity was obtained by at least three times of purification.

The whole-genome of the PRV variants were extracted and paired-end sequenced with a high-throughput Illumina HiSeq 2500 at Beijing Novogene Biotechnology Co., Ltd. PE150 sequencing was performed in the decibosome chain-specific library construction mode. Sequencing data were first filtered to obtain clean data, and decontamination of the clean data was performed. The sequence stitching software SOAP denovo (version 2.04) [33, 34] and MITOBim [35] were then used for stitching and assembly. Krskgf, Gapclose and other software were used to optimize and fill holes in the preliminary assembly results. The coding gene prediction software GeneMarkS [36], repeat sequence prediction software RepeatMasker [37] and TRF [38], MUMmer [39] alignment software, and genome homology alignment software clustal 2.1 were used for correction. The full-length gB, gC, gE, gI, gD and TK genes were amplified by PCR and verified by sequencing to obtain the final assembly results. The complete sequence data of the XJ5 strain was submitted to the GenBank database under accession number OP512542.

### Gene rearrangement analysis by Easyfig

Easyfig (Easy genome comparison figures) [40] software (developed at the Beatson Microbial Genomics Lab) was used to analyse the gene rearrangement of Ea, Bartha and XJ5 [41, 42]. The blast options parameter is min. identity value: 90 and retains the default settings for other settings.

### Comparative genome analysis by BRIG and Mauve

The comparative genome circle graph is drawn by the software BRIG (Blasting Image Generator) [43] and linear genome comparisons were performed using Mauve software [44]. Upper identity threshold: 90%, Lower identity threshold: 75%. Retain the default settings for other settings.

### Percentage of whole-gene homology

Genome homology was analysed by DNASTar software. The MegAlign subsoftware was used for comparison using the Martinez-NW method in one-pair mode.

### Phylogenetic tree constructed by MEGA X

The phylogenetic tree was drawn by MEGA X software [45]. All sequences were aligned using the ClustalW method and output to MEGA format. Phylogenetic trees of the whole genome and single genes gB, gC, gD, gE, gI and TK were constructed by Maximum Likelihood Method [46]. The phylogenetic bootstrap method was tested and replications were 1000. The Tamura-Nei model was used, and substitutions type was used to select nucleotide. In the tree inference option, use

Nearest-Neighbor-Interchange (NNI) as the ML heuristic Method, and make initial tree automatically (default-NJ/BioNJ), very strong option was chosen for branch swap filter. Number of threads was set to 1000.

### Mouse nasal infection test

Ten mice (8-week-old BALB/c, female) were randomly divided into 2 groups with 5 mice in each group. One group was infected with  $10^{6.0}$  TCID<sub>50</sub> XJ5 inoculum through nasal drip, and the control group was inoculated with cell maintenance solution. The death of the mice were observed and recorded every day after inoculation [16]. All of the experiments were performed following the animal ethics guidelines approved by the Institutional Animal Care and Ethics Committee of Yangzhou University.

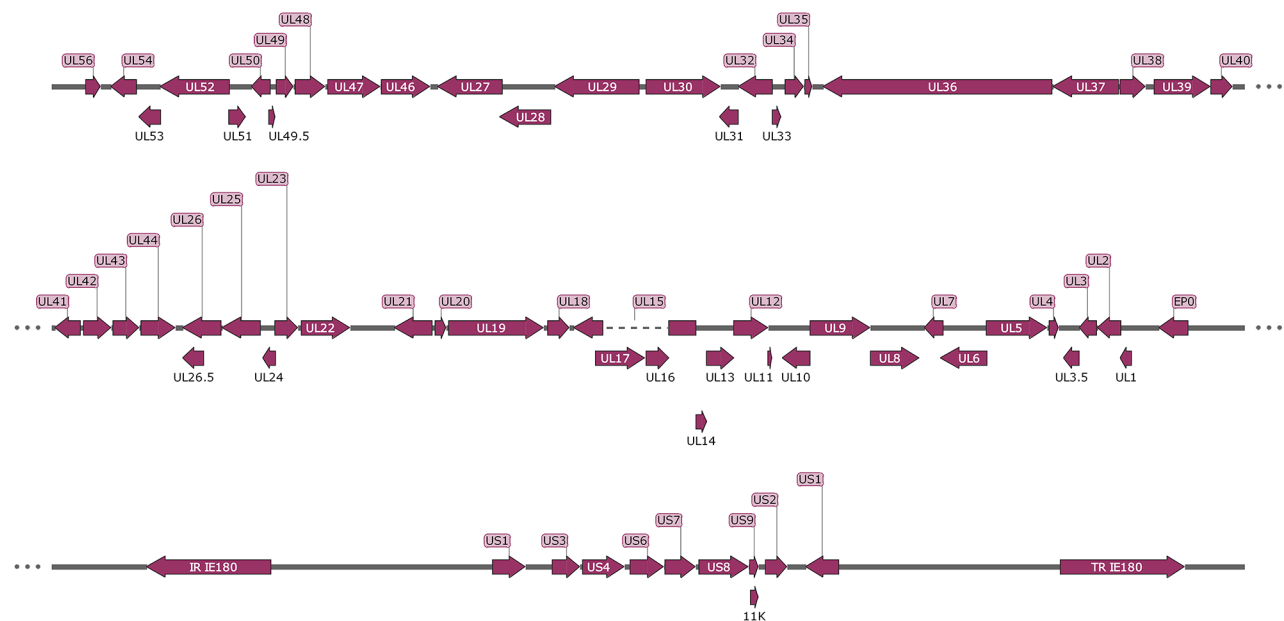
## Results

### Information on coding genes

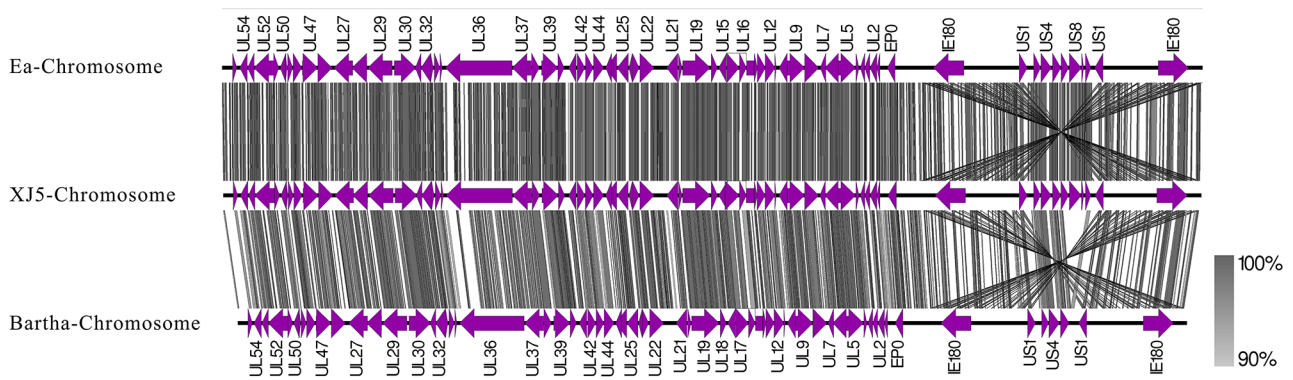
Through sequencing and gene annotation, we found that XJ5 has a sequence length of 141,955 bp, possesses 69 open reading frames (ORFs) in PRV, and has a GC content of 73.79% (73.6% for Ea, 73.71% for Bartha, 73.74% for Becker) of the whole genome.

Sequence comparison with the 1990 isolate Ea strain in China revealed that its gene position and direction basically did not change (Fig. 1).

Comparing the sequence of XJ5 after whole-genome sequencing with the gene positions of other reported strains in GenBank, we found that the overall order and

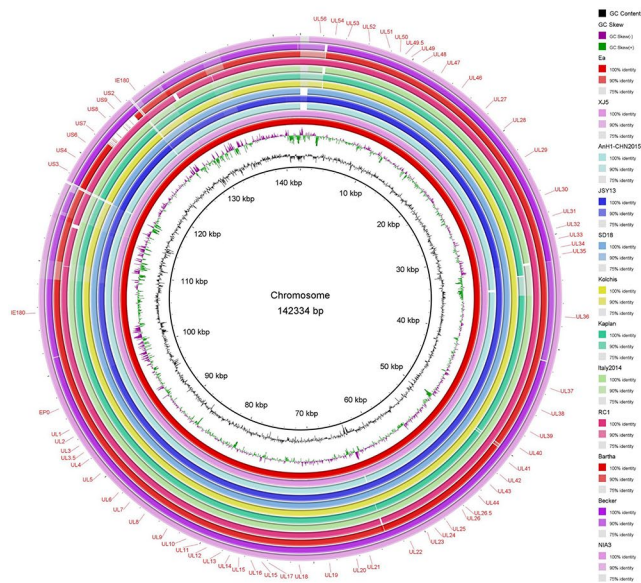


**Fig. 1** Gene annotation of the sequence of XJ5 obtained by sequencing. The gene annotation of XJ5 (OP512542) is annotated with reference to the Ea (KU315430.1) strain and Bartha (JF797217.1) strain. The direction of the arrow indicates the direction of translation

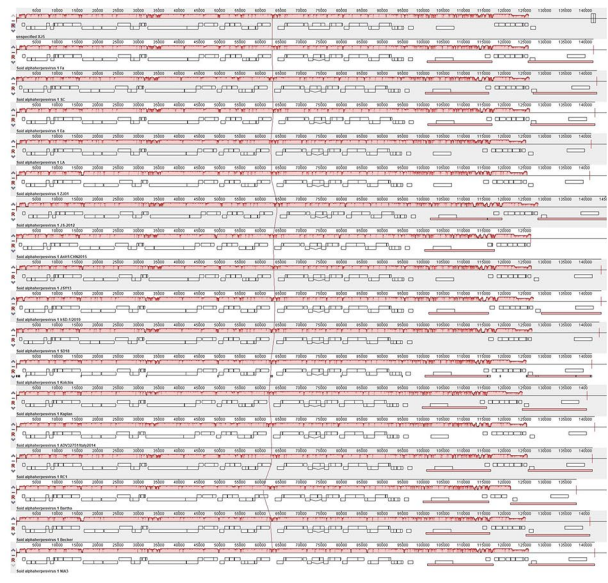


**Fig. 2** Comparison of reading frames between XJ5 and Ea strains as well as Bartha strains. The direction of the arrow indicates the direction of translation, and the position is in the whole genome. The two segments of IE180 are two repeated fragments, so the gene position exchange shown here is actually the original gene

**A**



**B**



**Fig. 3** Genome-wide comparison of XJ5 with different isolates. **A:** Whole-genome circle map constructed by Brig, where we selected representative strains from each region for whole-genome comparison with Ea strains as a reference. The depth of the color in the same color circle indicates the level of homology. **B:** Whole-genome linear comparison map constructed by Mauve, where we selected representative strains from each region for whole-genome comparison with Ea and other strains as reference. The level of the line shape at the same position indicates the level of homology. The middle correction line is located near the 63,000 bp position of XJ5

direction of the amino acids encoded by XJ5 had not changed (Fig. 2).

Comparing the whole genome sequenced XJ5 and the isolate Ea strain and Bartha strain reported in GenBank, we found that the overall order and direction of the amino acids encoded by XJ5 had not changed (Figs. 1 and 2).

The XJ5 strain shows genomic architecture similar to other virulent strains, such as the classical PRV strain Ea isolated from China in 1990, while the Bartha strain was an attenuated vaccine strain with deletion of the US8 gene by artificial gene editing, as shown in previous studies [47]. These results indicate that mutations in XJ5

should occur naturally and are not the result of laboratory editing.

**Genome-wide gene comparison (gene circle diagram)**

XJ5 exhibited no artificial gene deletion of common PRV vaccine strains, and there was no clear evidence of other gene sources. The overall structure was similar to that of other isolates (Fig. 3).

**Genetic characteristics of XJ5 (percentage of whole gene homology)**

The gene sequence of XJ5 was compared with other PRV complete genome sequences



**Table 1** Information on the PRV reference strains used for phylogenetic tree construction

Strains	GenBank accession number	Year isolation	Origin	Reference
BJ-YT	KC981239.1	2012	China	[49]
TJ	KJ789182.1	2012	China	[7]
ZJ01	KM061380.1	2012	China	[50]
HNX	KM189912.1	2012	China	[8]
Fa	KM189913.1	1980s	China	[9]
HNB	KM189914.3	2012	China	[10]
HeN1	KP098534.1	2012	China	[11]
JS-2012	KP257591.1	2012	China	[11]
HN1201	KP722022.1	2012	China	[12]
SC	KT809429.1	1986	China	[51]
HLJ8	KT824771.1	2013	China	[51]
Qihe547	KU056477.1	2014	China	[52]
Ea	KU315430.1	1990	China	[2]
DL14-08	KU360259.1	2014	China	[53]
LA	KU552118.1	1997	China	[54, 55]
Ea(Hubei)	KX423960.1	1993	China	[56]
GD0304	MH582511.1	2015	China	
HLJ-2013	MK080279.1	2013	China	[57]
AnH1-CHN2015	MK618718.1	2015	China	[58]
JX-CH-2016	MK806387.1	2016	China	[59]
JSY7	MT150583.1	2018	China	[48]
JSY13	MT157263.1	2018	China	[48]
GD-YH	MT197597.1	2014	China	[60]
HuBXY-2018	MT468549.1	2018	China	[13]
hSD-1-2019	MT468550.1	2019	China	[13]
HeNLH-2017	MT775883.1	2017	China	[61]
GD1802	MT949535.1	2020	China	[62]
SD18	MT949536.1	2020	China	[62]
HuB17	MT949537.1	2020	China	[62]
XJ5	OP512542	2015	China	isolated by our laboratory
Hercules	KT983810.1	2010	Greece	[63]
Kolchis	KT983811.1	2010	Greece	[63]
Kaplan	JF797218.1	1979	Hungary	[47]
Kaplan	JQ809328.1	2011	Hungary	[64]
DUL34gfp	JQ809329.1	2011	Hungary	[64]mutant derived from Kaplan strain; contains UL34 deletion; [64] expresses GFP
DUL34Pass	JQ809330.1	2011	Hungary	derived from DUL34gfp strain after passaging in cell culture approx. 100 times to regain replication competence; UL34 deletion mutant
Kaplan	KJ717942.1	1959	Hungary	[65]
PRV-MdBio	LT934125.1	2017	Hungary	[66]
ADV32751-Italy2014	KU198433.1	2014	Italy	[67]
MY-1	AP018925.1	2015	Japan	[68]
RC1	LC342744.1	2016	Japan	[69]
NIA3	KU900059.1	1971	Spain	[70]
Bartha	JF797217.1	1961	Hungary	[47, 71]
Becker	JF797219.1	1967	USA	[47]

published in GenBank (Table 1). The homology of XJ5 with Chinese isolate M189913.1(Fa), KT809429.1(SC), KU315430.1(Ea), KU552118.1(LA), KM061380.1(ZJ01), KP257591.1(JS-2012), KU360259.1(DL14-08) and MK618718.1(AnH1-CHN2015) were 96.6%, 96.2%, 96.8%, 94.9%, 95.6%, 95.4%, 95.3% and 97.5%, respectively. The homology with KT983811.1(Kolchis), JF797218.1(Kaplan), JF797217.1(Bartha) and JF797219.1(Becker) isolates was 92.4%, 93.1%, 89.9% and 92.1%, respectively. AnH1-CHN2015 isolated in 2015 showed high homology with XJ5 isolated by us in the same year.

#### Evolutionary analysis of the whole genome of XJ5 (whole gene evolutionary tree)

There are two main evolutionary branches of PRV published according to the phylogenetic tree constructed by the maximum likelihood (ML) method in MEGA X (Fig. 4). Most of the Chinese and Japanese isolates are the first branch, and some isolates from other countries and a few Chinese isolates are the second branch. Chinese isolates in the second branch have the highest homology with the Bartha strain.

#### Genetic evolution analysis of the gC gene (evolutionary tree of the gC gene)

From the phylogenetic tree of the gC gene of PRV, it is also divided into two clades: its first clade includes most Chinese and Japanese isolates, and the second clade is dominated by foreign isolates (Fig. 5). Some Chinese isolates have evidence of genetic recombination derived from Bartha vaccine strains such as JSY13 [48].

#### XJ5 was confirmed to be the gene mutation product of the virus after 2011 by different phylogenetic trees

The evolutionary tree of the gB, gD, gE, gI, and TK genes shows that XJ5 is still in the same branch as most Chinese isolates (Fig. 6). However, the GD1802 strain (137651 bp, from dog) isolated from Guangdong, China, in 2020 was more than 99.9% identical to the whole genome of the Bartha strain (137764 bp), since GD1802 has not been reported for virulence, it is unclear whether it was isolated in dogs due to environmental spread due to improper use of the Bartha vaccine strain, or whether the Bartha strain has a new mutation or recombination with other strains that leads to increased transmissibility.

#### Tracing possible sources of recombination

In the coding region, we could not find a relationship between different strains, but in the non-coding region, we found a large number of unique molecular markers displayed by some monarchs. The results of partial regions after collation are shown in Fig. 7. We randomly selected the non-coding region for analysis from scratch,

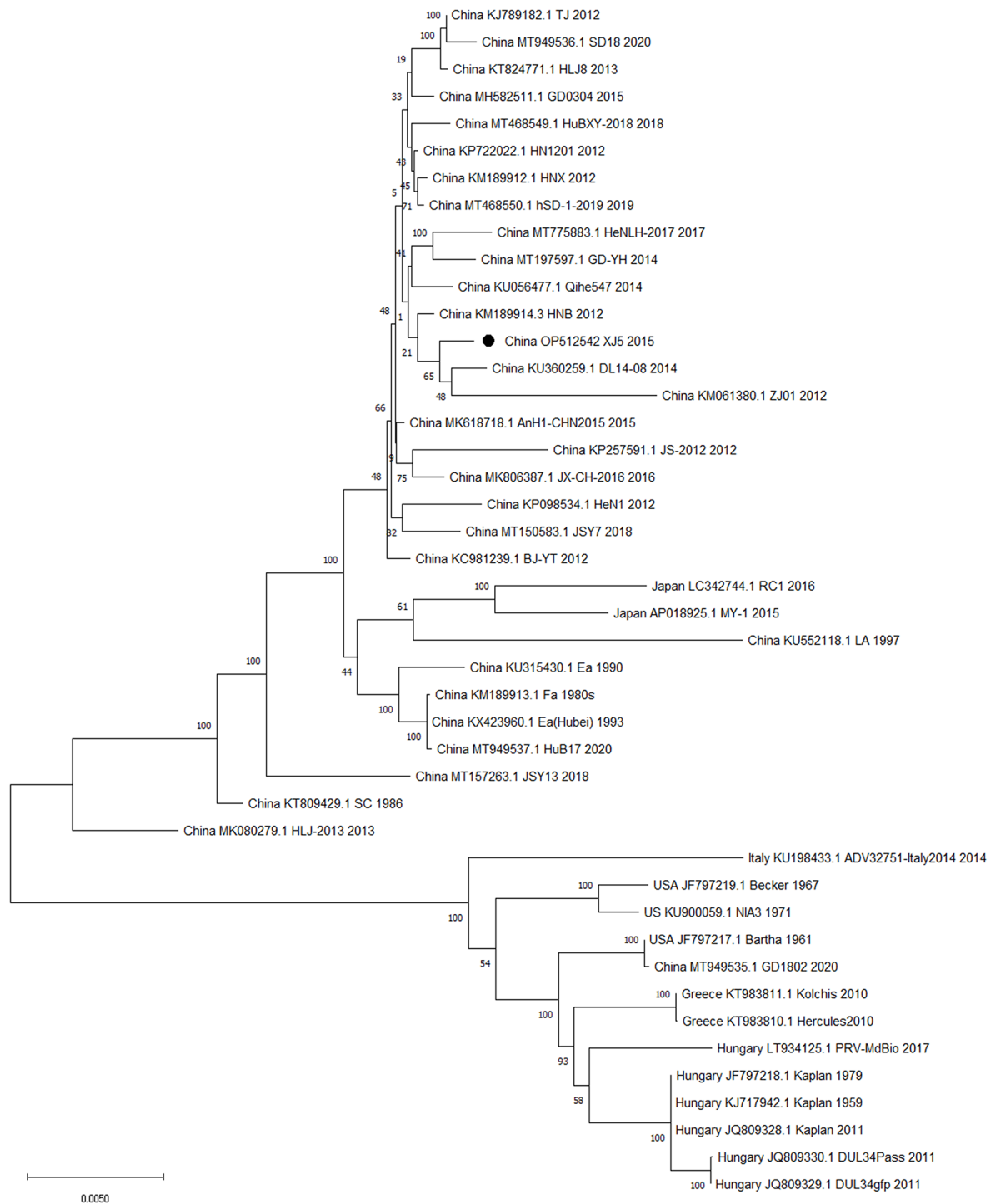
and the position of this position in XJ5 in the whole genome is provided in the upper left corner of the picture. In the results of our analysis, this is only a very small fraction of the regions that are significantly representative, and there are still a large number of regions that are not shown in the results. Other noncoding regions not shown have a similar evolutionary history.

In Fig. 7A, according to the nucleotide similarities and differences of each strain, we can clearly find that Fa in the 1980s, Ea in 1990, Ea (hubei) in 1993, HLJ-2013 in 2013, HuB17 in 2020 have almost the same molecular markers. GD0304 in 2015 was similar but slightly different, which might be the product of gene mutation. SC (1986), TJ and BJ-YT (2012), HLJ8 (2013), DL14-08 (2014), AnH1-CHN2015 (2015), hSD-1-2019 (2019), and SD18 (2020) had almost identical molecular markers. HN1201, HNB, HeN1, HNX, JS-2012 in 2012, GD-YH, Qihe547 in 2014, XJ5 in 2015, JX-CH-2016 in 2016, HeNLH-2017 in 2017, HuBXY-2018 and JSY13 in 2018 JSY7 has almost the same molecular markers, and ZJ01 in 2012 is similar but slightly different, which may be the product of gene mutation. Bartha strain isolated before 1961 and Becker strain isolated before 1967 form two similar but slightly different molecular marker groups.

In Fig. 7B, we can clearly find that the LA strain isolated in China in 1997 and Kaplan strain isolated in Hungary before 1959 have similar molecular markers. Unlike the above, XJ5 was similar to other Chinese strains Fa dating back to the 1980s. This suggests that reassortative strains with foreign strains may have emerged in China before 1997. LA strain was in the intermediate state of recombination at that time.

In Fig. 7C, LA strain in 1997, ZJ01 strain in 2012, HLJ-2013 strain in 2013, and GD1802 strain in 2020 have large deletions of molecular markers that are completely different from the Chinese strain. It seems that the above PRV viruses are also the evolutionary results of LA strain. The molecular marker of XJ5 in this segment can be traced back to Fa strain in the 1980s.

Figure 7D provides further evidence that LA strain may be an intermediate state of historical recombination of current epidemic strains in China, and in this region, the deletion of Fa strain isolated in the 1980s relative to foreign strains in this region was gradually inserted into SC strain isolated in 1986 and Ea strain isolated in 1990, and became a fixed part of current Chinese variants. Some molecular markers of XJ5 strain could be traced back to Bartha strain isolated before 1961. However, what puzzled us is that the Ea strain isolated in 1990 seems to be more in the intermediate state of evolution from Fa strain to SC strain. The GD1802 isolated in 2020 was the possibility of recombination between the foreign strain and the Chinese strain.

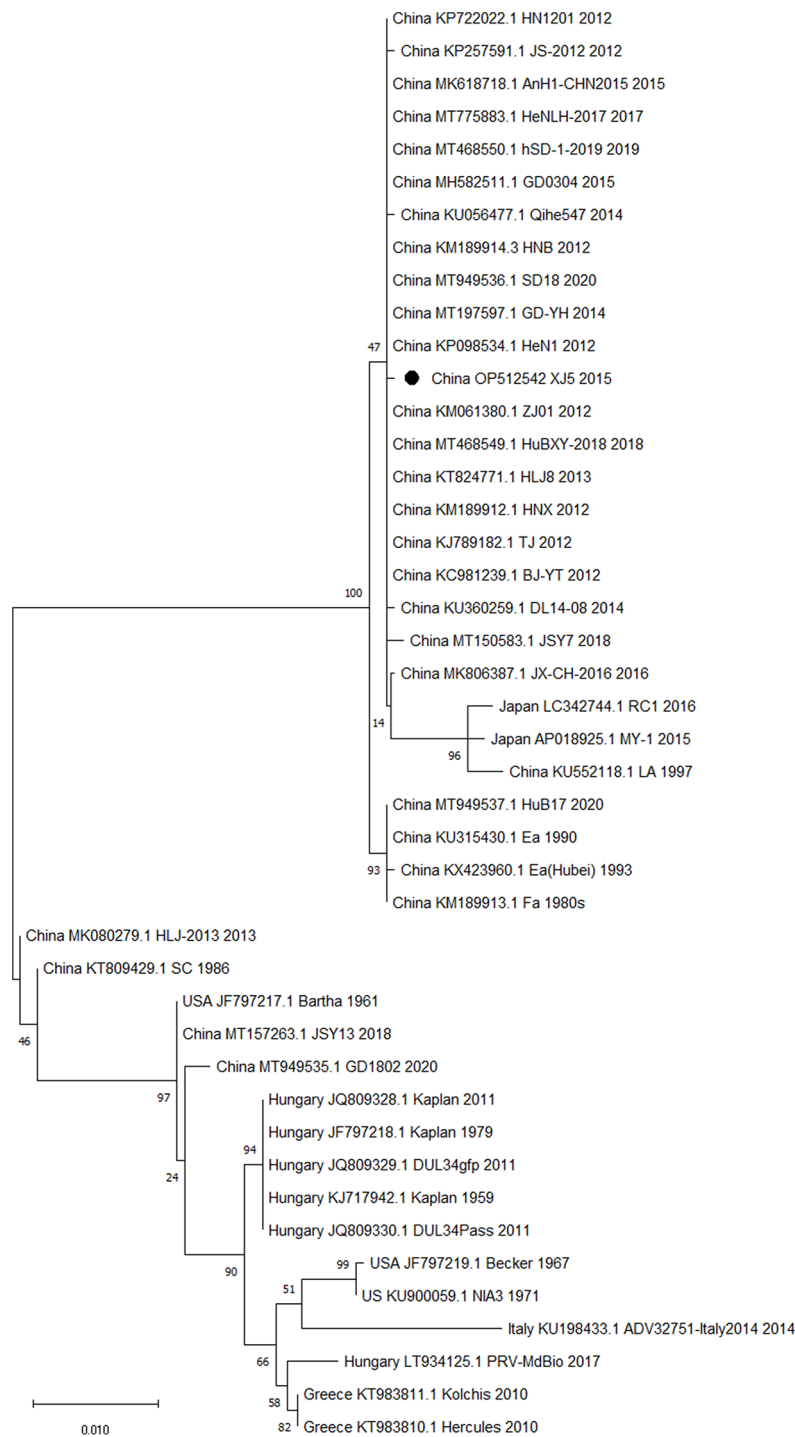


**Fig. 4** Evolutionary analysis of the whole genome of XJ5 and different isolates

In Fig. 7E, in the second segment marked, Fa strain seems to be gradually missing to the current epidemic strain, and the Ea strain isolated in 1990 seems to be in the intermediate state of evolution from Fa strain to SC strain, which seems to further deepen our doubts. We speculate that the Ea strain may have existed earlier than the SC strain, but it was isolated later. And this segment

also seems to provide a small part of the evidence that the Greece strain seems to be derived from the Bartha strain, which has the whole segment deletion.

In Fig. 7E, in the first segment of the marker, the evidence that the Greek strain originated from the Bartha strain and evolved locally is further strengthened. JS-2012 strain showed the same molecular markers as LA



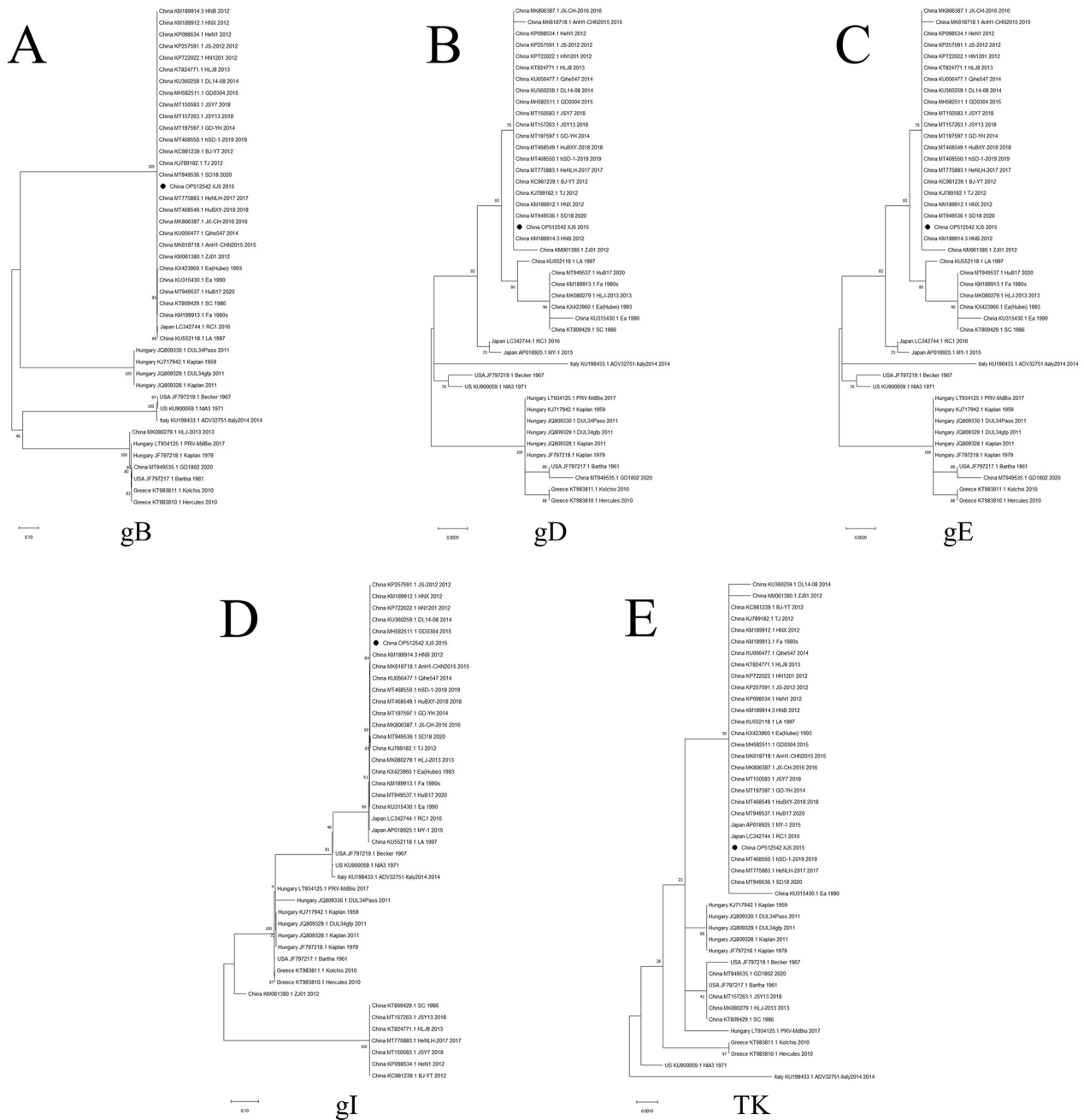
**Fig. 5** Evolutionary analysis of the gC gene of XJ5 and different isolates

strain, and some of the markers were observed in Becker strain. The molecular markers of XJ5 strain could be traced back to SC strain in 1986 and Ea strain in 1990.

Figure 7G further strengthens the possibility that JS-2012 strain may be derived from LA strain, where the same molecular markers as foreign strains appear. The LA strain can be traced back to the Bartha strain in 1961

or to the Berker or Kaplan strain in 1967 or 1959. The molecular markers of Fa strain, SC strain, Ea strain, Ea (Hubei) strain, HLJ-2013 strain and HuB17 strain could be clearly seen. XJ5 strains were similar to most of the PRV strains isolated in China since 2012.





**Fig. 6** Evolutionary analysis of the gB gD gE gI TK gene of XJ5 and different isolates

In Fig. 7H, in the fourth segment of the marker, the molecular markers of LA strain consistent with Fa strain, SC strain and Ea strain are further shown.

The position of this position in XJ5 in the whole genome is provided in the upper left corner of the picture.

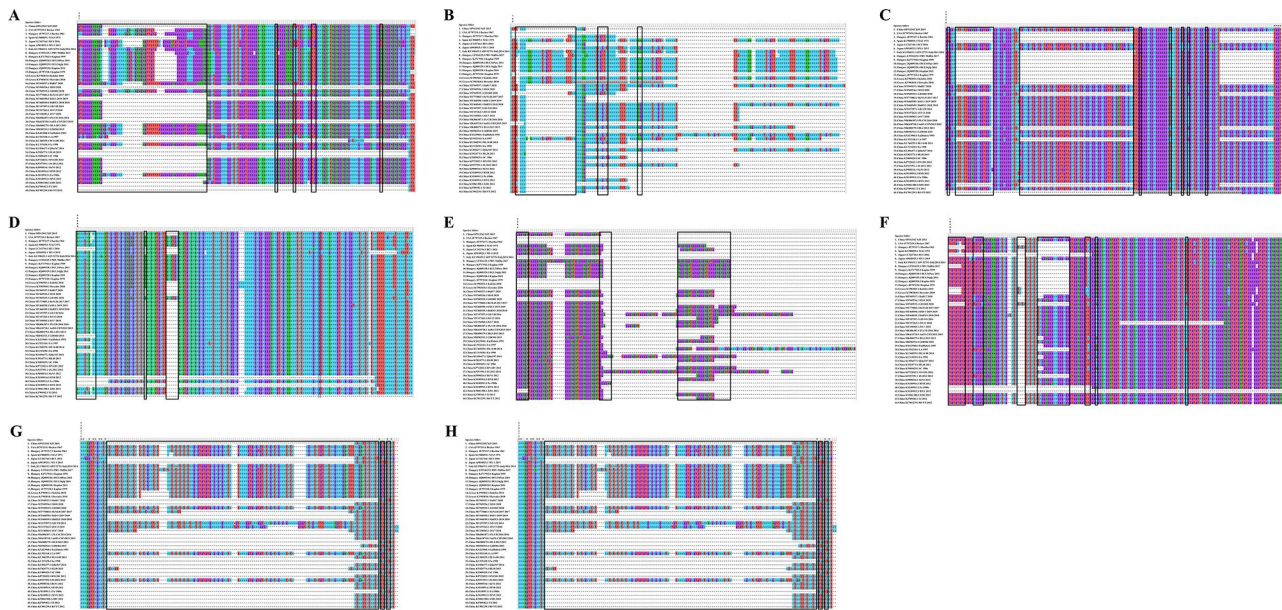
**Mouse mortality statistics**

After infection of BALB/c mice with the XJ5 virus strain, the mice began to die on day 3 after infection, and 100% of the infected group died by day 5 (Fig. 8). This means

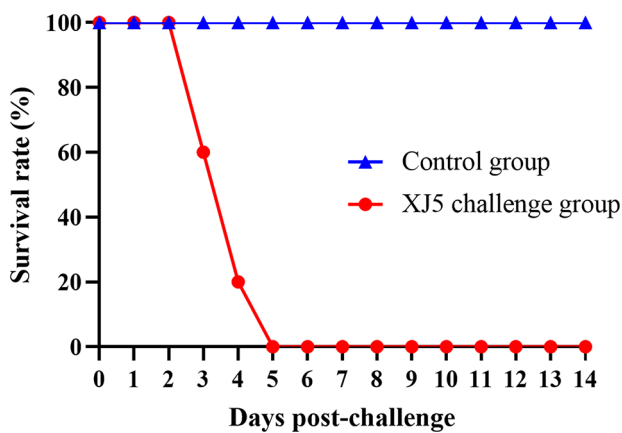
that XJ5 belongs to a variant strain branch with strong virulence, as shown by genotyping results such as a whole-genome evolutionary tree and gE.

**Discussion**

Based on the analysis of coding and non-coding regions in the whole genome of PRV, our findings provide a new hypothesis for the genomic evolution analysis of PRV. The nucleotide sequence of the partial noncoding region of XJ5 was traced back to the earliest known isolation of any strain (i.e., all possible strains were from before



**Fig. 7** Traceability diagram based on noncoding region



**Fig. 8** Survival rates of mice after challenge with PRV isolate XJ5

2012). We had a bold guess that Bartha, Becker, or Kaplan strains isolated in Hungary in the 1950s and 1960s May be one of the earliest ancestors of recombination, and that Fa strains isolated in China in the 1980s are also one of the ancestors of recombination. We speculated that there might be a natural evolution process of recombination and mutation as follows: Hungary strain had entered China around 1990s, and after a certain degree of recombination and evolution, it became LA strain isolated in 1997, while Fa strain in 1980s still evolved independently. With the passing of time and the frequent communication between different regions, some strains still maintain a certain evolution, and others continue to evolve naturally after recombination. For example, Fa strain may independently evolve into SC strain and Ea strain, and may further evolve into HLJ-2013 strain through recombination. The LA strain became ZJ01 (2012), JS-2012 and

HLJ-2013 strains during the process of recombination and evolution with local strains. Since a large number of Chinese PRV strains have been isolated and sequenced since 2011, there are probably several evolution patterns. They are Hub17 (isolated in 2020) with molecular markers similar to Fa strain, PRV strain with molecular markers similar to Becker/Bartha strain, Greece strain similar to Kaplan strain isolated in Hungary, and a large number of variants in China, etc. Our proposed conjecture is based solely on the data available to us so far. Since most of the recombinant intermediate state or natural evolution of PRV strains may have been submerged in the long river of history, we do not have complete evidence to support or confirm the above conjecture. This is primarily because during the time when these strains were prevalent, sequencing and diagnosis technologies were not as advanced and easily accessible as they are today. It is also possible that there may be some intermediate strains of the virus that have not yet been sequenced or uploaded onto any database. These missing pieces of information could alter our understanding of how the virus spreads and mutates over time. Despite these limitations, it is important for us to continue researching and analysing all available data to gain a better understanding of this complex virus. Although there is no further evidence of the origin of recombination, the present report suggests that the commercial vaccine strain Bartha K61 can recombine with the wild-type strain to form new strains and that this has occurred. The isolation of JSY13 (MT157263.1) [48] in 2018 was direct evidence of recombination between the wild-type strain JSY7 (MT150583.1) [48] and the vaccine strain Bartha. In addition, GD1802 (MT949535.1) isolated from the host

dog may also be the result of mutation or recombination of the vaccine strain Bartha, based on the nucleotide sequence of the non-coding region and the gene analysis of the whole genome phylogenetic tree.

In previous studies, we have found that all pigs began to develop high fever in the first day of vaccination after the XJ5 inoculation, and the rectal temperature was 42.3°C. Pigs from the XJ5 attack group showed a loss of appetite, depression and difficulty breathing. In the 3–10 days of inoculation of XJ5, the pig's weight was gradually reduced to death. After the examination and the observation of the histopathology, the dead pig had a typical PRV disease [41]. This is consistent with the toxicity shown in mice.

Therefore, in the prevention of PRV, in addition to the standardized use of high-quality vaccines, biosafety prevention and control measures should be implemented. How to further prevent the recombination of the PRV vaccine strain and wild-type strain is also one of the problems we need to consider.

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#### Author contributions

Authors' contributions: LYJ & JLC have contributed equally to this work. LYJ & JLC: Designing the experiments, and writing the original draft. HP: Providing advice. FY: Providing advice. XMZ: Resources. JYW: Resources. HCP: Resources. PY: Resources. JZZ: Resources. QQG: Modify the original draft. CCH: Modify the original draft, funding acquisition, writing review & editing. SG: Modify the original draft, funding acquisition, writing review & editing. All authors read and approved the final manuscript.

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#### Data availability

Whole genome data of JS-XJ5 is available at Genbank database: <https://www.ncbi.nlm.nih.gov/nuccore/2490825621>.

#### Declarations

##### Ethics approval and consent to participate

The experiments were conducted in accordance with the Regulations of the Chinese Council on Animal Care, approved by the Institutional Animal Care and Ethics Committee of Yangzhou University (approval ID: SYXK (Su) 2016–0009), and reported following the ARRIVE guidelines. All research protocols were approved by the Animal Ethical Committee of Yangzhou University. All surgery of mice was performed under pentobarbital sodium anesthesia with minimum fear, anxiety and pain.

##### Consent for publication

Not applicable.

##### Competing interests

The authors declare no competing interests.

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