ORIGINAL ARTICLE

Veterinary Research Forum. 2025; 16 (9): 493 - 497

doi: 10.30466/vrf.2024.2034665.4353

Journal Homepage: vrf.iranjournals.ir

Veterinary Research Forum

Isolation and molecular characteristics of a novel recombinant pseudorabies virus strain in Hunan province, China

Qi-wu Tang[1], Gui-hui Weng[1], Jie-feng Huang[2], Lei Zhang[3], Zao-fu Deng[1]*

[1] *Hunan Biological and Electromechanical Polytechnic, Changsha, China;* [2] *Loudi Agriculture and Rural Bureau, Loudi, China;* [3] *Subdistrict Office of Nanzhuangping Street, Yongding District, Zhangjiajie, China.*

Article Info

Article history:

Received: 06 July 2024
Accepted: 20 November 2024
Available online: 15 September 2025

Keywords:

Biological feature
China
Genetic characteristics
Nature recombination
Pseudorabies virus

Abstract

Pseudorabies (PR), an infectious disease caused by PR virus (PRV), has had a significant impact the development of the swine industry in China. Moreover, the public threat posed by PRV has raised widespread concern, with over 30 documented cases of PRV infection in humans in China. Various vaccines have been developed to prevent and control PR in China, primarily including inactivated vaccines and attenuated live vaccines. However, the extensive use of attenuated live vaccines and the presence of the field PRV strain in pig farm may increase the likelihood of recombination. In this study, we isolated a PRV variant strain (designated HuN-YY) from a piglet that had been immunized with the HB-98 vaccine strain in Hunan province and explored its genetic and biological features. The results showed that the *gE*, *TK* and *gD* genes of HuN-YY exhibited the highest sequence similarity with those of PRV variants, while the *gC* gene showed high homology with classical PRV strains. In addition, HuN-YY strain exhibited similar replication features compared to those of HB-98 and variant strains. Its pathogenesis in a mouse model was significantly lower than that of the variant strain. Collectively, these results provide clear evidence of genomic recombination of the HuN-YY strain, which will offer guidance for the future prevention and control of PR.

© 2025 Urmia University. All rights reserved.

Introduction

Pseudorabies (PR) virus (PRV), also known as Aujeszky's disease virus or Suid herpesvirus, belongs to the sub-family *Alphaherpesvirinae* within the family *Herpesviridae*.[1] Pigs and wild boars are the natural reservoirs for PRV, can also infect various mammals, such as goats, cattle, dogs, and foxes.[2-4] Importantly, an increasing number of cases of human encephalitis or endophthalmitis caused by PRV infection have been documented in China,[5,6] This indicates that the prevalence of this pathogen not only impacts the development of the pig industry but also poses a potential threat to public health.

According to the genomic characteristics of PRV strains prevalent worldwide, they can be divided into two genotypes: genotype I and genotype II. Genotype II consists of PRV classical and variant strains.[4] Among these, genotype I strains were mainly isolated from America, Europe, and some regions of Asia, while most genotype II strains were identified in China.[4] Similar to other *herpesviruses*, PRV has a large DNA genome with nearly 150 kbp in length that encodes more than 70 proteins.[7] Some PRV protein-encoding genes are involved in viral virulence, such as the *glycoprotein I (gI)*, *gE*, and *TK* genes, while deletions of these genes do not affect viral replication.[8] Genome-modified PRV strains with multiple gene deletions (including *gE*, *gI*, and *TK* genes) have been created to develop live attenuated PRV vaccines.[9,10]

Considering the significant threats of PRV to the global pig industry, various types of vaccines have been developed to prevent and control its spread. These vaccines mainly include live attenuated and inactivated PRV vaccines.[11] Currently, live attenuated PRV vaccines, such as Bartha-K61, HB-98, and SA215 strains, are widely used in the Chinese pig population. The HB-98 (Ea-△gG/TK) and SA215 (Fa-△gE/gI/TK) vaccine strains were derived from classical PRV strains.[11]

Due to the co-prevalence of different genotypes of viruses, and the administration of their corresponding live attenuated vaccines in the pig population, natural recombination has been frequently documented in the genomes of several swine viruses, including African Swine Fever virus,[12] Porcine Reproductive and Respiratory Syndrome Virus,[13] Porcine Circovirus (PCV) Type 2,[14] and

***Correspondence:**

Zao-fu Deng. DVM
Hunan Biological and Electromechanical Polytechnic, Changsha, China
E-mail: dengzaofu@hnbemc.edu.cn

This work is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International (CC BY-NC-SA 4.0) which allows users to read, copy, distribute and make derivative works for non-commercial purposes from the material, as long as the author of the original work is cited properly.

Porcine Epidemic Diarrhea Virus.[15] In recent years, natural recombination in the PRV genome derived from different genotypes or wild and live attenuated vaccines has been documented in China, resulting in modified viral virulence and accelerated genome evolution.[16-18]

In this study, a field PRV strain was isolated from a pig farm vaccinated with the PRV HB-98 strain. The *gC*, *gD*, *gE* and *TK* genes of this strain were amplified by polymerase chain reaction (PCR), and their genetic characteristics were further analyzed. Subsequently, it's *in vitro* biological features and pathogenicity in mice were explored in comparison with other PRV strains. This study facilitates our understanding of the evolution of PRV, which will further guide the development of vaccines for PR control and prevention in the future.

Materials and Methods

Cells, viruses, animals and ethics statement. Vero cells and PK15 cells were cultured in Dulbecco's Modified Eagle Medium (Gibco, Grand Island, USA) supplemented with 10.00% foetal bovine serum (Gibco), 100 U mL^{-1} penicillin, and 100 µg mL^{-1} streptomycin sulfate (Biosharp, Hefei, China) at 37.00 ˚C under 5.00% CO_2. HB-98 is a live attenuated vaccine strain derived from the Ea strain with two gene deletions (*gG* and *TK*). The field PRV strain (PRV-HuN) was isolated from a pig farm in Hunan province. Further investigation revealed that PRV-HuN was genetically similar to PRV variant strains that are prevalent in China. Specific-Pathogen-Free Kunming mice were purchased from Hunan SJA Laboratory Animal Co., Ltd, Changsha, China.

Virus isolation and identification of filed PRV. The brain and lymph node samples were obtained from a pig farm in Hunan province, where piglets vaccinated with HB-98 exhibited clinical symptoms suspected of PRV infection, including severe diarrhea, vomiting and neurological disorders, resulting in high mortality rates. The tissue samples were cut into pieces, placed in sterilized phosphate-buffered saline and subjected to three freezing-thawing cycles. The supernatants were obtained to extract the DNA/RNA genome using a commercial DNA/RNA isolation kit (Hunan Aikuirui Biotechnology Co. Ltd., Changsha, China). RNA was transcribed into cDNA using a Revert Aid First-Stranded cDNA Synthesis Kit (Hunan Aikuirui Biotechnology Co. Ltd.). Real-time PCR assays were performed to screen for the presence of potential viral pathogens, including PRV, PCV2, PCV3, Japanese encephalitis virus, Classical swine fever virus, Porcine Reproductive and Respiratory Syndrome Virus, and African Swine Fever virus using the corresponding commercial viral detection kits (Wuhan Keqian Biology Co., Ltd., Wuhan, China). The PRV-positive supernatants were incubated with PK15 cells in a 6-well plate at 37.00 ˚C for 1 hr. Then, the supernatants were removed and replaced with Dulbecco's Modified Eagle Medium supplemented with 2.00% foetal bovine serum. The infected cells and supernatants were collected when cyto-pathic effects were detected in 70.00 to 80.00% of infected cells. The plaque formation assay was performed to select single plaques for further molecular identification.[19]

Polymerase chain reaction amplification, sequencing, and genetic analysis. The viral genome of PRV-infected cells was extracted using a commercial viral DNA genome extraction kit (Hunan Aikuirui Biotechnology Co.). The full-length *gC*, *gD*, *TK*, and *gE* genes of the isolated PRV strain were amplified by PCR with corresponding primers (Table 1). Each PCR reaction (50.00 µL) comprises 25.00 µL of 2.00X PCR mix, 1.00 µL of each primer, 0.50 µL of dimethyl sulfoxide, 3.00 µL of DNA template, and 20.50 µL of nuclease-free water. The PCR reaction was subjected to a temperature cycle of 98.00 ˚C for 1 min, followed by 35 cycles of 98.00 ˚C for 1 min, 58.00 ˚C for 15 sec, 72.00 ˚C for 30 sec. This was followed by a 5-min incubation at 72.00 ˚C. The purified positive PCR fragments were then sent to a commercial company for sequencing.

The *gC*, *gD*, *TK*, and *gE* genes of this novel PRV strain along with the corresponding gene sequences of the representative PRV strains obtained from the GenBank database were aligned using DNAStar version 7.10 software (DNAStar, Inc, Wisconsin, USA). Phylogenetic trees were reconstructed based on these gene sequences using the neighbour-joining method in MEGA Software (version 7.0; Biodesign Institute, Tempe, USA).

Virus growth. The growth characteristics of the novel isolated virus (designated HuN-YY) were investigated by infecting monolayer Vero cells and PK15 cells seeded in a 6-well plate with HuN-YY, HB-98, or PRV-HuN strain at a multiplicity of infection of 0.05 for 1 hr. The cells and supernatants were harvested at 12 hr post-

Table 1. Primers used in this study.

Primers	Sequence (5'-3')	Length (bp)	Reference sequence
PRV-gC-F	CGCCACTAGCATTAAATCC	1791	KP257591
PRV-gC-R	TCTCGCAGATGATGTCCC		
PRV-gE-F	ATGCGGCCCTTTCTGCTGCGC	1740	KP257591
PRV-gE-R	TTAAGCGGGGCGGGACATCAAC		
PRV-TK-F	GCACCCCGAGGTTGACTTCA	1125	KP257591
PRV-TK-R	AGGGTCACACCCCCATCTCC		
PRV-gD-F	ATTTGAATTCCCCAGGTTCCCATAC	1579	KP257591
PRV-gD-R	CTTGAAGCTTGGCAGAGGTCGTAC		

infection (hpi), 24 hpi, 36 hpi, and 48 hpi, respectively. Following three rounds of freeze-thaw, the supernatants were collected to determine the virus titer in Vero cells.

Animal experiment in mice. Seventy-eight female Kunming mice (aged 6 weeks) were purchased from Hunan SJA Laboratory Animal Co. Ltd. They were randomly divided into 13 groups with six mice per group. Each mouse was then challenged with 10^2, 10^3, 10^4, or 10^5 tissue culture infectious dose 50% ($TCID_{50}$) of the HuN-YY, HB-98, or the PRV-HuN strain by hind footpad injection. The mice in the placebo group were injected with an equal volume of phosphate-buffered saline. The clinical symptoms and mortality rate of the mice in each group were monitored daily for 2 weeks. In addition, the viral copies in the brain, lung, heart and liver tissues of mice challenged with 10^4 $TCID_{50}$ of different PRV strains were quantified by real-time PCR.

Results

Isolation of HuN-YY and its biological characteristics. The results of PCR/ reverse transcription -PCR assays showed that the tissue samples obtained from PR-suspected piglets were positive for PRV-gE and PRV-gB nucleic acids, but negative for other potential causative pathogens, including Classical swine fever virus, African Swine Fever virus, Japanese encephalitis virus, PCV2, PCV3, and Porcine Reproductive and Respiratory Syndrome Virus, and bacteria (data not shown). This indicated that the issue in this pig industry was caused by field PRV. As illustrated in Figure 1, typical PR-specific cytopathic effects were observed in PK15 cells incubated with the supernatants of PRV-positive tissue samples, which exhibited similar characteristics to those of the HB-98 vaccine and PRV-HuN strains (Fig. 1). Further one-step growth assays demonstrated that the PRV-HuN and HuN-YY strains exhibited similar growth characteristics in both PK15 and Vero cells. However, they exhibited a faster replication rate than that of the HB-98 vaccine strain, as evidenced by the higher viral titers of the PRV-HuN and HuN-YY strains at the 48 hpi time point (Fig. 2).

Genetic features and phylogenetic analysis of HuN-YY strain. The full-length *gC* (1,464 bp), *gD* (1,209 bp), *gE* (1,740 bp), and *TK* (963 bp) genes of HuN-YY strain were successfully amplified by PCR (Fig. 3).

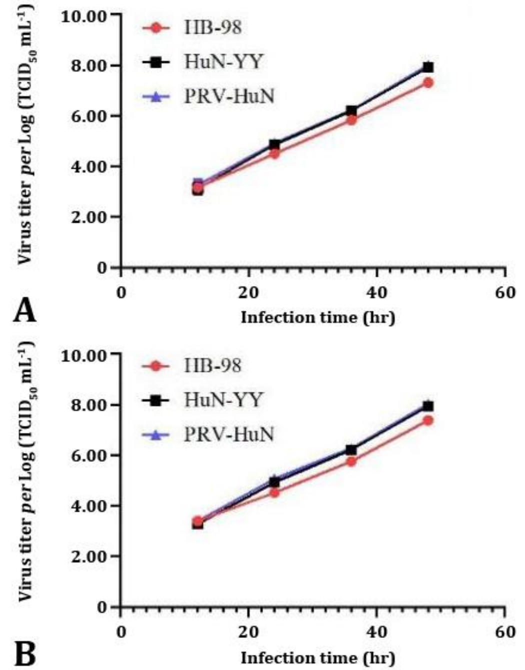

Fig. 2. One step growth curve of three the PRV strains in **A)** PK15 cells and **B)** Vero cells. TCID50: Tissue culture infectious dose 50.00%.

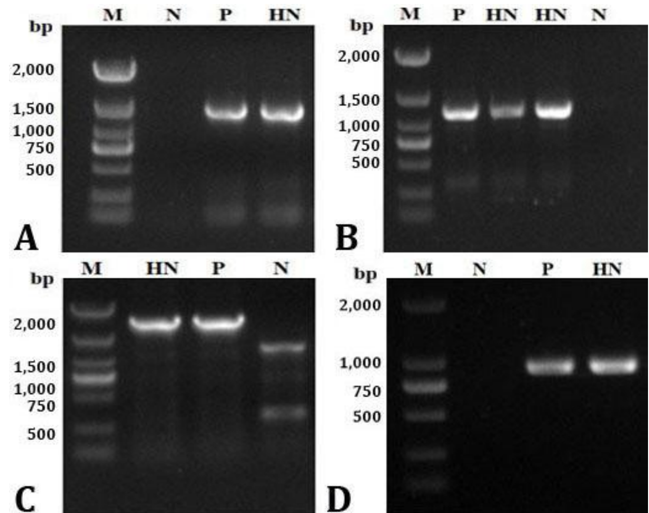

Fig. 3. Gel electrophoresis of: **A)** *gC* (1,464 bp), **B)** *gD* (1,209 bp), **C)** *gE* (1,740 bp), and **D)** *TK* (963 bp) gene products of HuN-YY strain. M: DL2000 DNA marker; N: negative control; P: positive control; HN: HuN-YY strain.

Fig. 1. The biological features of the HuN-YY strain *in vitro*, (100×). The cytopathic effects in **A)** PK15 cells infected with HuN-YY, **B)** Pseudorabies virus (PRV)-HuN and **C)** HB-98 strains.

The *gD*, *gE*, and *TK* genes of HuN-YY strain exhibited the highest nucleotide sequence homologies with variant PRV strains, including the HuN1 and JS-2012 strains. The *gC* gene shared a higher degree of sequence similarity with variant PRV strains (such as the Ea and Fa strains) than with PRV variants. Further phylogenetic analysis demonstrated that the *gE*, *gD*, and *TK* genes of the HuN-YY strain and other representative PRV variants constituted a single clade (Fig. 4A, 4B, and 4D), whereas the *gC* gene of this novel strain exhibited genetic proximity to classical PRV strains previously isolated in China, including the Ea and Fa strains (Fig. 4C).

The changed virulence of HuN-YY strain in mice. To assess the virulence of the HuN-YY strain *in vivo*, six-week-old Kunming mice were infected with different doses of PRV strains (HB-98, HuN-YY, and PRV-HuN) or an equivalent volume of Dulbecoo's Modified Eagle Medium. As shown in Table 2, all mice (6/6) exhibited 100% mortality following challenge with a high dose of the HuN-YY or PRV-HuN strain (10^4 and 10^5 $TCID_{50}$). All mice died within 96 hr after being challenged with 10^3 $TCID_{50}$ of PRV-HuN strain, while the mortality rate of mice was only 33.33% (2/6) when incubated with 10^3 $TCID_{50}$ of HuN-YY strain. However, all mice remained alive even when infected with 10^5 $TCID_{50}$ of HB-98 vaccine strain. Also, the viral copy number in the brain, heart, lung and liver tissues of PRV-HuN-infected mice was markedly higher than that in HuN-YY-infected mice (Fig. 5). These findings suggest that the virulence of the HuN-YY strain is less pronounced than that of the PRV-HuN strain in the murine model.

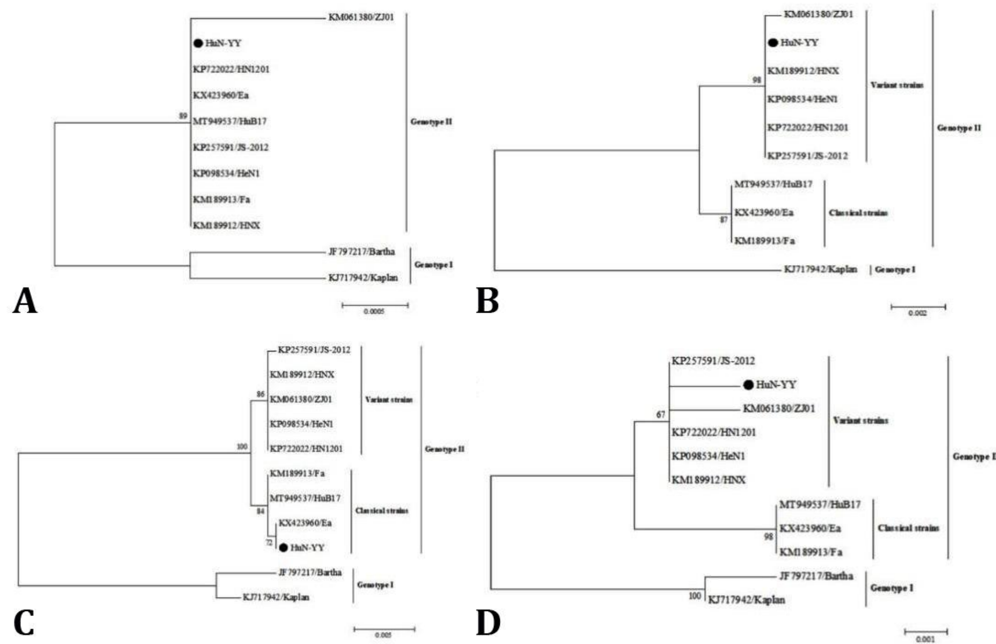

Fig. 4. Phylogenetic trees based on **A)** *TK*, **B)** *gE*, **C)** *gC*, and **D)** *gD* genes of pseudorabies virus strains, were generated by the neighbor-joining method using the MEGA Software (version 7.0; Biodesign Institute, Tempe, USA).

Table 2. The pathogenicity of different PRV strains in mice.

Strains	Dose (TCID50)	Morbidity	Mortality (Mean days of death)	LD_{50}
	10^5	6/6	6/6 (~2.90)	
	10^4	6/6	6/6 (~3.50)	
PRV-HuN	10^3	6/6	6/6 (~4.00)	$10^{3.0}$
	10^2	4/6	3/6 (~5.00)	
	10^1	2/6	0/6 (0)	
	10^5	6/6	6/6 (~3.50)	
	10^4	6/6	6/6 (~4.00)	
HuN-YY	10^3	4/6	2/6 (~4.60)	$10^{3.7}$
	10^2	1/6	1/6 (~5.80)	
	10^1	0/6	0/6 (0)	
	10^5	0/6	0/6 (0)	
	10^4	0/6	0/6 (0)	
HB-98	10^3	0/6	0/6 (0)	-
	10^2	0/6	0/6 (0)	
	10^1	0/6	0/6 (0)	
DMEM	-	0/6	0/6 (0)	-

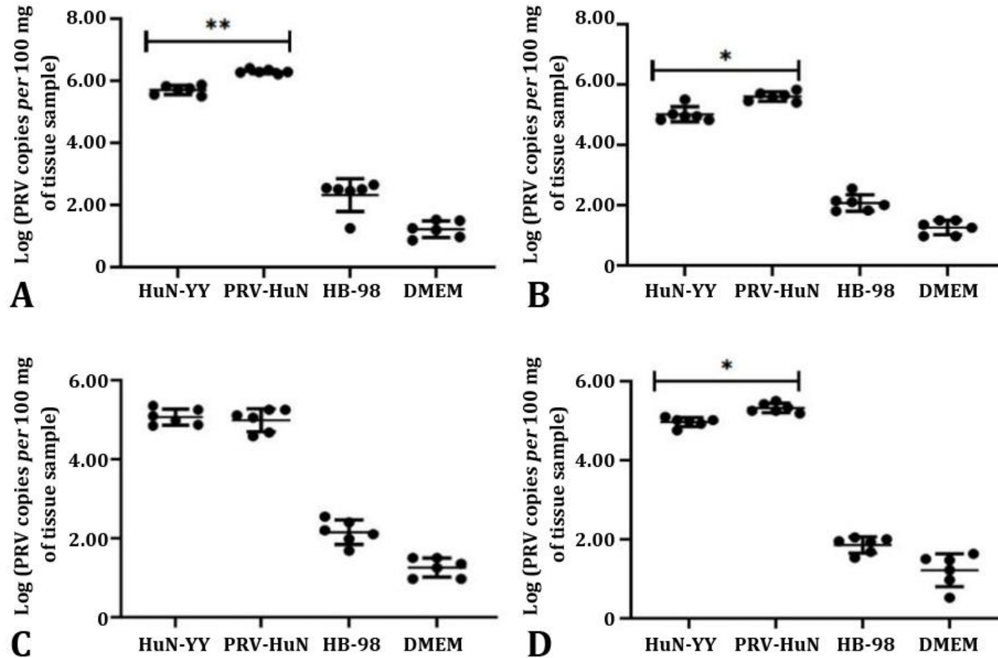

Fig. 5. Virus copy number of three pseudorabies virus (PRV) strains in **A)** brain, **B)** liver, **C)** heart and **D)** lung tissue samples of mice. DMEM: Dulbecco's Modified Eagle Medium. * $p < 0.05$ and ** $p < 0.01$.

Discussion

Since the first outbreak of variant PRV infection in China in 2011, the prevalence of these novel strains has attracted widespread attention. Traditional vaccines derived from the Bartha-K61 strain could not provide complete protection against variant PRV strains.[20,21] In addition, the cross-species transmission ability of PRV infecting other mammalian species, including humans, has been reported in recent years.[22] In light of this, several live attenuated PRV vaccines derived from classical PRV strains have been created and applied in the Chinese pig industry for PRV eradication. In recent years, the prevalence of novel PRV strains with inter- or intra-genotype recombination has been monitored in China, which is important for its genomic evolution.[23]

In this study, we isolated a field PRV strain from an HB-98-vaccinated pig farm in China, and further investigated its biological and genetic characteristics. The results showed that the HuN-YY strain might be a natural recombinant strain derived from variant and classical PRV strains or from variant PRV strain and the HB-98 live attenuated vaccine strain (Fig. 6). The supported evidence is as follows: 1) The *gC* gene of the HuN-YY strain shared nearly 100% sequence homology with Ea and Fa strains, while its *gD*, *gE* and *TK* genes showed higher sequence similarities to PRV variants. 2) Similarly, the *gC* gene of the HuN-YY strain was classified within the group containing classical PRV strains, while the *gD* and *gE* genes of this novel strain had closer genetic relationships with variant PRV strains than with other PRV strains. 3) The HuN-YY strain displayed similar replication characteristics in Vero and PK15 cells, while the virulence of the former was lower than that of the latter.

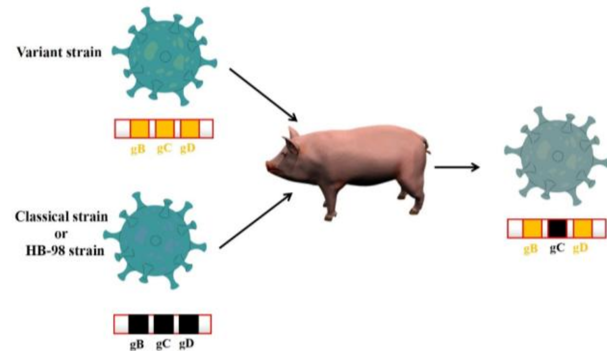

Fig. 6. Model of recombinant event occurred at the *gC* gene of the HuN-YY strain, which might be recombinant from the HB-98 and variant pseudorabies virus (PRV) strain or from classical and variant PRV strains.

The gC protein encoded by the PRV *UL44* gene is involved in viral attachment to the cell surface.[24] Additionally, this protein acts as a major antigen that can trigger the production of neutralizing antibodies against PRV infection.[25] Wu et al. discovered that replacing the *gC* gene of a variant PRV strain with that of a classical PRV strain led to a significant decrease in viral adsorption.[26] Another study showed that a single substitution of the *gC* gene of the Bartha-K61 strain with that of a PRV variant strain HB1201 could provide complete protection against the lethal challenge by PRV variant strain HB1201.[27] Consistent with these findings, our study revealed that the

mortality rate of mice exposed to 10^3 $TCID_{50}$ of PRV-HuN strain was significantly higher than that of HuN-YY strain. Further experiments will be performed to assess the pathogenicity of this novel recombinant strain in the porcine model.

In recent years, several novel recombinant PRV strains have been documented in China,[23] the recombination sites were mainly located in the TK,[11,18] gB,[7,28] and other regions.[23] Undoubtedly, the pathogenicity of these recombinant strains has been altered compared to the parental strains, the prevalence of these strains in swine farms cannot be ignored.[11] In view of this, the findings in this study suggest that the use of live attenuated vaccines or the co-prevalence of multiple genotypes could increase the recombination frequency of the viral genome.[11]

It should be noted that the biological and genetic characteristics of the HuN-YY strain have not been fully investigated. Firstly, we have only amplified the *gC*, *gD*, *TK* and *gE* genes of this novel strain and analyzed their genetic characteristics, further studies will be carried out to explore its complete genomic features. Secondly, it can be concluded that the HuN-YY strain is genetically recombinant from variant and classical PRV strains, or from a variant PRV strain and live attenuated strain derived from classical PRV strains. Future efforts will focus on the molecular epidemiology of PRV to confirm whether classical PRV strain was prevalent in this pig farm.

In conclusion, a novel recombinant PRV strain was isolated and identified in this study. It may have originated from a recombination of classical and variant PRV strains or from a combination of the HB-98 strain and PRV variants. Furthermore, its pathogenicity in mice was found to be different from that of the variant PRV strain. These findings highlight that it is necessary to continuously monitor the epidemiological characteristics, including recombination events, of field PRV strains.

Acknowledgments

This research was supported by a grant from the 2022 Hunan Vocational Education (Zhuzhou, China) “Chuyi” Action High Level Teacher Team Construction Project (Xiangjiaotong, 2023; No. 211).

Conflict of interest

All authors declare no conflict of interest.

References

1. Mettenleiter TC. Aujeszky's disease (pseudorabies) virus: the virus and molecular pathogenesis--state of the art, June 1999. Vet Res 2000; 31(1): 99-115.
2. Abbate JM, Giannetto A, Iaria C, et al. First isolation and molecular characterization of pseudorabies virus in a hunting dog in Sicily (southern Italy). Vet Sci 2021; 8(12): 296. doi: 10.3390/vetsci8120296.
3. Cheng Z, Kong Z, Liu P, et al. Natural infection of a variant pseudorabies virus leads to bovine death in China. Transbound Emerg Dis 2020; 67(2): 518-522.
4. Tan L, Yao J, Yang Y, et al. Current status and challenge of pseudorabies virus infection in China. Virol Sin 2021; 36(4): 588-607.
5. Hou Y, Wang Y, Zhang Y, et al. Human encephalitis caused by pseudorabies virus in China: a case report and systematic review. Vector Borne Zoonotic Dis 2022; 22(7): 391-396.
6. Zhang Y, Fang L, Zhou Y, et al. A case report of long-delayed diagnosis of pseudorabies virus encephalitis with endophthalmitis: lessons from metagenomic next generation sequencing. BMC Neurol 2023; 23(1): 192. doi: 10.1186/s12883-023-03227-1.
7. Zhai X, Zhao W, Li K, et al. Genome characteristics and evolution of pseudorabies virus strains in eastern China from 2017 to 2019. Virol Sin 2019; 34(6): 601-609.
8. Xu M, Zhu L, Ge A, et al. Construction of pseudorabies virus variant attenuated vaccine: codon deoptimization of *US3* and *UL56* genes based on PRV gE/TK deletion strain. Front Microbiol 2023; 14: 1248573. doi: 10.3389/fmicb.2023.1248573.
9. Ma Z, Jiang C, Liu D, et al. Pathogenicity and immunogenicity of a quadruple gene-deleted pseudorabies virus variant as a vaccine candidate. Vet Microbiol 2024; 288: 109931. doi: 10.1016/j.vetmic.2023.109931.
10. Lv L, Liu X, Jiang C, et al. Pathogenicity and immunogenicity of a *gI/gE/TK/UL13*-gene-deleted variant pseudorabies virus strain in swine. Vet Microbiol 2021; 258: 109104. doi: 10.1016/j.vetmic.2021.109104.
11. Tan L, Yao J, Lei L, et al. Emergence of a novel recombinant pseudorabies virus derived from the field virus and its attenuated vaccine in China. Front Vet Sci 2022; 9: 872002. doi: 10.3389/fvets.2022.872002.
12. Zhao D, Sun E, Huang L, et al. Highly lethal genotype I and II recombinant African swine fever viruses detected in pigs. Nat Commun 2023; 14(1): 3096. doi: 10.1038/s41467-023-38868-w.
13. Pan J, Zeng M, Zhao M, et al. Research progress on the detection methods of porcine reproductive and respiratory syndrome virus. Front Microbiol 2023; 14: 1097905. doi: 10.3389/fmicb.2023.1097905.
14. Zhan Y, Wang N, Zhu Z, et al. In silico analyses of antigenicity and surface structure variation of an emerging porcine circovirus genotype 2b mutant, prevalent in southern China from 2013 to 2015. J Gen Virol 2016; 97(4): 922-933.
15. Chen J, Tian L, Liu Y, et al. Molecular characterization and phylogenetic analysis of porcine epidemic diarrhea

virus in Xinjiang, China, from 2020 to 2022. Arch Virol 2024; 169(5): 96. doi: 10.1007/s00705-024-06029-z.

16. Bo Z, Miao Y, Xi R, et al. Emergence of a novel pathogenic recombinant virus from Bartha vaccine and variant pseudorabies virus in China. Transbound Emerg Dis 2021; 68(3): 1454-1464.
17. Lian Z, Liu P, Zhu Z, et al. Isolation and characterization of a novel recombinant classical pseudorabies virus in the context of the variant strains pandemic in China. Viruses 2023; 15(9): 1966. doi: 10.3390/v15091966.
18. Qin Y, Qin S, Huang X, et al. Isolation and identification of two novel pseudorabies viruses with natural recombination or *TK* gene deletion in China. Vet Microbiol 2023; 280: 109703. doi: 10.1016/j.vetmic. 2023.109703.
19. Tang YD, Liu JT, Fang QQ, et al. Recombinant pseudorabies virus (PRV) expressing firefly luciferase effectively screened for CRISPR/Cas9 single guide RNAs and antiviral compounds. Viruses 2016; 8(4): 90. doi: 10.3390/v8040090.
20. An TQ, Peng JM, Tian ZJ, et al. Pseudorabies virus variant in Bartha-K61-vaccinated pigs, China, 2012. Emerg Infect Dis 2013; 19(11): 1749-1755.
21. Tong W, Liu F, Zheng H, et al. Emergence of a pseudorabies virus variant with increased virulence to piglets. Vet Microbiol 2015; 181(3-4): 236-240.
22. Bo Z, Li X. A Review of pseudorabies virus variants: genomics, vaccination, transmission, and zoonotic potential. Viruses 2022; 14(5): 1003. doi: 10.3390/ v14051003.
23. Liu Q, Kuang Y, Li Y, et al. The epidemiology and variation in pseudorabies virus: a continuing challenge to pigs and humans. Viruses 2022; 14(7): 1463. doi: 10.3390/v14071463.
24. Yao J, Li J, Gao L, et al. epidemiological investigation and genetic analysis of pseudorabies virus in Yunnan province of China from 2017 to 2021. Viruses 2022; 14(5): 895. doi: 10.3390/v14050895.
25. Zuckermann FA, Zsak L, Mettenleiter TC, et al. Pseudorabies virus glycoprotein gIII is a major target antigen for murine and swine virus-specific cytotoxic T lymphocytes. J Virol 1990; 64(2): 802-812.
26. Wu H, Qi H, Wang B, et al. The mutations on the envelope glycoprotein D contribute to the enhanced neurotropism of the pseudorabies virus variant. J Biol Chem 2023; 299(11): 105347. doi: 10.1016/j.jbc. 2023.105347.
27. Ren J, Wang H, Zhou L, et al. Glycoproteins C and D of PRV strain HB1201 contribute individually to the escape from Bartha-K61 vaccine-induced immunity. Front Microbiol 2020; 11: 323. doi: 10.3389/ fmicb.2020.00323.
28. Huang J, Zhu L, Zhao J, et al. Genetic evolution analysis of novel recombinant pseudorabies virus strain in Sichuan, China. Transbound Emerg Dis 2020; 67(4): 1428-1432.