Analysis of genetic variations of a recombinant strain derived from the three lineages of porcine reproductive and respiratory syndrome virus-2

Limei Zheng[1], Xiaoyan Wu[2], Chang Liu[2], Chen Li[2], Jianli Shi[2], Mengjie Huang[3], Yingru Ma[1], Hong Han[2], Shaojian Xu[2], Nataliia Hrabchenko[2], Jianhua Qiu[3], Jianxin Wen[1], Jun Li[1,2] and Xianjie Han[1*]

[1]Qingdao Agricultural University, Qingdao, China.
[2]Shandong Provincial Key Laboratory of Animal Disease Control and Breeding, Institute of Animal Science and Veterinary Medicine Shandong Academy of Agricultural Sciences, Jinan, China
[3]College of Animal Science and Veterinary Medicine Shandong Agricultural University, Taiwan, China

---

ABSTRACT

Since the rise of the porcine reproductive and respiratory syndrome virus (PRRSV) in China, gene mutations have frequently occurred. To understand the current prevalence and evolution of PRRSV in Shandong Province, 1,528 samples suspected of PRRSV were collected from local pig farms of different sizes. The complete genome sequence of the PRRSV strain SDLY-27 was determined by next-generation sequencing (NGS) technology. The genomic sequence of SDLY-27 was 15,363 nucleotides (nt) in length, comparative analysis of the whole genome sequence suggested that the homology between SDLY 27 and 81 PRRSV strains from China and other countries in genbank was 61.9 ~ 96.4%. This study is the first to detect recombinants from multiple recombination events among the Lineage 8 (JXA1-like strains), Lineage 5 (RespPRRSV-MLV and VR2332 strains) and Sublineage 1.5 (NADC34-like strains) in Shandong, China, and provides new data for the epidemiological study of PRRSV in China. This study enriches the epidemiological data on PRRSV in Shandong Province, China. It provides an important reference for the development of new vaccines and for the prevention and control of PRRSV in China.

Key words: PRRSV; recombination strains; GP5; NSP2; genetic evolutionary analysis; next-generation sequencing

---

Introduction

Porcine reproductive and respiratory syndrome (PRRS) is a highly infectious disease caused by the porcine reproductive and respiratory syndrome virus (PRRSV) (MONTANER-TARBES et al., 2019). The disease often causes infected pigs to have blue and purple ears. In the affected pigs, this disease mainly leads to spontaneous abortion in late pregnancy, stillbirth, mummified fetuses or weak piglets. It also causes congenital dysplasia, respiratory distress, interstitial pneumonia and immunosuppression in piglets. In addition, it often occurs in mixed infection with other pathogens,

---

such as porcine circovirus and resistant bacterial species (YAN et al., 2020; ZHANG et al., 2022). PRRSV is one of the most serious pathogens threatening global pig production. After it began to spread in Europe and America in the 1990s, it began to spread around the world.

The porcine reproductive and respiratory syndrome virus (PRRSV) is a small, enveloped, single-stranded positive-sense RNA virus, belonging to the order *Nidovirales*, family *Arteriviridae* (NELSEN et al., 1999). PRRSV is mainly divided into two genotypes: PRRSV-1 (European type, a prototype strain Lelystad virus) and PRRSV-2 (American type, a prototype strain VR-2332). PRRSV2 strains have been predominant in Chinese swine herds since their initial emergence in 1996, and are further divided into nine lineages based on the ORF5 sequence (PAPLOSKI et al., 2019). To fully understand the evolutionary patterns and dynamics of PRRSV, and to aid prevention and control policies against the disease, a genomic scale analysis was necessary.

Materials and methods

Sample collection and sample handling. In this study, suspected cases of PRRS were collected from pig farms of different sizes in some cities of Shandong Province in 2020-2022. Blood and nasal cotton swabs were mainly collected from affected pigs, while lymph nodes and lung tissues were collected from dead pigs. The lymph nodes and pulmonary tissues were collected aseptically, excised and crushed in a suspension. The syringe of the received blood samples was placed on the experimental table at an angle of 45° and left to stand at room temperature for 30 min. After the serum was precipitated, the samples were transferred to a 1.5mL centrifuge tube using a micropipette, and centrifuged at room temperature at 6,000 rpm for 3 min. Red-blood cell precipitation at the bottom of the tube was visible to the naked eye, and the upper layer was a light-yellow clarified liquid. The supernatant was taken for subsequent testing, and the backup was stored in the -80°C refrigerator. The nasal swabs obtained were centrifuged at 6,000 rpm at room temperature for 3 min, and the supernatant was taken for subsequent testing. The backup was stored in the refrigerator at -80°C.

Viral RNA extraction, reverse transcription and ORF5 determination. The viral solution was added to the RNA isolater, and the total RNA was extracted according to the instructions of the RNA isolater Total RNA Extraction Reagent (Vazyme, Nanjing, China). With the use of a HiScript III 1st Strand cDNA Synthesis Kit (Vazyme, Nanjing, China), the resulting total RNA was reverse transcribed into the cDNA in accordance with the instructions. PCR amplification was performed using the primers (Table 1). The primer NSP2-F/R was as reported in previous research (YIN et al., 2021). In the experiment, we used positive and negative controls. The positive control was positive serum that had already been stored in our laboratory for the experiment. The amplification reactions were carried out in a 25 μL reaction volume containing 12.5 μl of EmeraldAmp® PCR Master Mix (2×Premix), 0.5 μM of the forward primer, 0.5 μM of the reverse primer, 1 μL of cDNA, and an appropriate volume of double-distilled (dd) H2O. The cycling parameters were: 35 cycles of 98°C for 5 s, different annealing temperature for 30 s, and 72°C for 30 s, followed by a final extension at 72°C for 10 min. The different annealing temperatures for different primers are detailed in Table 1. The PCR products were analyzed by electrophoresis in a 1.5% agarose gel under Gel Doc™XR+ (Bio Rad, USA), and the samples with positive results were recorded. After the amplification, products were purified using the E.Z.N.A Gel Extraction kit (Omega, USA), and cloned into a P-Easy-Blunt Zero cloning kit (Tran, Beijing, China). Each fragment was sequenced at least three times. All nucleotide sequences generated in this study were submitted to the GenBank database.

The complete genome sequence of the PRRSV strain SDLY-27 was determined by next-generation sequencing (NGS) technology (Shanghai Tan Pu Biotech Yao Yan Company). The next-generation sequencing, also known as high throughput sequencing, is a DNA sequencing technology developed on the basis of PCR and gene chips. All samples with positive RT-PCR findings were sequenced for the ORF5 gene.

Table 1. List of primers used in this study

Name	Primer sequence (5'-3')	Length of the amplicon (bp)	Annealing temperature (°C)
ORF5 F	AGGTGGGCAACTGTTTTAGC	697	53.5
ORF5 R	TTTGTGGAGCCGTGCTATCA		
Nsp2-F	GAAGGGAATTGTGGTTGGCA	2175	57.6
Nsp2-R	AGACCCAGAAAACACACCCA		
PRRSV F	GAGTTTCAGCGGAACAATGG	451	58.6
PRRSV R	GCCGTTGACCGTAGTGGAG		

F represents forward PCR primer; R represents reverse PCR primer

Sequence comparison and evolutionary analysis. Phylogenetic trees were constructed with MEGA-X (Version10.1) using the maximum-like hood method. Bootstrap values were calculated on 1,000 replicates of the alignment. The evolutionary trend of PRRSV in China was analyzed based on nucleotide sequences of CN/Q9/2021 and other known isolates of China and other countries. Multiple sequence alignments were generated with the MegAlign software DNAstar 7.0 software (DNASTAR Inc., Madison, WI, USA). To explore the genetic variation of PRRSVs of China and other countries, comparison of GP5 and of the SDLY-27 isolate with 81 other strains of China and other countries were performed. The detailed information of the selected PRRSV reference strains is shown in Supplementary Table 2.

Recombination analysis. In this study, the full-length genome of SDLY-27 was sequenced for recombination analysis. Alignment was screened using RDP4 (v3.5.1, JHK University, Baltimore, MD, USA), implementing the RDP, GENECONV, Bootscan, Chimaera, SiSan, MaxChi, and 3 Seq algorithms. At least seven of the above methods can identify a recombination event. In addition, if the breakpoint region of the recombination event is larger than 100 nt, the region can be regarded as a recombination region. Recombination events were only considered significant (P-value $< 1 \times 10^{-6}$) when supported by at least five of the seven detection methods. To visualize the recombinant signal and inferred breakpoint locations, a similarity analysis between the presumptive recombinant sequences and the parental lineages was implemented in Simplot v3.5.1. The window size was set to 200 nt and the step size to 20 nt.

Virus isolation and electron microscopy. Marc-145 cells were propagated in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum. Lymph node homogenates were suspended in DMEM (10% v/v) and then subjected to centrifugation. The supernatant was filtered (0.22 μm filter) and applied to inoculate MARC-145 cells. Then, the isolated viruses were amplified at 37°C with 5% CO_2 and monitored daily for cytopathic effects (CPEs). The culture supernatants were harvested after 60 h and stored at −80°C as the virus stock until use.

For visualization of the virion particles in infected-cell culture medium, PRRSV-infected MARC-145 cell culture media were clarified by centrifugation at 1,847 g for 30 min at 4°C. After filtration through 0. 22 μm filters, the virus medium was further ultracentrifuged at 106,750 g for 3h at 4°C using an ultracentrifuge (Beckman Coulter, Miami, FL, USA). The virus pellets were resuspended in DMEM. The prepared cell culture sample was stained with an equal volume of 3% phosphotungstic acid (PTA) (pH 7.0) in 0.4% sucrose for 1 min and then applied onto a 300-mesh

Formvar- and carbon-coated copper grid for 5 min. After blotting and drying, the grids were examined with an H7500 electron microscope (Hitachi High Technologies, Tokyo, Japan).

Results

RT-PCR results, virus isolation and electron micrographs. Throughout Shandong Province, a total of 1,528 suspected PRRS-positive samples were gathered in the years 2020–2022. From the virus fluid that had been cultivated, total RNA was isolated and then converted into cDNA. cDNA was used as the template for the PCR. According to the findings, there were 76 samples that tested positive for PRRSV, and the detection rate was 6.04%. Marc-145 cells injected with PRRSV SDLY-27 are shown on electron micrographs. There are spikes that resemble a crown. At 60 hours, SDLY-27 was injected into Marc-145 cells that had no CPE (Fig. 1).

Genomic characteristics of the PRRSV SDLY-27 isolate. Using Marc-145 cells, one PRRSV strain was successfully identified; the isolate was found in a Shandong sample. The genome sequence of the SDLY-27 strain was submitted to GenBank under the accession number OP805381. It was 15,363 nucleotides (nt) in length and comprised an 181 nt 5' UTR and a 200 nt 3' UTR (Fig. 3 B). A highly pathogenic PRRSV named SDLY-27 has been discovered.

Analysis on homology of PRRSV and other countries in China. After a previous analysis of the whole genome sequence, it was found that the homology between SDLY 27 and 80 PRRSV strains from China and other countries in the GenBank was 61.9 ~ 96.4%. The genome sequences of the isolate SDLY-27 showed the highest nucleotide identities (96.4%, respectively) with the highly-pathogenic strain CN/Q9/2021 (Table 2).
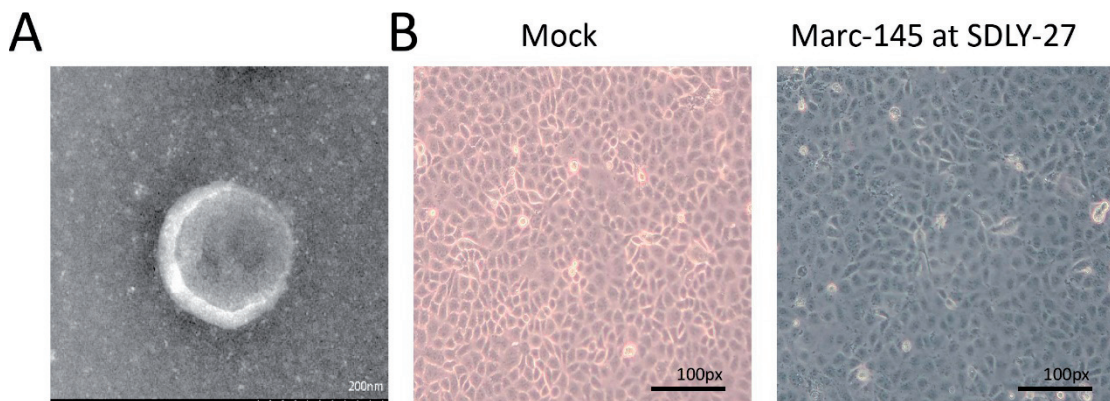

Fig. 1. (A) Electron micrographs of PRRSV SDLY-27 inoculated Marc-145 cells. Crown-shaped spikes are visible. The samples were negatively stained with 3% phosphotungstic acid. Bar, 200 nm. (B) Cytopathic effect (CPE) in Marc-145 cells at 60h post-infection with SDLY-27. Scale bar = 100×

Phylogenetic analysis of the PRRSV isolates SDLY-27. The sequences related to PRRSV Sublineage 1.5, Sublineage 1.8, Lineage 3, Lineage 5, and Lineage 8 were downloaded from the NCBI database as reference strains. These were analyzed using MEGA X and MegAlign. The ORF5 genomic sequences of the three PPRSV strains were deposited in the GenBank database under the respective accession numbers: OP730529 (SDLY-27), OP730530 (SDLY-28), OP730531(SDLY-32). The NSP2 genomic sequences of the four PPRSV strains were deposited in the GenBank database under the respective accession numbers: OP785695 (SDLY-28), OP785696 (SDLY-32), OP785693 (SDLY-176), OP785694 (SDLY-177). The whole genomic sequences of the PPRSV strains were deposited in the GenBank database under the accession number: OP805381.

The strains circulating in China mainly cluster into four lineages: lineage 8 strains which are also called highly-pathogenic PRRSV strains represented by TJ (Accession no. EU860248), JXA1 (Accession no. EF112445), HuN4 (Accession no. JF268674), and CH-1a (Accession no. AY032626); lineage 5 strains which are also called classic PRRSV strains represented by VR2332; lineage 3 strains which are also called QYYZ-like strains represented by QYYZ (Accession no. JQ308798) and GM2 (Accession no. JN662424); and lineage 1 strains which are also called NADC30-like strains represented by NADC30 (Accession no. JN654459) and NADC34 (Accession no. MF326985) (SHI et al., 2010; ZHOU et al., 2018) (Fig. 2A). According to the phylogenetic tree generated on the basis of the whole genome sequences, SDLY-27 isolates clustered in lineage 8 with highly-pathogenic PRRSVs like CH-1a, TJ, JXA1, and HuN4. Interestingly, SDLY-27 clustered into lineage 5 together with VR2332, RespPRRS MLV, in the phylogenetic tree generated on the basis of the ORF5 gene, indicating that these strains might undergo recombination events (Fig. 2 B).

Recombination analysis of the PRRSV isolate. BLAST analysis showed that the SDLY-27 sequence had high homology with JXA1-like strains. RDP4 and SimPlot (version 3.5.1) were used to test for recombinations of SDLY-27. The RDP4 analysis results showed that SDLY-27 was a recombinant strain from JXA1 and RespPRRSV-MLV strains, with a potential crossover event spanning NSP 2b and ORF 4. The recombinant events in the PRRSV genomes were confirmed by a Bootscan analysis in Simplot software (v3.5.1, JHK University, Baltimore, MD, USA). For SDLY-27, four recombination breakpoints located in ORF2(nt 12197, nt 12736), ORF3 (nt 12736, nt 13256), ORF4 (nt 13256), and ORF5 (nt 13756) were identified in its genome (Fig. 3A). The breakpoints in SDLY-27 divided its genome into five fragments (Fig. 3B). The phylogenetic trees showed that fragment A (5′UTR-ORF2, nt1-12197) was closely related to the JXA1-like strains. Fragment C (ORF2-ORF4, nt 12737-13256) and fragment E (ORF5-3′UTR, nt 13756-15363) were closely associated with the RespPRRSV-MLV strains. The phylogenetic trees showed that fragment B (ORF2-ORF3, nt 12198-12736) and fragment D (ORF4- ORF5, nt 13256-13736) were closely related to the NADC34-like strains. These results indicated that the SDLY-27 strain was likely to have originated from multiple recombination events among Lineage 8 (JXA1-like strains), Lineage 5 (RespPRRSV-MLV and VR2332 strains) and Sublineage 1.5(NADC34-like strains).

ORF5 sequence analysis of the three PRRSV isolates. Sequence comparison of ORF5 genes showed that the nucleotide sequence identities of the ORF5 genes among the three isolates ranged from 98% to 99%, and the amino acid similarities of the GP5 proteins ranged from 98.5% to 99.7%. Compared to the GP5 protein of RespPRRS MLV, as shown in Fig. 4, three isolates (SDLY-27, SDLY-28, and SDLY-32) had mutations at sites 35 (S→N). In addition, three isolates (SDLY-27, SDLY-28, and SDLY-32) had mutations at sites 13 (Q→R) in the signal peptide, sites 58 (Q→D) in the HVR 2, sites 51 (K→R) in the 151, sites 170 (E→G) and sites 171 (G→S) in the unknown region, two isolates (SDLY-27 and SDLY-28) had mutations at sites 6 (L→W) in the signal peptide and sites 59 (K→R) in the HVR 2, the isolates SDLY-27 had mutations at sites 16 (S→F). However, the three isolates (SDLY-27, SDLY-28, and SDLY-32) showed no amino acid substitution of the decoy epitope, the primary neutralizing epitope (PNE), the transmembrane regions 1 (TM1), the transmembrane regions 2 (TM2), the transmembrane regions 3 (TM3), T cell epitope 1, T cell epitope 2, or B cell[3rd] epitope in this region.

Table 2. PRRSV strains used in this study

NO.	Isolate	Identity(%)a	Access no.	Year	Region
1	15HEB1	94.4	KX815411	2016	Jiangsu China
2	15HEN1	85.8	KX815413	2016	Jiangsu China
3	15LN3	87.2	KX815425	2016	Jiangsu China
4	2020Acheng1	83.2	MW079495	2020	Jilin China
5	BJ4	88.8	AF331831	2000	Beijing China
6	BJEU061	61.9	GU047344	2009	Beijing China
7	CG	95	EU864231	2008	Guangdong China
8	CHGX2019	95	MZ219272	2022	Guangxi China
9	CH1a	92.4	AY032626	1998	Heilongjiang China
10	CH1R	92.3	EU807840	2008	Heilongjiang China
11	CH2018NCV Anheal1	83	MH370474	2018	Beijing China
12	CHsx1401	83.80	KP861625	2015	Beijing China
13	CHWH20191	84.8	MK450333	2019	Hubei China
14	Em2007	91	EU262603	2007	Hubei China
15	FJ0908	83.3	MK202794	2018	Fujian China
16	FJFS	86.4	KP998476	2015	Fujian China
17	FJM4	83.8	KY412888	2017	Fujian China
18	FJW05	92.8	KP860911	2015	Fujian China
19	FJXS151	93.1	KX758250	2016	Fujian China
20	GM2	86.3	JN662424	2011	Guangzhou China
21	GZ1101	88.1	KF771273	2013	Beijing China
22	HB1sh2002	93.5	AY150312	2002	Beijing China
42	JS2021NADC34	82.6	MZ820388	2021	Jiangsu China
43	JXA1	95.4	EF112445	2006	Beijing China
44	rJXA1-R	95.7	MT163314	2020	Jiangsu China
45	KP	95.4	GU232735	2009	Guangdong China
46	KUN1606	83.5	MK057530	2018	Korea
47	LNCH1604	83.5	MH651741	2018	Heilongjiang China
48	LNDZD101806	82.8	MN648054	2019	Heilongjiang China
49	LNWK96	83	MG860516	2018	Heilongjiang China
50	LNWK130	83.1	MG913987	2018	Heilongjiang China
51	Minnesota7	84.6	KP283409	2014	USA
52	MN6	84.6	KP283401	2014	USA
53	MN414	84.1	KT581982	2015	USA
54	NADC30	84.8	JN654459	2011	USA
55	NCV1	83.4	ON950548	2022	USA
56	NVDCSD22012	95.4	KP771768	2015	Beijing China
57	QHD2	83.4	MH167387	2018	Hebei China
58	QYYZ	86.2	JQ308798	2011	Guangdong China
59	Resp-MLV	88.9	AF066183	2015	USA
60	RFLP	83.4	MW887655	2021	USA
61	S022	95.7	OM201198	2022	Heilongjiang China
62	SCcd17	87.1	MG914067	2018	Sichuan China
63	SCnj16	89.2	MF196906	2017	Sichuan China

Table 2. PRRSV strains used in this study (continued)

NO.	Isolate	Identity(%)a	Access no.	Year	Region	NO.	Isolate	Identity(%)a	Access no.	Year	Region
23	HeNanA9	92.4	KJ546412	2014	Heilongjiang China	64	SD1602	86.7	MH651743	2018	Heilongjiang China
24	HENZMD9	86.9	KU950374	2016	Henan China	65	SDbz162	84.6	MH588710	2018	Zhejiang China
25	HLHDZD321901	82.8	MN648449	2019	Heilongjiang China	66	SDhz1512	91.7	KX980392	2016	Shandong China
26	HLJB1	93.2	KT351740	2015	Henan China	67	SDYG1606	88.3	KY053458	2016	Shandong China
27	HLJZD221812	82.8	MN648450	2019	Heilongjiang China	68	SHB	93.1	EU864232	2008	Guangdong China
28	HNhx	85.3	KX766379	2016	Henan China	69	SP	87.7	AF184212	1999	Singapore
29	HNjz15	83.8	KT945017	2015	Henan China	70	TJbd14	95.1	KP742986	2015	Henan China
30	HUB1	95.2	EF075945	2006	Beijing China	71	TJ	95.5	EU860248	2008	Jilin China
31	HuN4	95.5	EF635006	2007	Heilongjiang China	72	TJnh1501	92.8	KX510269	2016	Beijing China
32	IA2014NADC34	83.6	MF326985	2017	USA	73	TP	95.4	EU864233	2008	Guangdong China
33	IA2015ISU14	83.4	MF327001	2017	USA	74	VR2332	88.4	EF536003	2007	USA
34	IA14737	83.9	MF663706	2017	USA	75	XJ175	94.8	MK759853	2019	Jiangsu China
35	ISU17	83.3	KT257967	2015	USA	76	XW007	87.9	KF724404	2013	USA
36	ISU30	83.6	KT257977	2015	USA	77	CN/Q9/2021	96.4	OL422843	2021	Fujian China
37	JBNU20N01	82.6	MZ287313	2021	Korea	78	GDST	95.6	KY498542	2017	Guangdong China
38	JL580	86.2	KR706343	2007	Heilongjiang China	79	rJXwn06	95.5	MF187956	2017	USA
39	13V117	62.00%	KT159249	2015	Belgium	80	PR402014	50.90%	MF346695	2017	Italy
40	HK10	62.60%	KF287131	2013	Hongkong China	81	WestSib13	62.10%	KX668221	2016	Russia
41	LNEU12	61.70%	KM196101	2014	Liaoning China						

The strains in the table are the reference strains used in this article. The identity of SDLY-27 from each of the different reference strains is shown in Table 1. Sequences information are retrieved from GenBank and obtained using BLAST algorithm.
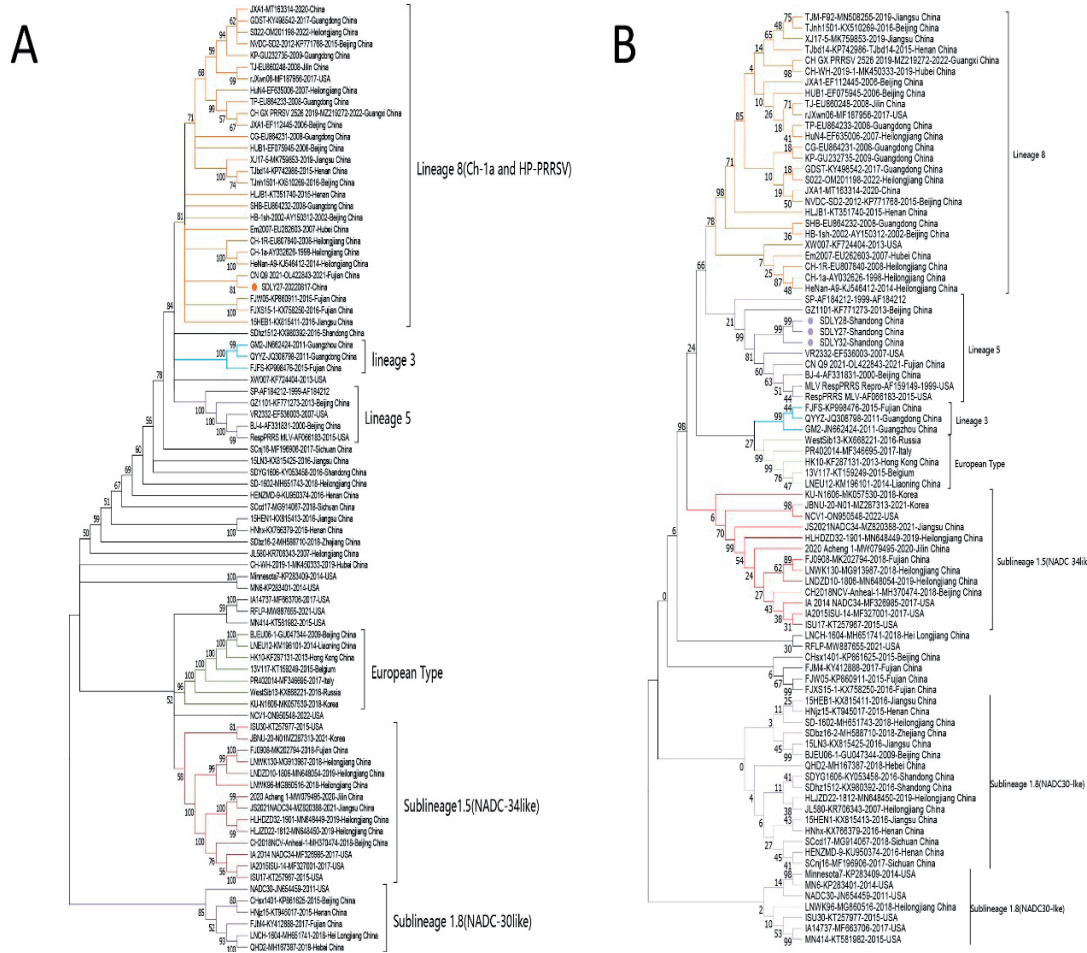
Fig. 2. They included similar sequences obtained using the BLAST algorithm. The categorization of the sub-lineage was based on the description by SHI et al. (2010). Phylogenetic trees based on the complete genome and ORF5 of PRRSV. (A) Complete genome-based tree. (B) ORF5-based tree. The phylogenetic tree was constructed using the Mega X distance-based maximum-likelihood method, with a total of 1000 replicates. The isolates recovered in this study are indicated by orange circles and purple circles.
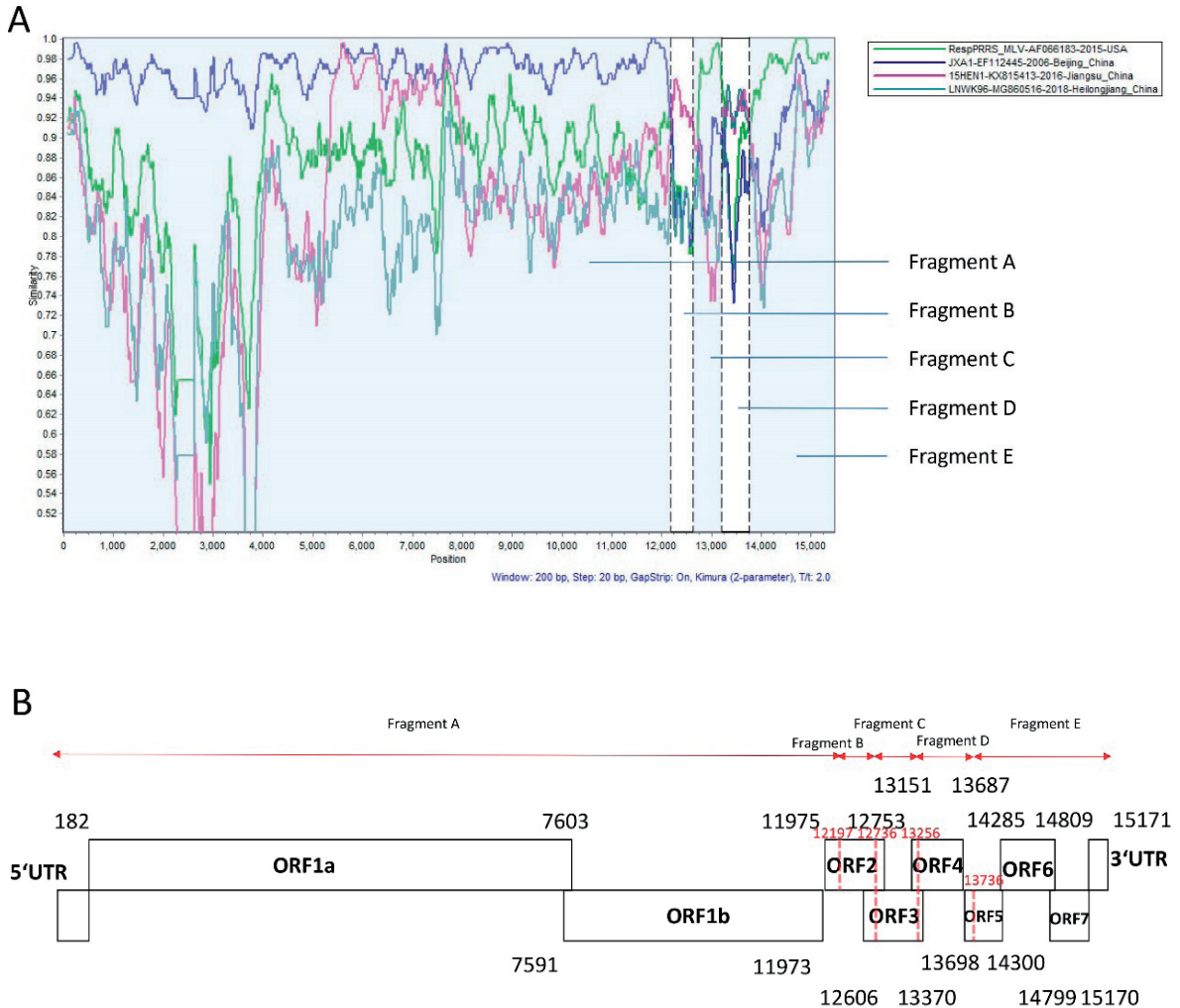
Fig. 3.（A）Simplot was used to compare the similarities of SDLY-27 Recombinant breakpoints are displayed as black virtual lines. RespPRRS-MLV, JXA1, 15HEN1 and LNWK96 are depicted by green, blue, pink and atrovirens colorations in the plotted similarity curves, respectively. (B）Below the similarity plot is a full genome structure of PRRSV, with reference to SDLY-27, that shows the position of the seven open reading frames.
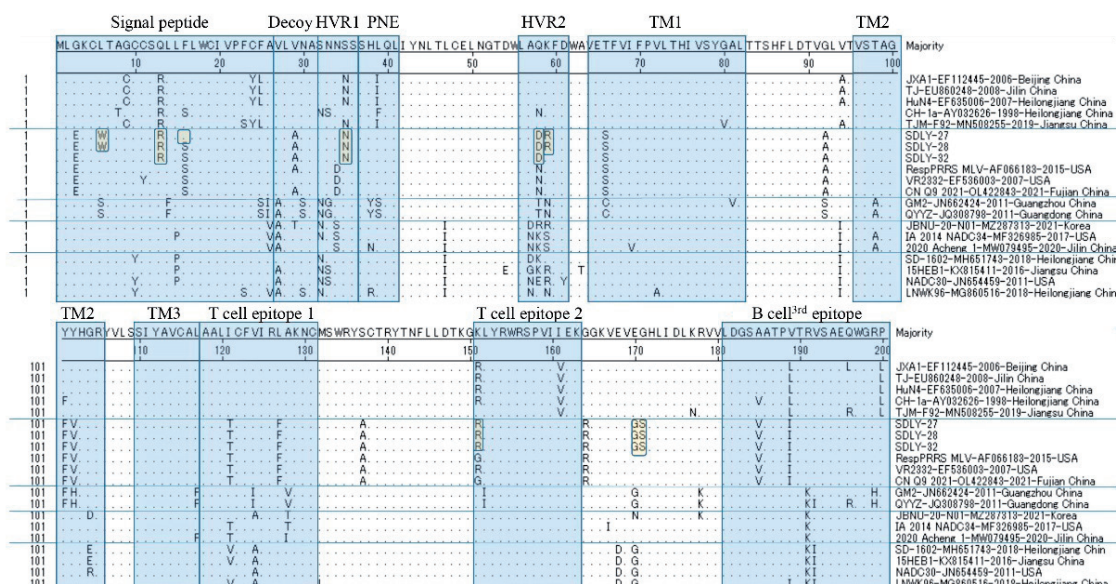
Fig. 4. Alignment of the ORF5 translated amino acid sequence. The signal peptide, the decoy epitope, HVR 1, PNE, HVR 2, TM1, TM2, TM3, T cell epitope 1, T cell epitope 2, B cell[3rd] epitope is denoted with those blue boxes. Compared to the GP5 protein of RespPRRS MLV, the variable region is shown in the yellow box.

NSP 2 sequence analysis of the five PRRSV isolates. The host immune response and viral replication are mostly regulated by the NSP 2 genes, which largely express some protease. Comparing the 15 PRRSVs from Central China's NSP2 amino acid sequences, we found 47.9-97.1% identity between five strains (SDLY-27, SDLY-28, SDLY-32, SDLY-176 and SDLY-177) and the 15 other PRRSVs of Central China. In this study, we compared the NSP2 of SDLY-27 with 15 strains from China and abroad, and we found that the NSP2 of five strains (SDLY-27, SDLY-28, SDLY-32, SDLY-176 and SDLY-177) were identified as a continuous 19-aa deletion from position 446 to 464 and included a discontinuous 20-aa deletion (including the usually missing 1-aa at position 432 and 19-aa at positions from 446-464), but two of them exhibited special mutations in NSP2, such as a continuous 120 deletion at positions from 446-464 in SDLY-176 and SDLY-177 (denoted with yellow boxes in Fig. 5).

Discussion

In particular, the emergence of HP-PRRSV has significantly increased the pathogenicity of PRRSV. It has brought huge economic losses to the Chinese pig farming industry. In the present study, a novel PRRSV-2 strain, SDLY-27, was found during epidemiological surveillance of PRRSV-2 in Shandong Province in China (KIM et al., 2022). Phylogenetic tree analysis based on the ORF5 gene showed that the three PRRSV isolates were divided into lineage 5, along with the representative VR2332 strains, but they were classified as JXA1-like strains with respect to the whole genome evolutionary trees. This study indicated that the SDLY-27 strains was likely to have originated from multiple recombination events among the Lineage 8, Lineage 5 and Sublineage 1.5. ZHAO et al. (2017) reported that nine of 28 isolates and one isolate from another laboratory were potential complicated recombinants between the vaccine JXA1-R strains and predominant circulating strains, and the PRRSV recombination rate increased under the current
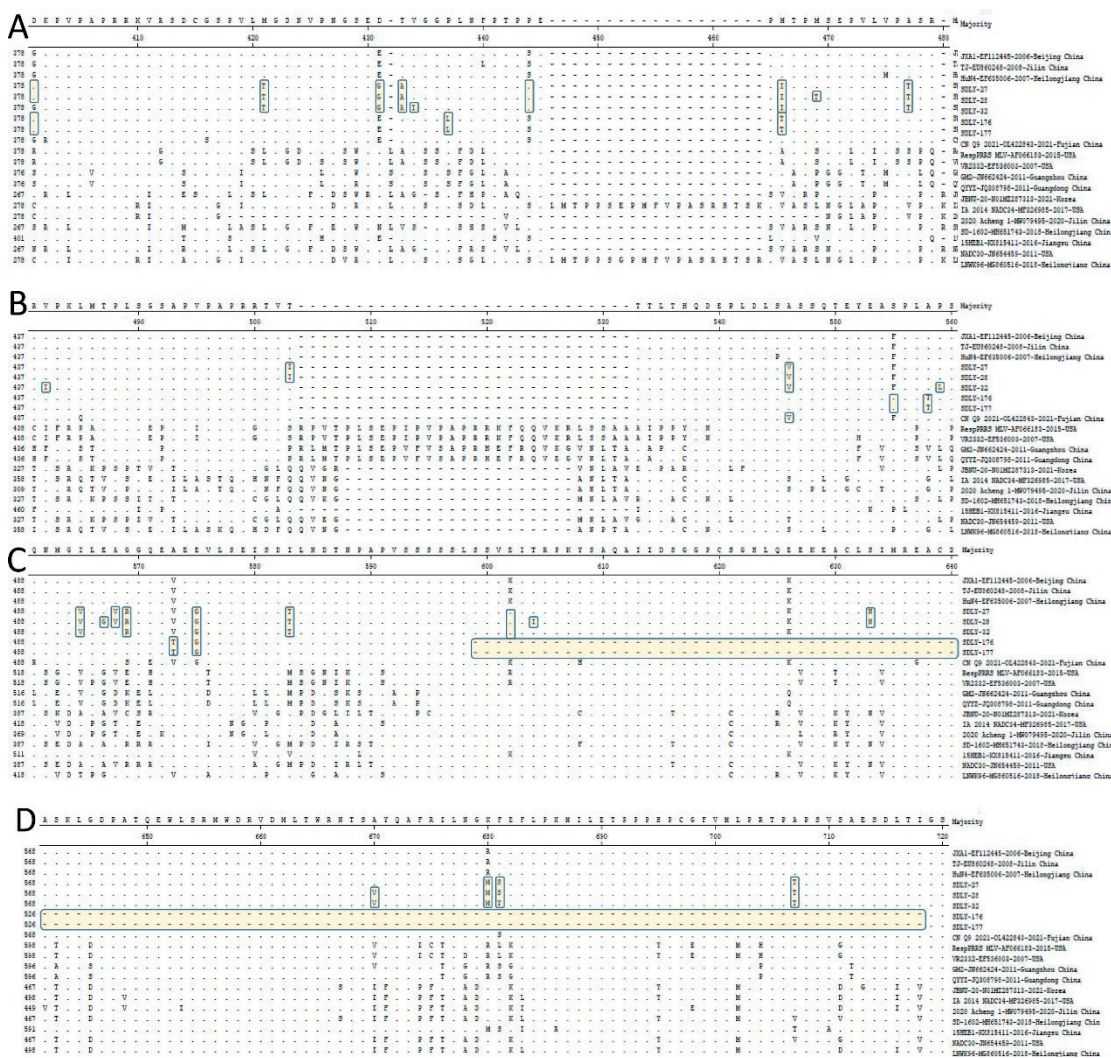
Fig. 5. Alignment of the partial NSP2 translated amino acid sequence. Compared to the GP5 protein of TJ, the variable region is shown in the yellow box.

vaccination pressure. LIU et al. (2018) reported that not only NADC30-like strains but also recombinant strains of NADC30 with classical PRRSV and/or HP-PRRSV have become the current popular trend.

The results of ZHANG et al. (2018) first confirmed the emergence of ORF5 RFLP 1-7-4 Like ((NADC34-like) PRRSVs in China. This makes it very important to monitor PRRSV and understand its prevalence in Shandong Province. The GP5 protein, as an extremely important PRRSV protein, has become an important indicator for the

identification and analysis of PRRSV. GP5 is the most heterogeneous structural protein of PRRSV, and it could induce IFN-β production in host cells, and plays a significant role during viral attachment and internalization (GAO et al., 2014). As shown in Fig. 4, the most variable regions of the GP5 protein were located in the hypervariable region 1 (HVR1), the hypervariable region 2 (HVR2) and the signal peptide. The variable N-linked glycosylation motif (30NASNDS35) is contained in the hypervariable region 1 (HVR1). The potential N-glycosylation

sites (NGSs) at N44 and N51 were conserved in all isolates, but the potential NGSs located upstream of N44 were relatively variable. This amino acid mutation pattern at site 35 (S→N) in the HVR 1, sites 58 (Q→D) in the HVR 2 which also occurred in the GP5 proteins of the TJM-F92 strains. Protein N-glycosylation modification is a common post-translational way for organisms to regulate the location, function, activity, lifespan and diversity of proteins in tissues and cells (XIE et al., 2021). It is also one of the important prerequisites for understanding the function of sugar chains. Therefore, the change of N-glycosylation will have a certain impact on the function of GP5 protein.

The Nsp2-coding region is recognized as one of the most variable proteins with different deletions and insertions. Additionally, this gene that has a highest genetic diversity in the whole sequences of PRRSV is used as a molecular marker for monitoring the molecular epidemiology and evolution of PRRSV (WANG et al., 2015). Previous research has also shown that Nsp2 contains many immunogenic epitopes, suggesting that it is an immunogenic protein in PRRSV that can cause the production of specific antibodies during the infection process.

Still, JXA1-like strains of PRRSV are the most common in Shandong Province. This study found a novel recombinant strain in Shandong, China, and adds fresh information to the epidemiological analysis of PRRSV there. The epidemiological information on PRRSV in Shandong Province, China, is improved by this study. It provides an important reference for the development of new vaccines and for the prevention and control of PRRSV (LI et al., 2022). Continuous surveillance of the dynamics of PRRSV evolution in Shandong China should be further performed.

Among all the PRRSV-positive samples, one recombination strain (SDLY-27) was isolated, and their complete genomic sequences were determined. RT-PCR was used to detect pathogenic bacteria, the ORF5 gene and the NSP2 gene were sequenced and analyzed. The complete genome sequence of the PRRSV strain SDLY-27 was determined by next-generation sequencing (NGS) technology. The recombination of SDLY-27 was quite complex and demonstrated different degrees of recombination formed by three strains (HP-PRRSV, NADC34 and classical PRRSV). Moreover, the findings play an important role in preventing and controlling the occurrence and spread of PRRSV.

Conclusions

In summary, a PRRSV strain, SDLY-27, was isolated from the serum of piglets in eastern China. SDLY-27 inoculated Marc-145 cells at 60h showing no CPE. Recombination analysis revealed that the newly emerged PRRSV isolate exhibits a novel recombination pattern. They both likely resulted from multiple recombination events among lineage 8 (JXA1-like), lineage 1 (NADC34-like) and lineage 5 (RepsPRRS MLV-like) strains that have circulated in China recently. Our study highlights the importance of continuous monitoring of PRRSVs in China and the necessity for new vaccine development. Finally, we also identified several vaccine-derived recombinant strains, which once more raises the question of the safety of these vaccines.

Author contributions

Limei Zheng, Xiaoyan Wu,Chang Liu, and Jun Li contributed equally to this work

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

Ackowledgements

References

GAO, J., P. JI, M. ZHANG, X. WANG, N. LI, C. WANG, S. XIAO, Y. MU, Q. ZHAO, T. DU, Y. SUN, J. A. HISCOX, G. ZHANG, E. M. ZHOU (2014): GP5 expression in Marc-145 cells inhibits porcine reproductive and respiratory syndrome virus infection by inducing beta interferon activity. Vet. Microbiol. 174, 409-418.
https://doi.org/10.1016/j.vetmic.2014.09.030

KIM, S. C., S. H. MOON, C. G. JEONG, G. S. PARK, J. Y. PARK, H. Y. JEOUNG, G. E. SHIN, M. K. KO, S. H. KIM, K. K. LEE, H. S. CHO, W. I. KIM (2022): Whole-genome sequencing and genetic characteristics of representative porcine reproductive and respiratory syndrome virus (PRRSV) isolates in Korea. Virol. J. 19, 66.
https://doi.org/10.1186/s12985-022-01790-6

LI, P., Y. SHEN, T. WANG, J. LI, Y. LI, Y. ZHAO, S. LIU, B. LI, M. LIU, F. MENG (2022): Epidemiological survey of PRRS and genetic variation analysis of the ORF5 gene in Shandong Province, 2020-2021. Front. Vet. Sci. 9, 987667.
https://doi.org/10.3389/fvets.2022.987667

LIU, Y., J. LI, J. YANG, H. ZENG, L. GUO, S. REN, W. SUN, Z. CHEN, X. CONG, J. SHI, L. CHEN, Y. DU, J. LI, J. WANG, J. WU, J. YU (2018): Emergence of Different Recombinant Porcine Reproductive and Respiratory Syndrome Viruses, China. Sci. Rep. 8, 4118.
https://doi.org/10.1038/s41598-018-22494-4

MONTANER-TARBES, S., H. A. DEL PORTILLO, M. MONTOYA, L. FRAILE (2019): Key Gaps in the Knowledge of the Porcine Respiratory Reproductive Syndrome Virus (PRRSV). Front. Vet. Sci. 6, 38.
https://doi.org/10.3389/fvets.2019.00038

NELSEN C. J, M. P. MURTAUGH, K. S. FAABERG (1999): Porcine reproductive and respiratory syndrome virus comparison: divergent evolution on two continents. J. Virol. 73, 270-280.
https://doi.org/10.1128/JVI.73.1.270-280.1999

PAPLOSKI, I. A. D., C. CORZO, A. ROVIRA, M. P. MURTAUGH, J. M. SANHUEZA, C. VILALTA, D. C. SCHROEDER, K. VANDERWAAL (2019): Temporal Dynamics of Co-circulating Lineages of Porcine Reproductive and Respiratory Syndrome Virus. Front. Microbiol. 10, 2486.
https://doi.org/10.3389/fmicb.2019.02486

SHI, M., T. T. LAM, C. C. HON, M. P. MURTAUGH, P. R. DAVIES, R. K. HUI, J. LI, L. T. WONG, C. W. YIP, J. W. JIANG, F. C. LEUNG (2010): Phylogeny-based evolutionary, demographical, and geographical dissection of North American type 2 porcine reproductive and respiratory syndrome viruses. J. Virol. 84, 8700-8711.
https://doi.org/10.1128/JVI.02551-09

WANG, F. X., L. T. QIN, Y. LIU, X. LIU, N. SUN, Y. YANG, T. CHEN, H. W. ZHU, J. Q. REN, Y. J. SUN, S. P. CHENG, Y. J. WEN (2015): Novel Nsp2 deletion based on molecular epidemiology and evolution of porcine reproductive and respiratory syndrome virus in Shandong Province from 2013 to 2014. Infect. Genet. Evol. 33, 219-226.
https://doi.org/10.1016/j.meegid.2015.05.006

XIE, C. Z., Z. WANG, Z. HA, Y. ZHANG, Y. B. XIE, H. ZHANG, F. L. NAN, J. Y. ZHANG, G. Y. ZHAO, Z. X. LI, C. H. LI, C. D. YU, P. ZHANG, H. J. LU, N. Y. JIN (2021): Genetic characterization of a new NSP2-deletion porcine reproductive and Respiratory Syndrome Virus in China. Microb. Pathog. 150, 104729.
https://doi.org/10.1016/j.micpath.2021.104729

YAN, X., J. ZENG, X. LI, Z. ZHANG, A. U. DIN, K. ZHAO, Y. ZHOU (2020): High incidence and characteristic of PRRSV and resistant bacterial Co-Infection in pig farms. Microb. Pathog. 149, 104536.
https://doi.org/10.1016/j.micpath.2020.104536

YIN, B., S. QI, W. SHA, H. QIN, L. LIU, J. YUN, J. ZHU, G. LI, D. SUN (2021): Molecular Characterization of the Nsp2 and ORF5 (ORF5a) Genes of PRRSV Strains in Nine Provinces of China During 2016-2018. Front. Vet. Sci. 8, 605832.
https://doi.org/10.3389/fvets.2021.605832

ZHANG, J., P. WANG, C. XIE, Z. HA, N. SHI, H. ZHANG, Z. LI, J. HAN, Y. XIE, X. QIU, Y. TAO, N. JIN, H. LU (2022): Synergistic Pathogenicity by Coinfection and Sequential Infection with NADC30-like PRRSV and PCV2 in Post-Weaned Pigs. Viruses 14, 193.
https://doi.org/10.3390/v14020193

ZHANG, H. L., W. L. ZHANG, L. R. XIANG, C. L. LENG, Z. J. TIAN, Y. D. TANG, X. H. CAI (2018): Emergence of novel porcine reproductive and respiratory syndrome viruses (ORF5 RFLP 1-7-4 viruses) in China. Vet. Microbiol. 222, 105-108.

https://doi.org/10.1016/j.vetmic.2018.06.017

ZHAO, H., Q. HAN, L. ZHANG, Z. ZHANG, Y. WU, H. SHEN, P. JIANG (2017): Emergence of mosaic recombinant strains potentially associated with vaccine JXA1-R and predominant circulating strains of porcine reproductive and respiratory syndrome virus in different provinces of China. Virol. J. 14, 67.

https://doi.org/10.1186/s12985-017-0735-3

ZHOU, L., R. KANG, Y. ZHANG, M. DING, B. XIE, Y. TIAN, X. WU, L. ZUO, X. YANG, H. WANG (2018): Whole Genome Analysis of Two Novel Type 2 Porcine Reproductive and Respiratory Syndrome Viruses with Complex Genome Recombination between Lineage 8, 3, and 1 Strains Identified in Southwestern China. Viruses 10, 328.

https://doi.org/10.3390/v10060328

ZHENG, L., X. WU, C. LIU , C. LI, J. SHI, M. HUANG, Y. MA, H. HAN, S. XU, N. HRABCHENKO, J. QIU, J. WEN, J. LI, X. HAN: Analiza genskih varijacija rekombinantnog soja dobivenog iz triju linija virusa-2 reproduktivnog i respiratornog sindroma svinja.Vet. arhiv 94, 205-218, 2024.

SAŽETAK

Usporedno sa širenjem virusa reproduktivnog i respiratornog sindroma svinja (PRRSV) u Kini, sve su češće bile i njegove genske mutacije. Kako bi se ustanovila trenutačna prevalencija i evolucija PRRSV-a u pokrajini Shandong, s lokalnih farmi prikupljeno je 1528 uzoraka svinja različitih kategorija za koje je postojala sumnja na zarazu PRRSV-om. Kompletan genomski slijed soja SDLY-27 PRRSV-a određen je tehnologijom sekvenciranja sljedeće generacije (NGS). Slijed je imao dužinu od 15 363 nukleotida (nt), a komparativna analiza cijeloga genomskog slijeda uputila je na to da je homolognost između sojeva SDLY 27 i 81 PRRSV-a iz Kine i uzoraka u banci gena iz drugih zemalja 61,9~96,4%. Ovo je prvo istraživanje koje je otkrilo rekombinantne sojeve iz višestrukih rekombinacija među linijama 8 (sojevi nalik na JXA1), 5 (sojevi RespPRRSV-MLV i VR2332) i podlinije 1,5 (sojevi nalik na NADC34) u Shandongu, Kina.Kao takvo, istraživanje pruža nove podatke o epidemiologiji PRRSV-a u Kini, posebno u pokrajini Shandong, a ujedno predstavlja i važnu referenciju za razvoj novih cjepiva te prevenciju i kontrolu bolesti uzrokovane navedenim virusom.

Ključne riječi: PRRSV; rekombinantni sojevi; GP5; NSP2; genetička evolucijska analiza; sekvenciranje sljedeće generacije