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Genetic evolution analysis of PRRSV ORF5 gene in five provinces of Northern China in 2024

Yanyin Jian[1†], Chun Lu[1†], Yuan Shi[1], Xiangyu Kong[1], Jun Song[1] and Jintao Wang[1*]

Abstract

Background Porcine reproductive and respiratory syndrome (PRRS) was first discovered in North America in 1987, and since then it has been spread widely all over the world. The prevalence of PRRS has caused significantly economic losses to pig industry in many countries.

Objectives Investigate the prevalence and genetic evolution of porcine reproductive and respiratory syndrome virus (PRRSV) in five provinces of northern China.

Methods 190 samples suspected of PRRS were collected from 28 pig farms in five provinces of northern China. The PRRSV ORF7 and ORF5 gene were detected by RT-PCR, and the ORF5 gene were sequenced for the homology and genetic evolution analysis.

Results The positive samples of ORF7 gene were 50, and its positive rate was 26.32%. The positive samples of ORF5 gene were 48, and its positive rate was 25.26%. The sequenced results of the ORF5 gene showed that 48 positive samples all belonged to PRRSV-2. Among them, 26 samples were NADC34-like strains, 17 samples were NADC30-like strains, and 5 samples were classical strains. The amino acid sequence analysis of PRRSV GP5 indicated that there was a deletion at the 37th amino acid in 4 NADC30-like strains. The amino acids of the transmembrane region 1 in all positive strains are relatively conserved, and multiple amino acid mutations were observed in the signal peptide, transmembrane region 2, and B cell epitope. The amino acid mutations were different in different strains and regions. The above results demonstrated that the complexity and diversity of PRRSV genetics.

Conclusion The strains from lineage 1 became the dominant strains in five provinces of northern China in 2024. The positive rate of NADC34-like strains was the highest in Heilongjiang Province and the NADC30-like strains were the most prevalent in these regions. The genetic evolution of PRRSV presented a complex trend. This study provided the data support for understanding PRRSV variation and for PRRS prevention and control in five provinces of northern China.

Keywords PRRSV, ORF7 gene, ORF5 gene, Genetic evolution analysis, NADC30-like strain, NADC34-like strain

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) caused by porcine reproductive and respiratory syndrome virus (PRRSV) is an infectious disease characterized by reproductive disorders in sows and respiratory difficulties in piglets [1]. This disease was first discovered in North America in 1987 and since then it has been spread widely all over the world [2]. PRRSV, a member of the *Arterivirus* family, is an enveloped positive-sense single-stranded RNA virus with approximately 1.5 kb genome and at least 10 ORFs [2]. PRRSV contains 8 structural proteins (GP2, GP3, GP4, GP5, GP5a, E, M, and N) and 16 non-structural proteins (nsp1α, nsp1β, nsp2-6, nsp2TF, nsp2N, nsp7a, nsp7b, and nsp8-12) [3, 4]. Among them, the ORF7 gene encoded the conserved N protein is usually used as the test target [5]. The GP5 contains the important neutralization epitope, but their homology among different strains ranges from 88 to 99%, which makes it the key protein for PRRSV research [6]. At the same time, the ORF5 gene encoded the GP5 is often used as the gene target for PRRSV epidemiological investigation and genetic evolution analysis due to its high degree of variation [7].

PRRSV is defined as two different pathogens: PRRSV-1 and PRRSV-2 by the International Committee on Taxonomy of Viruses in 2021 [8]. The nucleotide homology between them is only about 60%, and their pathogenicity and antigenicity is also different [9, 10]. PRRSV-1 is mainly prevalent in Europe, and PRRSV-2 is primarily dominant in Americas and Asia [11]. PRRSV-2 is divided into 11 lineages and 21 sublineages according to the ORF5-based lineage classification, and the lineage 1, 3, 5, and 8 are the mainly prevalent lineages in China [9, 12]. PRRSV CH-1a strain isolated by Guo et al. in 1996 was classified as the lineage 8 and was a sign that PRRSV became prevalent in China [13]. In 1997, PRRSV BJ-4 strain classified as the lineage 5 was isolated in China [14]. Before 2006, the two strains were mainly prevalent in China, and they were closely related to North American isolates [15]. In 2006, the 'high fever syndrome' characterized by the high fever and high mortality outbroke in some pig farms in southern China and caused a devastating blow to the pig industry. It was later confirmed that the pathogen was the highly pathogenic PRRSV strains, which was classified as the lineage 8 [16, 17]. In 2010, QYYZ strain classified as the lineage 3 was first discovered in China, and these strains were mainly prevalent in central and southern China [18]. Later, the NADC30-like strains classified as the sublineage 1.8 has gradually become the dominant strains in China, these strains share the highest homology with the NADC30 strain from USA [19, 20]. In 2017, two NADC34-like strains belonged to the sublineage 1.5 were identified for the first time in China, and BLAST analysis indicated that the two strains were likely derived from the IA/2014/NADC34 strain, and then their detection rate increased year by year [21]. The common feature of NADC34-like strains is the abortion in sows, but it is relatively mild for piglets. The different recombination modes of the NADC34-like strains may lead to differences in the virulence and clinical symptoms [22, 23].

The PRRSV strains showed the diversified development after the outbreak of African swine fever in China, so there was great significance in improving production performance of the pig industry through the effective prevention and control of PRRS [24]. In recent years, there were some reports on the epidemiological investigation and variation analysis of PRRSV in some regions of China [7, 9, 21]. However, the reports were still lack in the northern region, especially the northeast region. Therefore, this study collected the tissue and blood samples suspected of PRRS in five provinces of northern China in 2024 and conducted the genetic evolution analysis of PRRSV ORF5 gene in order to provide the data support for PRRS prevention and control in these regions.

Table 1 Sample distribution in five provinces in 2024

Province	Number of farms	Number of samples
Inner Mongolia	4	12
Heilongjiang	8	83
Jilin	2	10
Liaoning	8	45
Shandong	6	40
Total	28	190

Materials and methods

Sample collection

190 tissue and blood samples suspected of PRRS were collected from 28 pig farms in Inner Mongolia Autonomous Region, Heilongjiang, Jilin, Liaoning and Shandong Province during 2024 (Table 1), and stored at -80 ℃ for use. The sources and types of the samples were described in detail in supplementary Table S1. All samples in this study were friendly provided by the pig farm. The animals were euthanized through the ear vein injection of sodium pentobarbital at a dose of 150 mg per kg body weight. The euthanasia took place in a soundproof room to prevent the distress in the remaining pigs. After the samples were collected, the animals were transferred to the harmless treatment center for conducting the harmless treatment.

Primer design and synthesis

The nucleotide sequences of the PRRSV ORF5 gene were downloaded from NCBI, and compared by MegAlign software. The primers of the ORF5 gene were designed by Oligo7.0 software (Table 2), and the primers of the PRRSV ORF7 gene were from GB/T 18,090 – 2023 [25]

Table 2 Primer sequence of PRRSV ORF7 and ORF5 gene

Primer	Primer sequence (5′-3′)	Product (bp)
PRRSV-ORF7-F	ATGGCCAGCCAGTCAATCA	PRRSV-1: 398
PRRSV-ORF7-R	TCGCCCTAATTGAATAGGTGACT	PRRSV-2: 433
PRRSV-ORF5-F	ATGTTGGGGAAATGCTTGAC	603
PRRSV-ORF5-R	CTAGAGACGACCCCATTGTTC	

(Table 2). Both of them were synthesized by Sangon Biotech (Shanghai) Co., Ltd.

Sample Preparation and PCR amplification

100 mg tissue sample was ground with liquid nitrogen, and the tissue homogenate was prepared with 300 μL PBS in a 1.5 mL sterile EP tube. Total RNA was extracted from the homogenate or blood sample by TRIzol Universal Reagent (TIANGEN, Beijing, China) and transcribed into cDNA (Takara Bio, Dalian, China). The PCR reaction system of ORF7 gene is 50 μL, including 5 μL of PCR buffer (Takara Bio, Dalian, China), 0.5 μL of Taq enzyme (Takara Bio, Dalian, China), 1 μL of dNTPs (Takara Bio, Dalian, China), 3 μL of $MgCl_2$ (Takara Bio, Dalian, China), 1μL of forward and reverse primers each, 10 μL of template and 28.5 μL of RNase-free dH_2O. The reaction condition is pre-denaturation at 94 ℃ for 5 min; denaturation at 94 ℃ for 30 s, annealing at 55 ℃ for 40 s, extension at 72 ℃ for 50 s, 35 cycles; extension at 72 ℃ for 10 min. The PCR reaction system of ORF5 gene is 50 μL, including 25 μL of PreminxTaq™ (Takara Bio, Dalian, China), 2.5 μL of forward and reverse primers each, 5 μL of template, and 15 μL of RNase-free dH_2O. The reaction condition is pre-denaturation at 95 ℃ for 5 min; denaturation at 94 ℃ for 1 min, annealing at 54 ℃ for 30 s, extension at 72 ℃ for 40 s, 35 cycles; extension at 72 ℃ for 10 min. PCR products were detected by 1.5% agarose gel electrophoresis. The results were analyzed with full-automatic gel imaging analysis system (Bio Rad, USA) and used for the statistical analysis. At the same time, the positive products of ORF5 gene were sequenced by Sangon Biotech (Shanghai) Co., Ltd.

Homology and phylogenetic tree analysis of PRRSV ORF5 gene

The reference sequences of PRRSV ORF5 gene were downloaded from NCBI (supplementary Table S2), and the homology and phylogenetic tree analysis of PRRSV ORF5 gene were performed by MegAlign and MEGA11 software.

The amino acid sequence analysis of PRRSV GP5

30 positive samples from 22 farms were chosen to perform the full-length sequencing of the ORF5 gene for the amino acid sequence analysis. 30 sequencing results and 7 reference sequences of the ORF5 gene were inputted into MEGA11 software. The nucleotide sequences were translated into the amino acid sequences, which was followed by the multiple sequence alignment. Finally, the amino acid sequences of PRRSV GP5 were analyzed by Jalview software.

Results

RT-PCR results

The RT-PCR results of ORF7 and ORF5 gene was shown in Fig. 1. The results indicated that the primers can be used for subsequent experiments.

Statistics and analysis of RT-PCR results of PRRSV ORF7 and ORF5 gene

190 samples from five provinces were used to RT-PCR amplification of the ORF7 and ORF5 gene. The results indicated that the positive number of the ORF7 gene was 50, and the positive rate was 26.32%. The positive number of the ORF5 gene was 48, and the positive rate was 25.26% (Table 3). The consistency rate of RT-PCR results of ORF7 and ORF5 gene was 98.95% (Table 4).

Homology and evolutionary tree analysis of PRRSV ORF5 gene

The ORF5 gene sequences from different lineages as the reference sequences, the homology and evolutionary tree analysis based on the obtained 48 ORF5 gene sequences were performed by MegAlign and MEGA11 software. The results of the homology (Fig. 2) and evolutionary tree analysis (Fig. 3) showed that 48 ORF5 gene sequences all belonged to PRRSV-2. Among them, 26 sequences possessed the highest homology with the IA/2014/NADC34 strain from 92.7 to 96.9%, and belonged to sublineage 1.5. 17 sequences possessed the highest homology with the NADC30 strain from 90.9 to 93.1%, and belonged to sublineage 1.8. 5 sequences possessed the highest homology with CH-1a strain from 99.1 to 99.3%, and belonged to lineage 8. No lineage 3 or lineage 5 strains were detected in all five provinces.

Geographical distribution of the positive PRRSV strains

Geographical distribution of the positive PRRSV strains (Fig. 4) indicated that 26 NADC34-like strains were distributed in 3 provinces, including 19 strains in Heilongjiang Province, 5 strains in Liaoning Province, and 2 strains in Jilin Province. 17 NADC30-like strains were distributed in 5 provinces, including 5 strains in Liaoning Province, 4 strains in Inner Mongolia Autonomous Region, 4 strains in Heilongjiang Province, 2 strains in Jilin Province and 2 strains in Shandong Province. 5 classical PRRSV strains were only distributed in Shandong Province.

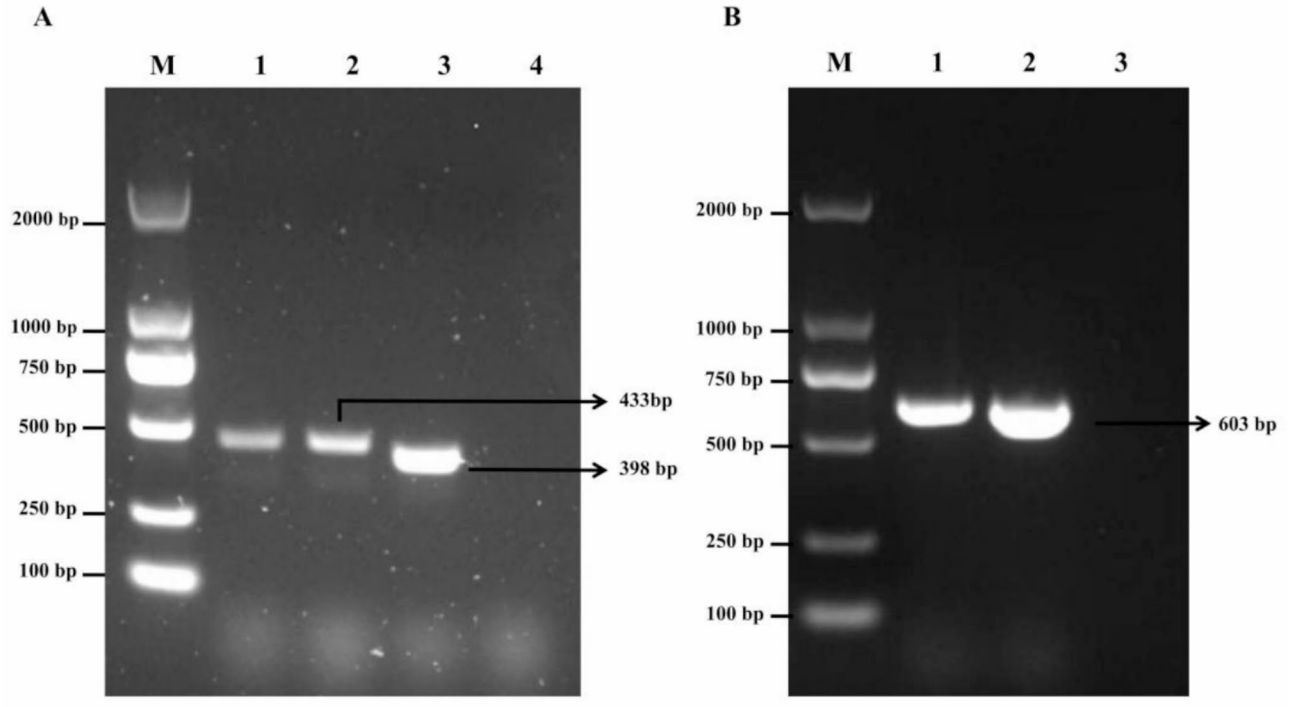

Fig. 1 Results of PRRSV ORF7 and ORF5 gene by RT-PCR. (**A**) Results of PRRSV ORF7 gene (M: DL2000 DNA Marker; 1: Sample; 2: PRRSV-2 positive control; 3: PRRSV-1 positive control; 4: Negative control); (**B**) Results of PRRSV ORF5 gene (M: DL2000 DNA Marker; 1: Sample; 2: Positive control; 3: Negative control)

Table 3 Positive number and positive rate of RT-PCR results of PRRSV ORF7 and ORF5 gene

Province	Sample number	RT-PCR results of the ORF7gene		RT-PCR results of the ORF5gene	
		Positive number	Positive rate (%)	Positive number	Positive rate (%)
Inner Mongolia	12	4	33.33	4	33.33
Heilongjiang	83	24	28.92	23	27.71
Jilin	10	4	40.00	4	40.00
Liaoning	45	10	22.22	10	22.22
Shandong	40	8	20.00	7	17.50
Total	190	50	26.32	48	25.26

Table 4 Consistency rate of RT-PCR results of PRRSV ORF7 and ORF5 gene

		RT-PCR results of the ORF7 gene			Consistency rate (%)
		+	-	Total	
RT-PCR results of the ORF5 gene	+	48	0	48	98.95
	-	2	140	142	
	Total	50	140	190	

Consistency rate = (true positive + true negative) / total × 100%, +: positive, -: negative

Amino acid sequence analysis of PRRSV GP5

As shown in Fig. 5; Table 5, there was a deletion at the 37th amino acid in 4 NADC30-like strains from 3 provinces. There were individual amino acid mutations in the main neutralizing epitope, transmembrane region 1, T cell epitope 1 and T cell epitope 2, and multiple amino acid mutations in the signal peptide, transmembrane region 2 and B-cell epitope [26, 27]. In addition, some amino acid site mutations were more complex, such as $57^{A}\rightarrow57^{N/D/K}$, $58^{N}\rightarrow58^{E/K/D/G}$ and so on. The above results showed that the complexity of the amino acid variation of PRRSV GP5.

Moreover, the strains from different lineages exhibited the different amino acid site mutations, for example, the NADC34-like strains existed the amino acid mutations at the 19th, 25th, 69th, 98th, 128th and 146th sites. In which, the 98th and 128th amino acid mutations were consistent with the IA2014 NADC34 strain, the 25th amino acid mutation was consistent with JXA1 and HUN4 strains, and the 19th, 69th, and 146th amino acid mutations were inconsistent with any reference strains. The NADC30-like strains exhibited the amino acid mutations at 10th, 90th, 95th, 124th, 158th, 168th and 192nd sites. In which, the 10th, 124th, 168th, and 192nd amino acid mutations were consistent with the NADC30 strain, and the 90th, 95th, and 158th amino acid mutations were inconsistent with any reference strains. In addition, the mutations at different amino acid sites also existed among strains from the same lineage in different regions.

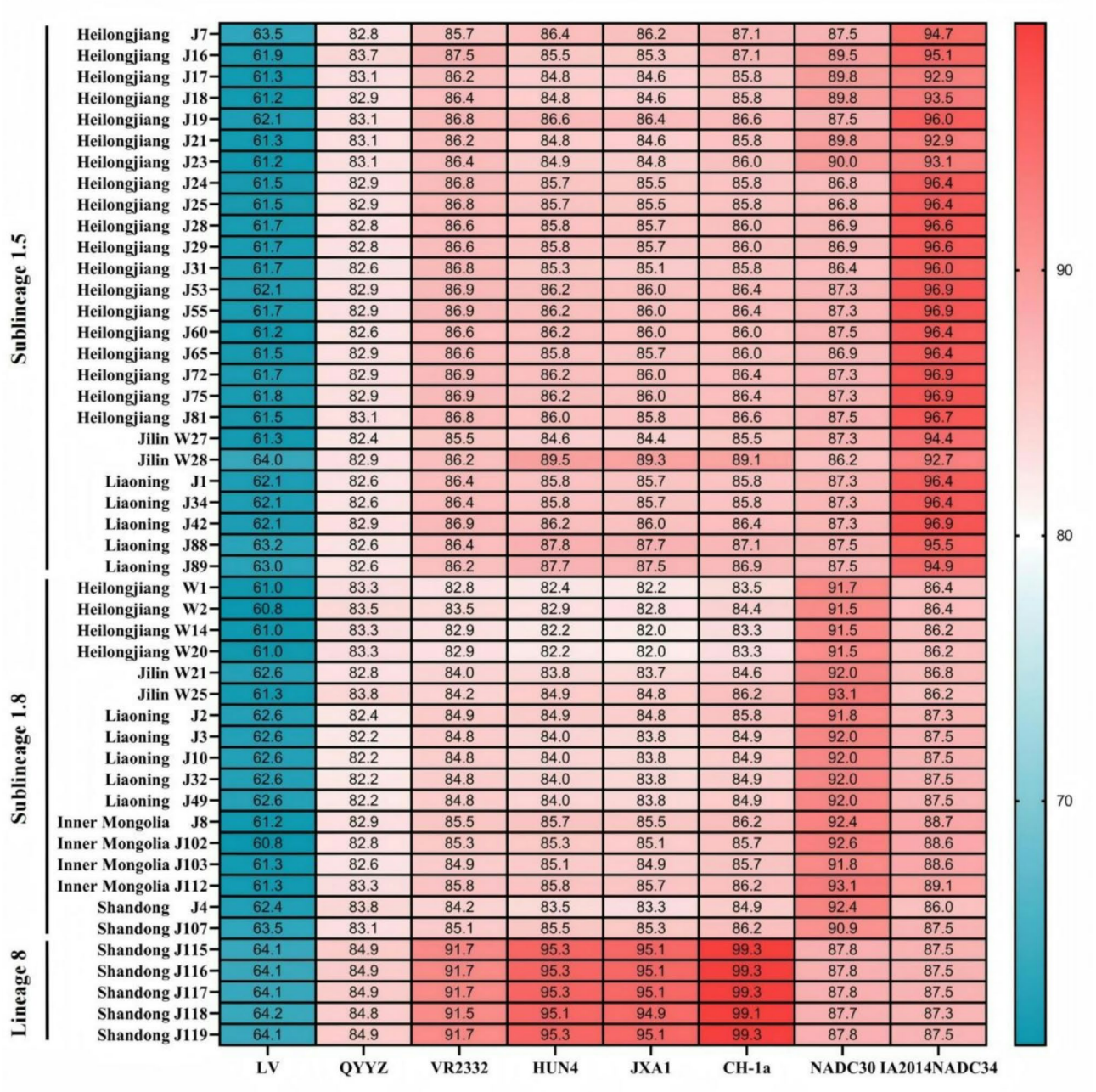

Fig. 2 Homology analysis of PRRSV ORF5 gene

For example, 3 NADC30-like strains from Inner Mongolia existed mutations at the 72nd, 120th, 166th and 172nd amino acid sites, while NADC30-like strains from other regions exhibited the mutations at the 20th and 90th amino acid sites. The above results indicated that there were strain and region differences in the amino acid variation of PRRSV GP5, and their variation possessed the diverse characteristics.

Discussion

PRRS is one of the important infectious diseases that affect the pig industry, and the complexity of PRRSV strains is also highly concerned. Zhai et al. showed that the positive rate of PRRSV were 6.27% and 27.82% respectively in Hunan and Hebei Province in 2021, and the dominant strains in both provinces belonged to lineage 1 and lineage 8 [28]. A study reported the prevalence of PRRSV in five provinces of China from 2020 to 2021, and the results indicated that the strains from the sublineage 1.8 have become the main prevalent strains [26].

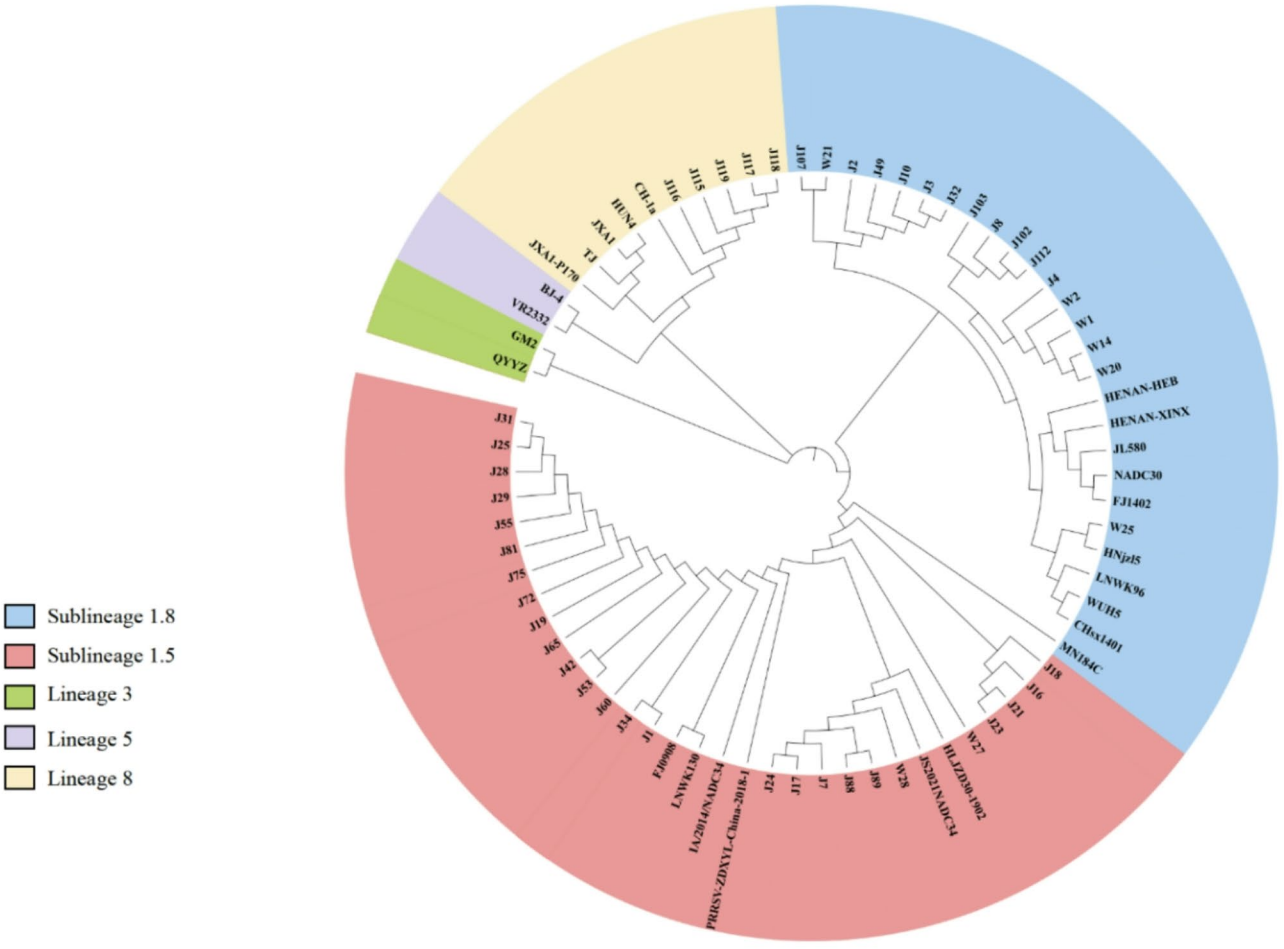

Fig. 3 Phylogenetic tree analysis based on PRRSV ORF5 gene. The phylogenetic tree was constructed using Neighbor-Joining method implemented in MEGA 11.0 software with Maximum Composite Likelihood model and 1,000 bootstrap replicates. Different colors represent different lineages or sublineages of PRRSV

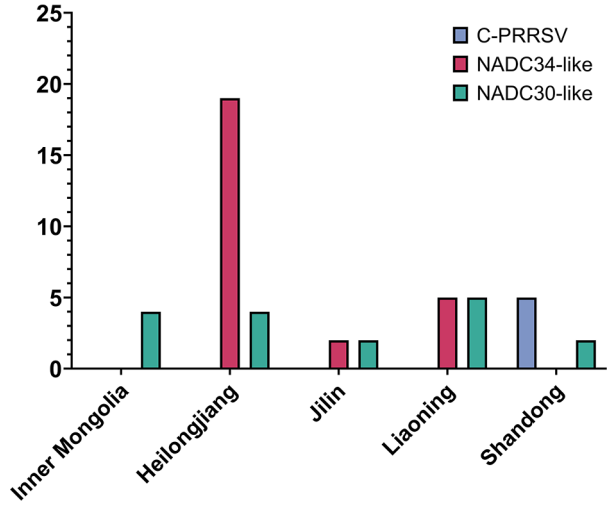

Fig. 4 Geographical distribution of the positive PRRSV strains

Li et al. also indicated that the NADC30-like strain was the dominant strain in Shandong Province from 2020 to 2021 [1]. This study investigated the prevalence of PRRS in five provinces of northern China, and the results indicated that the positive rate of PRRSV ORF7 and ORF5 gene were 26.32% and 25.26% respectively. The difference between them might be caused by the differences in the transcription level of the two genes or the frequent variation of ORF5 gene from different strains. The sequencing results of ORF5 gene showed that the prevalent strains was the NADC34-like and NADC30-like strains in these regions, and their positive rate were 13.7% and 8.9% respectively. Among them, the NADC34-like strains were mainly prevalent in Heilongjiang Province, and the NADC30-like strains were prevalent in all five provinces. The positive samples belonged to the classical PRRSV strains only existed in Shandong Province, and their homology with the CH-1a strain were 99.1–99.3%. After investigation, it was found that this pig herd has been vaccinated with the attenuated vaccine containing the

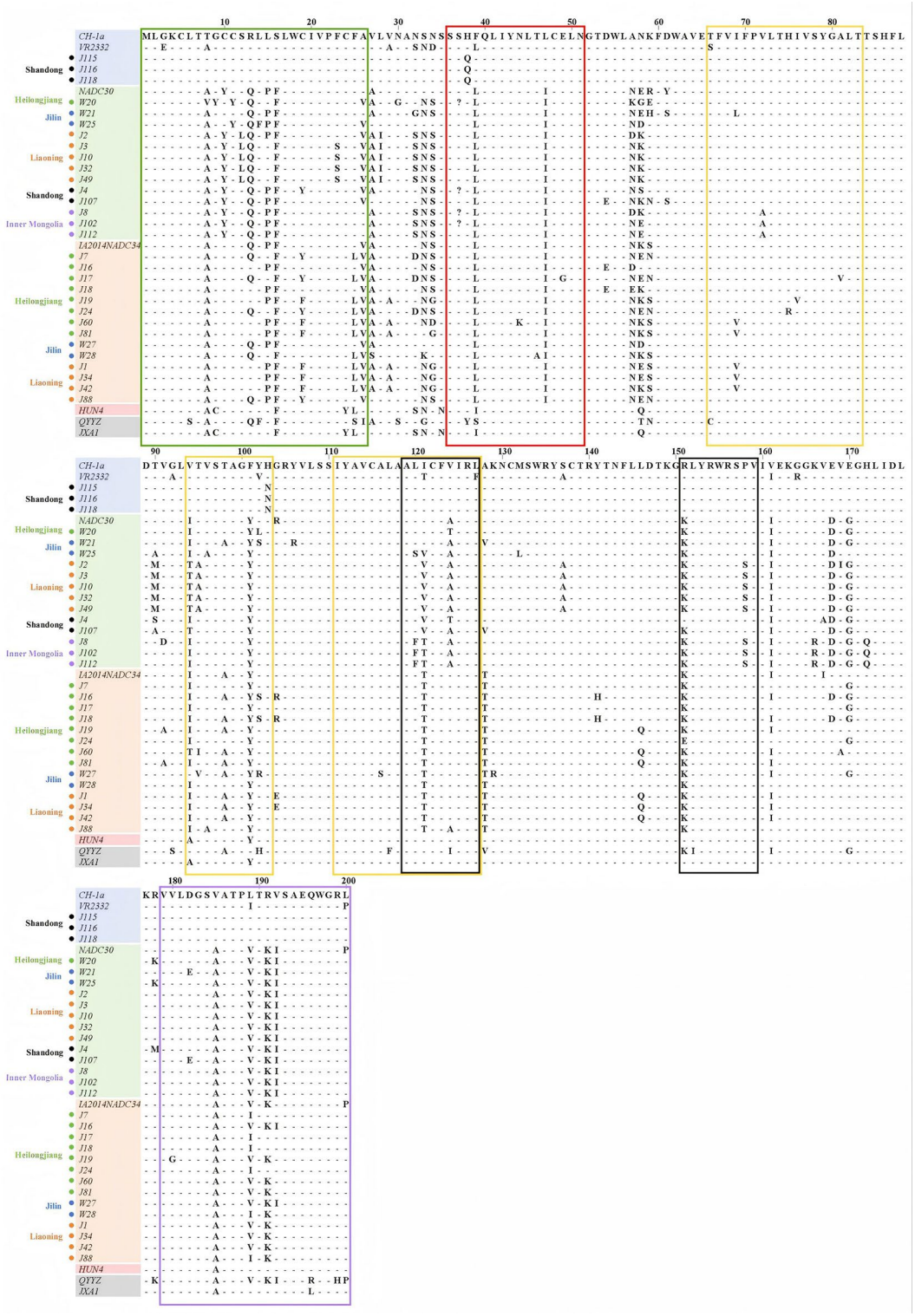

Fig. 5 Amino acid sequence analysis of PRRSV GP5. Green box: signal peptide; red box: main neutralizing epitope; yellow box: transmembrane region; black box: T cell epitope; purple box: B cell epitope

Table 5 Main amino acid mutation site of PRRSV GP5

Amino acid position	Main amino acid mutation site						
Signal peptide (1-26aa)	8	10	11	12	13	15	16
	T→A	C→Y	C→Y	S→L	R→Q	L→P	S→F
	19	23	25	26			
	C→Y/F	F→S	F→L	A→V			
Main neutralizing epitope (36-51aa)	38	39	47				
	H→Q	F→L	L→I				
Transmembrane region 1 (66-83aa)	69	72					
	I→V	V→A					
Transmembrane region 2 (94-103aa)	94	95	96	98	101	102	103
	V→I/T	T→A	V→A	T→A	F→Y	Y→S	H→N
Transmembrane region 3 (111-127aa)	120	121	124				
	L→F	I→V/T	V→A/T				
T cell epitope 1 (119-127aa)	120	121	124				
	L→F	I→V/T	V→A/T				
T cell epitope 2 (151-159aa)	151	158					
	R→K	P→S					
B cell epitope (179-200aa)	182	185	189	191	192		
	D→E	V→A	L→V/I	R→K	V→I		

CH-1a strain before sampling. This result indicated the importance of the PRRSV strains identification.

PRRSV GP5 was related to the virus replication, virus assembly and neutralizing antibody production, so the deletion, insertion or mutation of the GP5 amino acid could cause the change in the virus replication and vaccine protection [6, 7]. The 13th and 151st amino acids of the GP5 are the main virulence sites of PRRSV, and the highly pathogenic strains was R at both sites [29]. This study showed that 6 NADC34-like strains and all NADC30-like strains mutated from R to Q at the 13th amino acid site, and almost all strains exhibited the mutation from R to K at the 151st amino acid site. These mutations might lead to the decrease of the strain pathogenicity [1, 30]. The pigs infected by them didn't manifest the apparent clinical symptoms, and this made these strains easier to spread in the pig herd. The results also indicated that there were mutations at the 39th amino acid of NADC30-like and NADC34-like strains compared to CH-1a, HUN4 and JXA1 strains, and the 39th, 40th and 41st amino acid of the GP5 were the binding sites of neutralizing antibodies [31, 32]. So the mutation at the 39th amino acid might interfere with the binding of neutralizing antibodies, and reduce the cross protection of vaccines to the wild strains [33]. With the increasing reports about the recombination between the NADC30-like or NADC34-like strains and other strains, studies indicated that there were significant differences in pathogenicity of the NADC30-like or NADC34-like strains within and between lineages [34, 35]. In this study, it was also found that the amino acid mutations of the NADC30-like and NADC34-like strains were different, and it needs further research whether the different mutation sites are related to the pathogenicity differences.

In summary, NADC34-like and NADC30-like strains have become the dominant strains in five provinces of northern China, and NADC30-like strains were the most widely prevalent strains. The differences in sites and quantities of PRRSV GP5 amino acid mutation from different strains demonstrated that the genetic diversity and complexity of PRRSV. Although the commercial vaccine strains included CH-1a, VR2332, R98, JXA1-R, TJM-F92, GDr180 and so on, these strains all belonged to the lineage 5 and lineage 8. So the commercial vaccines possessed the limited protection against the NADC30-like and NADC34-like strains [36]. In addition, the proportion of mixed infections between the PRRSV and other pathogens (such as PCV, CSFV and PRV) also increased in field [37, 38]. Therefore, the best way to prevent and control PRRS was to apply multiple measures, such as the establishment of the bio-safety system, the healthy management of the pig herd, the safe and reliable vaccines, and the routine monitoring and so on. This study provided the data support for the development of PRRS prevention and control strategies in five provinces of northern China.

Conclusion

This study indicated that the NADC34-like and NADC30-like strains were the dominant strains in five provinces of northern China. The amino acid mutations of the PRRSV GP5 were different in different strains and regions, and this increased the genetic diversity of PRRSV.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12917-025-04679-y.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

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Author contributions
JY and WJ designed the study and collected the samples. JY, LC, SY and KX performed the experiment and analyzed data. JY and LC prepared the manuscript. SJ and WJ revised the manuscript. All authors were responsible for data integrity and accuracy, and approved the submitted version.

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Data availability
The data presented in this study can be found in the manuscript or supplementary file.

Declarations

Ethics approval and consent to participate
The collection and handling of the samples were approved by the Animal Experiment Ethical Committee of Heilongjiang Bayi Agricultural University (approval number: DWKJXY2023135) and the farm owner got our notification and informed consent to the collection of the samples has been taken.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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