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Epidemiology and Pathogenicity of Porcine Reproductive and Respiratory Syndrome Virus NADC30-like Strains in China

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Abstract

Porcine reproductive and respiratory syndrome (PRRS) is a swine-specific immunosuppressive disease caused by PRRS virus (PRRSV). Because PRRSV mutates rapidly and recombines frequently, current vaccines provide insufficient cross-protection against emerging recombinant strains. From late 2023 to early 2024, several large-scale pig farms in Shaanxi and Anhui Provinces, China, experienced severe outbreaks marked by widespread maternal abortions and elevated stillbirth rates. In this study, a recombinant PRRSV strain (designated SS) was isolated from clinical samples. Phylogenetic analysis of its complete genome and *GP5* gene classified strain SS into Lineage 1.8 (NADC30-like). Recombination analysis confirmed that the isolate is a natural recombinant between the NADC30 sublineage and a highly pathogenic PRRSV-like strain. The SS strain's biological characteristics were assessed using piglet pathogenicity and pregnant sow challenge models. Results showed that it causes severe symptoms and mortality in piglets and sows, with higher virulence than most low-pathogenic NADC30-like strains. In the immunized pregnant sow model, sows given four doses of PRRSV modified live virus vaccine exhibited pyrexia and anorexia following SS challenge, despite having high antibody levels; the litter abortion rate reached 50%. Postchallenge antibody

kinetics showed a transient decline followed by a sharp rise, indicating an anamnestic response and suggesting strong immune evasion. Overall, this research characterizes the pathogenicity of a novel NADC30-like recombinant in piglets and immunized sows, explores the immune evasion mechanisms of this sublineage, and offers insights to support epidemiological surveillance and targeted control of PRRSV.

Keywords: PRRSV, NADC30-like strain, Virus isolation, Pathogenicity, Sow abortion, Whole-genome sequencing, Immune evasion, Heterologous challenge

1 Introduction

Porcine reproductive and respiratory syndrome (PRRS) is a viral disease that has caused substantial economic losses in the swine industry since its first report in the United States in 1987 (Keffaber, 1989). The causative agent, PRRS virus (PRRSV), an enveloped, single-stranded, positive-sense RNA virus with a 15.1–15.5 kb genome (Cavanagh, 1997), is currently classified under the order Nidovirales, family *Arteriviridae*, and genus *Porartevirus* (Adamset al., 2017). PRRS primarily causes reproductive failure in sows, characterized by abortions, fetal mummification, and stillbirth, as well as respiratory disease and congenital abnormalities in piglets (Butler, 2014). Two distinct PRRSV genotypes are recognized globally: Genotype I (*Eurpobartevirus Betaarterivirus suid 1*), typified by the Lelystad strain; and Genotype II (*Ampobartevirus Betaarterivirus suid 2*), represented by the VR2332 strain.

In China, PRRSV Genotype II (PRRSV-2) remains widespread in the field and has been a major concern for the swine industry for over two decades. According to the global classification system, most Type 2 PRRSV strains in China fall into five lineages: Lineage 1.8 (NADC30-like), Lineage 1.5 (NADC34-like), Lineage 3 (QYYZ-like), Lineage 5.1 (VR2332-like), and Lineage 8.7 (JXA1-like) (Shi et al., 2010; Guo et al., 2018). In recent years, Lineage 1 variants, especially the NADC30-like sublineage, have gradually replaced highly pathogenic PRRSV (HP-PRRSV) as the dominant circulating lineage in China (Zhang et al., 2024). NADC30-like PRRSV consists of viruses closely related to the NADC30 strain, which was identified in 2012. Genetically, NADC30-like strains differ from other PRRSV strains by three discontinuous deletions totaling 131 amino acids (aa) in nonstructural protein 2 (Nsp2): an 111-aa deletion at positions 322–432, a 1-aa deletion at 483, and a 19-aa deletion at 504–522 (Zhou et al., 2015a). These strains currently dominate China's swine industry, and recombination events drive their pathogenic variability and genetic diversity. The pathogenicity of these recombinant strains often falls between that of their parental strains, resulting in a highly heterogeneous clinical phenotype (Yu et al., 2021).

In 2023–2024, a PRRSV strain, designated SS, was isolated from lung tissue in suspected clinical

cases at a large-scale pig farm in Shaanxi Province. Sequence and phylogenetic analyses identified the SS isolate as NADC30-like (Sublineage 1.8). Its pathogenicity was initially evaluated in piglets and pregnant sows, but its impact on hosts with high pre-existing immunity remains unclear. The present study evaluated SS pathogenicity in multiparous sows at 85 days of gestation, with the animals undergoing a rigorous vaccination program involving four consecutive doses of a commercial PRRS modified live virus (MLV) vaccine. By evaluating the strain's capacity to induce pyrexia, breach the placental barrier, and cause reproductive failure under the combined pressure of high antibody levels and established cellular immune barriers, we assessed its immune evasion potential under extreme clinical conditions. The findings improve our understanding of the virulence and transmission dynamics of emerging PRRSV strains in vaccinated populations.

2 Materials and Methods

2.1 Sample Collection and Screening

In 2023, a suspected PRRSV outbreak of PRRSV was reported at a swine farm in Shaanxi Province, marked by sow abortions, stillbirths, and respiratory disease in fattening pigs. With permission, we collected lung tissue samples from affected pigs and transported them to our laboratory to confirm PRRSV infection. Lung tissues were then placed in Dulbecco's Modified Eagle Medium (DMEM; Gibco), homogenized using a freeze mill (Jingxin Instrument Equipment Co., Ltd., Shanghai), and the homogenates were employed for RNA extraction and virus isolation procedures.

2.2 Molecular Detection of PRRSV RNA

Total RNA was isolated from the supernatant using the RNeasy Kit (Meagen Bio, Shanghai, China), following the manufacturer's instructions. The extracted RNA was analyzed using real-time reverse transcription polymerase chain reaction (RT-PCR) with the 2× Ace Q Universal U Probe Master V2 PCR Detection Kit (Vazyme Biotech, Nanjing, China) and *GP5*-specific primers. To confirm the presence of viral RNA, PCR products were analyzed via agarose gel electrophoresis.

2.3 Virus Isolation

Tissue homogenates were centrifuged at 12,000 × *g* for 20 min. The supernatant was filtered through a 0.22-μm membrane (e.g., Millipore) and then inoculated onto MARC-145 cell monolayers. After incubating at 37°C for 1 h to allow viral adsorption, the inoculum was removed and replaced with fresh DMEM containing 2% fetal bovine serum (Gibco) and antibiotics (50 μg/mL penicillin, 50 μg/mL streptomycin, and 100 μg/mL neomycin). The cells were cultured at 37°C in an incubator with 5% CO_2 for 3–5 days. Once cytopathic effects (CPEs) appeared, the supernatant was harvested and subjected to three freeze–thaw cycles. The supernatant was then transferred onto fresh MARC-145 cells for viral amplification. Finally, the virus stock was aliquoted and stored at −80°C.

2.4 Indirect Immunofluorescence Assay

For the indirect immunofluorescence assay (IFA), Marc-145 cells were infected with the PRRSV isolate. At 72-h postinfection, cells were washed with phosphate-buffered saline (PBS) and fixed with 4% paraformaldehyde for 15 min. They were then permeabilized with 0.2% Triton X-100 (Solarbio, Beijing, China) for 15 min and blocked with 3% bovine serum albumin (Solarbio, Beijing, China) at room temperature for 1 h to prevent nonspecific binding. Next, cells were incubated overnight at 4°C with a primary antibody against the PRRSV N protein. Following three PBS washes, an anti-mouse IgG secondary antibody (CST, USA) was applied and incubated at room temperature for 1 h. Finally, nuclei were counterstained with DAPI in the dark, and fluorescence signals were captured and analyzed using an inverted fluorescence microscope.

2.5 Genome Sequencing and Analysis

The PRRSV-SS strain's complete genome was amplified in overlapping fragments using primers designed and maintained in our laboratory. Following PCR, the products were purified using a gel extraction kit (TransGen Biotech, Beijing, China) and sequenced by Sangon Biotech (Shanghai, China). Sequence homology and identity were assessed through multiple sequence alignment using DNASTAR software. The SS isolate's full genome sequence was aligned with representative PRRSV reference strains from GenBank. A phylogenetic tree was constructed using the neighbor-joining method in MEGA 11, with bootstrap analysis used to assess the reliability of branching patterns.

Recombination events were detected using the RDP4 software, integrating seven algorithms: RDP, Chimaera, BootScan, 3Seq, GENECONV, MaxChi, and SiScan. A recombination event was confirmed only if at least six methods supported it. To refine and map the recombination breakpoints between the SS isolate and reference strains, similarity plotting was performed via Sim Plot (v3.5.1), employing a 500-bp sliding window and 20-bp step size.

2.6 Animal Experiments

All animal experiments received approval from the Animal Ethics Committee of South China Agricultural University (Approval No.: SCAU-AEC-2023C043) and adhered strictly to the 3R principles and animal welfare standards. To evaluate the basic pathogenicity of the SS isolate, 16 35-day-old PRRSV-antigen and antibody-negative piglets were randomly assigned to the SS-infected group and control group (n = 8 each). Piglets in the SS group were inoculated with 2 mL of viral suspension (10^5 $TCID_{50}$) through intramuscular injection and intranasal instillation; the control group received an equivalent volume of DMEM. Concurrently, eight PRRSV-negative pregnant sows at 85 days of gestation were randomly divided into two groups (n = 4 each) and inoculated with the same dose via the same routes. Postinoculation, daily monitoring included rectal temperature, appetite, and clinical signs. Serum and oropharyngeal swabs were collected at specified intervals [piglets: 0, 4, 7, 10, 14 days postinoculation (dpi); sows: 0, 1, 4, 7, 10, 14, 21, 28, 40, and 49 dpi] for antibody testing using an IDEXX ELISA Kit (IDEXX Laboratories, USA) and viral load assessment via quantitative RT-PCR. Farrowing data, including total litter size, healthy neonate count, and abortion rates, were systematically recorded. At 14 dpi, piglets were euthanized and necropsied. Tissue samples from the heart, liver, spleen, lungs, kidneys, and lymph nodes were collected to determine viral load. Lung and submandibular lymph node tissues were fixed in 4% paraformaldehyde and stained with hematoxylin and eosin for histopathological examination.

To evaluate the SS strain's capacity to overcome pre-existing immunity, eight PRRSV-negative multiparous sows were tested. These sows received four doses of a commercial PRRSV MLV vaccine before breeding and during gestation to establish high immunity levels. At 85 days of gestation, they

were randomized into either the SS-challenge or control group (n = 4 each) and inoculated with 10^5 $TCID_{50}$ of the SS isolate or an equal volume of DMEM, respectively. Rectal temperatures and clinical phenotypes were monitored until farrowing. Serum samples were collected at 0, 7, 14, 21, and 35 dpi to assess N-specific antibody kinetics, farrowing performance, and the SS strain's pathogenic risk in immunized populations.

2.7 Statistical Analysis

All experimental data were analyzed for significance using GraphPad Prism 5.0 and presented as means ± standard deviations. $P < 0.05$ was considered statistically significant.

3 Results

3.1 Epidemiology and Geographical Characteristics of PRRSV

In late 2023 and early 2024, several swine farms in Shaanxi and Anhui Provinces experienced sudden, widespread outbreaks characterized by a high rate of sow abortions and increased incidence of stillbirths and mummified fetuses, indicating an ineffective farrowing rate (Figure 1). The SS farm was most severely affected, with its abortion rate peaking at 69.12% in October (Figure 1A). Additionally, the ineffective farrowing rate at this farm increased monthly from October, peaking at 43.00% in January 2024 (Figure 1B).

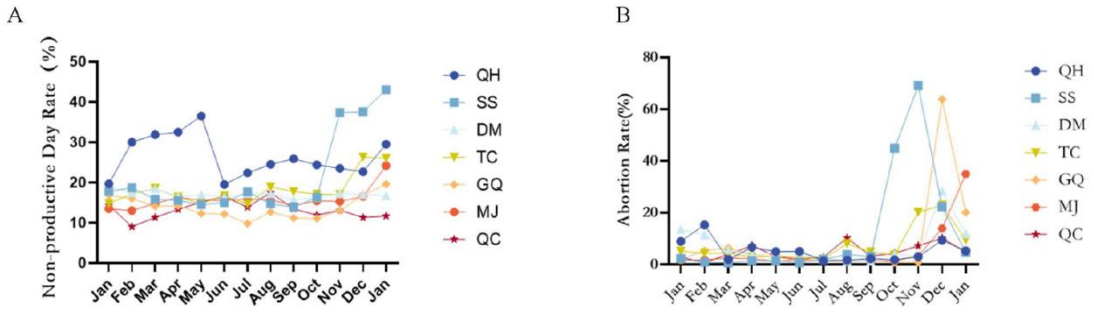


Figure 1. Reproductive failure in virus-affected swine farms (late 2023–early 2024). (A) Monthly (A) abortion rates and (B) ineffective farrowing rates at the SS farm.

3.2 Virus Isolation and Identification

The pathological material was inoculated onto healthy porcine alveolar macrophages (PAMs) to assess CPEs and perform IFAs. PAMs inoculated with PRRSV-positive samples exhibited significant CPEs after 72 h, and IFAs revealed red fluorescence (Figure 2A), confirming PRRSV presence. The supernatant from these CPE-positive PAMs was then subjected to freeze–thaw lysis and inoculated onto well-conditioned Marc-145 cells for virus propagation. CPEs appeared at 24-h postinoculation, with IFA results showing red fluorescence. CPE severity increased over time (Figure 2B). Collectively, these results indicate successful isolation of the PRRSV-SS strain. Nucleic acids from SS-infected cells were extracted and RT-PCR–amplified using *GP5*-specific primers. Gel electrophoresis showed a single clear amplification band of the expected gene-fragment length in all samples (Figure 2C). Following ultracentrifugation, viral particles were observed using electron microscopy (Figure 2D).

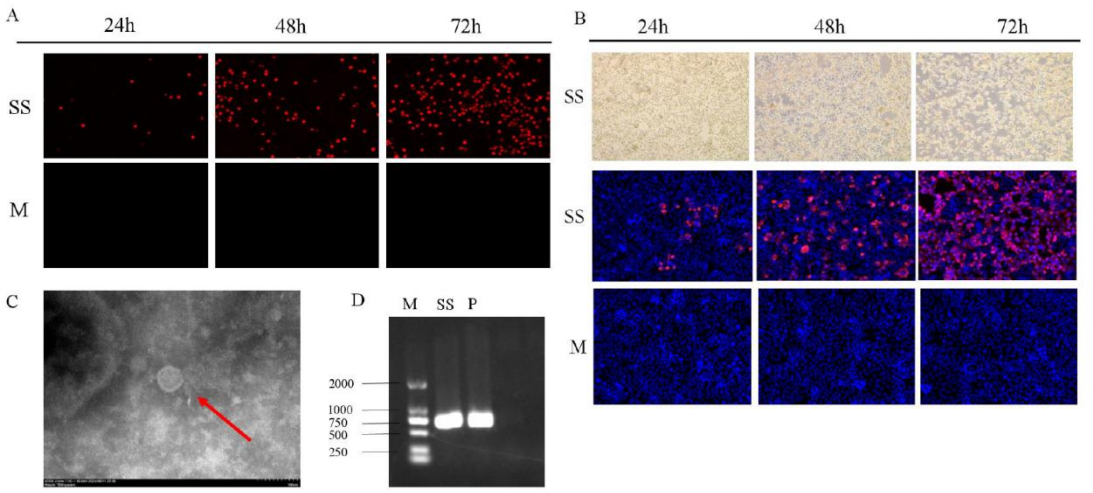


Figure 2. (A, B) Immunofluorescence staining of PRRSV N protein (red) in PAMs and Marc-145 cells at various time points. Nuclei were counterstained with DAPI (blue). (C) Ultrastructure of PRRSV-SS strain virions observed under transmission electron microscopy. (D) Agarose gel electrophoresis of the *GP5* gene amplified via RT-PCR.

3.3 Genomic Characterization and Analysis of PRRSV Strain SS

The SS isolates' complete genome sequences were assembled using DNASTAR. To investigate its evolutionary relationship, phylogenetic trees were constructed using the *GP5* gene and the complete genome. Results revealed that the isolate clustered with the NADC30 reference strain in the same

branch, with both classified under Lineage 1.8 (Figures 3A and B). Further analysis of the NSP2 aa sequence revealed the SS isolate's characteristic "111 + 1 + 19" aa deletion pattern in the NSP2 region, identical to that of the NADC30 strain (Figure 4). Collectively, these genomic characteristics confirm that the SS isolate is a NADC30-like PRRSV.

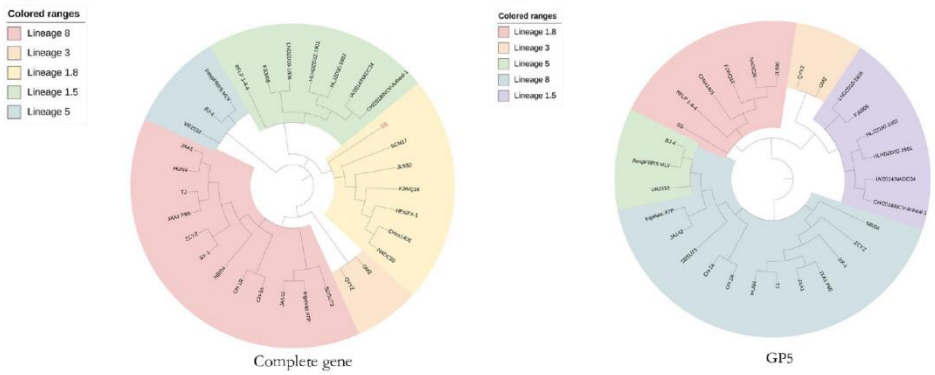


Figure 3. Genomic characterization and recombination analysis of the PRRSV-SS strain. (A) Whole-genome phylogenetic analysis. (B) *GP5* gene–based phylogenetic analysis.

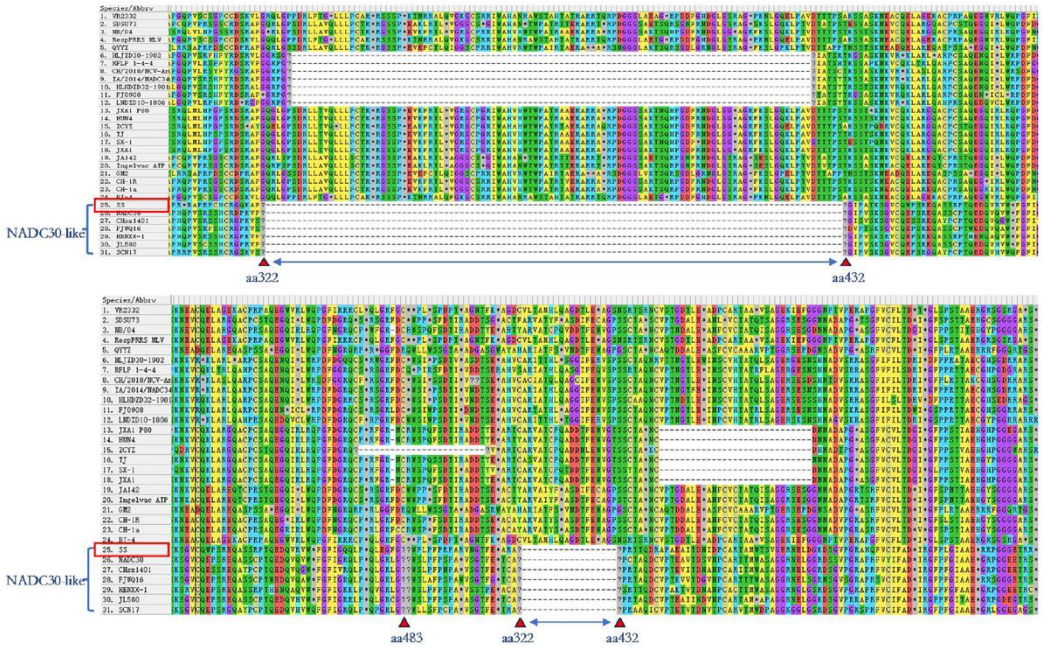


Figure 4. Schematic of genomic organization and characteristic deletions.

Recombination analysis using RDP4 and Sim Plot identified five recombination breakpoints in the SS isolate's full-length genome. These breakpoints were mapped to NSP1 [nucleotide (nt) 520], NSP2 (nt 1451 and 1971), NSP3 (nt 5308), and NSP9 (nt 9492), partitioning the genome into six recombination regions: Region A (5′-UTR–NSP1: nt 1–520), Region B (NSP1–NSP2: nt 520–1451), Region C (NSP2–NSP2: nt 1451–1971), Region D (NSP2–NSP3: nt 1971–5308), Region E (NSP3–NSP9: nt 5308–9492), and Region F (NSP9–3′-UTR: nt 9492–15,011). NADC30 was identified as the main parental strain, whereas the HP-PRRSV representative strain JXA1 was the minor parental strain (Figure 5B). Segmental phylogenetic analysis showed that recombination regions A, C, and E clustered with the JXA1 strain, belonging to Lineage 8, supporting the mosaic composition of the SS genome (Figure 5C).

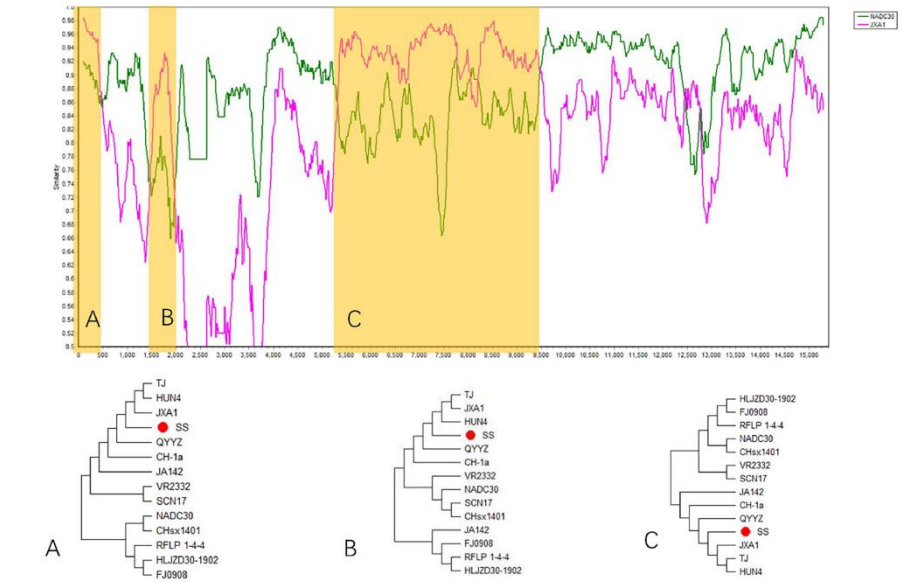


Figure 5. Detailed recombination landscape and segmental phylogenetic analysis of the PRRSV-SS isolate.

3.4 Pathogenicity of the PRRSV-SS Isolate in Piglets

Clinical observations revealed that piglets inoculated with the NADC30-like SS (P5) strain exhibited severe symptoms, including tachypnea, lethargy, and anorexia. These pigs displayed typical signs of porcine high fever syndrome, such as depression, dyspnea, conjunctivitis, skin cyanosis, and progressive emaciation. Febrile responses began at 2 dpi, with mean rectal temperatures

consistently >40°C throughout the experimental period and peaking at 8 dpi (Figure 6A). Percentage body weight gain in the inoculated group was significantly lower than in the control (Figure 6B).Viral shedding and viremia were assessed using oral swabs and cranial vena cava blood samples collected at 0, 3, 7, 10, and 14 dpi. Viral shedding was detectable via oral swabs from 3 dpi and peaked at 10 dpi (Figure 6A), whereas viremia peaked at 7 dpi (Figure 6D). Serological analysis showed that all surviving piglets in the SS group developed PRRSV N-protein antibodies by 14 dpi, with 50% seropositivity observed at 10 dpi. During the study, two piglets in the SS-challenge group died at 7 and 10 dpi, respectively; no deaths occurred in the control group (Figure 6C). All surviving piglets were euthanized at 14 dpi for tissue viral load analysis, with viral loads highest in the lungs (10^6 copies/mL), followed by the submandibular and inguinal lymph nodes. No PRRSV was detected in control tissues (Figure 6G).

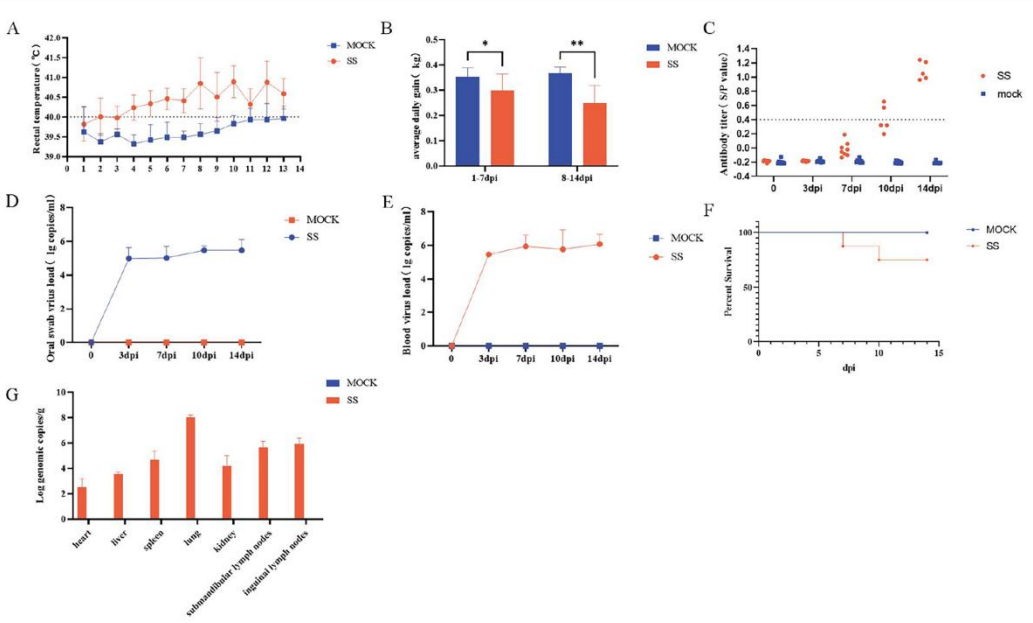


Figure 6. NADC30-like SS strain pathogenicity in piglets. (A) Clinical symptoms and febrile responses. (B) Growth performance. (C) PRRSV N-protein antibody seroconversion. (D, E) Viral kinetics in blood and oral swabs. (F) Survival analysis. (G) Viral load in tissues at 14 dpi.

In histopathological analysis, all piglets infected with the SS strain exhibited classic signs of interstitial pneumonia, including thickening of the alveolar septa and extensive inflammatory

infiltration. The alveolar walls were infiltrated with many macrophages, with fewer lymphocytes and neutrophils present (Figure 7A, arrows). Conversely, the control group had normal pulmonary architecture with clear, intact alveoli.

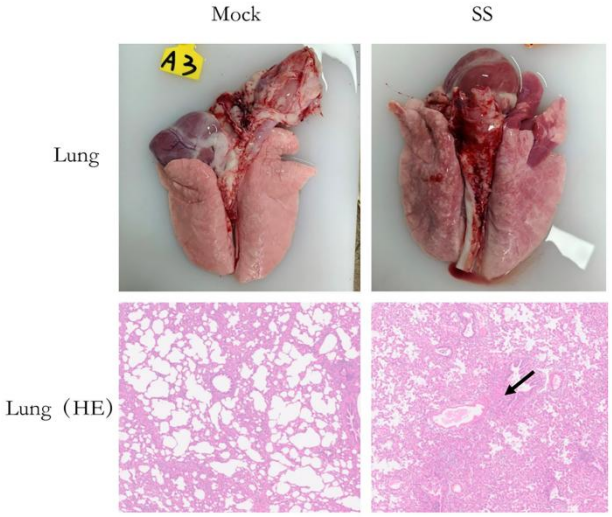


Figure 7. Histopathological analysis of lung tissues from piglets infected with the PRRSV-SS strain.

3.5 Pathogenicity of the PRRSV-SS Isolate in Pregnant Sows

The SS isolate's pathogenicity was evaluated via experimental challenge in pregnant sows. After inoculation, the sows showed increased body temperature, which normalized after approximately three weeks (Figure 8A). They also experienced reduced appetite during late gestation (Figure 8B). Viral shedding, viremia, and N-protein antibody levels were monitored using oral swabs and cranial vena cava blood samples collected at 0, 3, 7, 10, 14, 21, 28, and 35 dpi. Results revealed that viral shedding from the pharynx peaked at 10 dpi and then decreased gradually. Viremia levels in peripheral blood also peaked at 10 dpi and before declining (Figure 8D and F). Serological testing for PRRSV N-antibodies indicated that all infected sows seroconverted by 10 dpi, reaching 100% seropositivity by 14 dpi (Figure 8C). Throughout the experimental period, no clinical abnormalities appeared in the control group, whereas one sow in the SS-challenged group died at 7 dpi (Figure 8F).

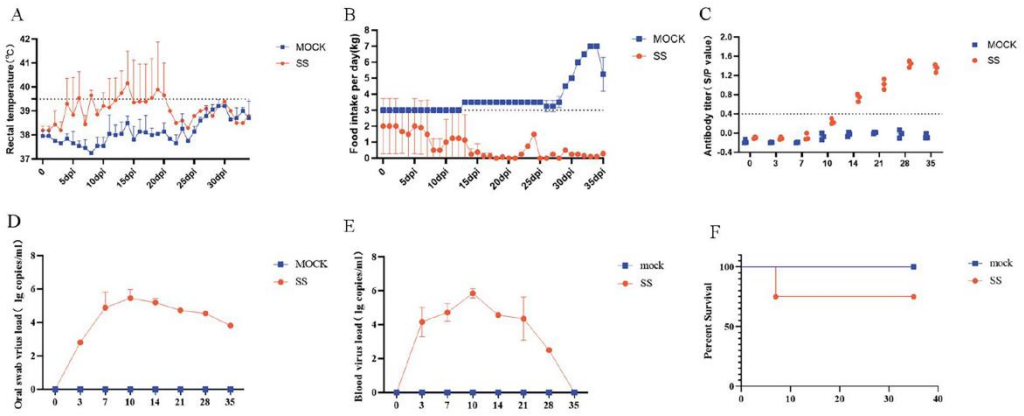


Figure 8. Clinical evaluation and viral kinetics of the PRRSV-SS isolate in pregnant sows. (A) Thermogram of rectal temperatures. (B) Mean daily feed intake. (C) Serological responses. (D) Viral shedding kinetics. (E) Viremia profiles. (F) Survival analysis and clinical outcomes.

The farrowing performance of each experimental group was analyzed statistically. In the control group, four sows delivered 15, 14, 16, and 15 piglets, with 13, 11, 15, and 14 surviving, respectively. Conversely, the SS-challenged group had farrowing outcomes of 16, 0, 14, and 12 piglets per sow, with zero survivors in all litters. Notably, SS-infected sows showed severe reproductive failure, with 16, 0, 14, and 0 aborted fetuses, and 5, 0, 2, and 9 mummified fetuses per litter (Table 2).

Vertical transmission was evaluated by testing the umbilical cord blood of newborns. PRRSV-SS RNA was detected in 16, 0, 14, and 12 piglets from challenged sows, demonstrating vertical transmission rates of 100%, 0%, 100%, and 100%, respectively (Table 2). Overall, the SS isolate induced severe clinical symptoms in pregnant sows, resulting in high abortion and fetal mummification rates, and was associated with highly efficient vertical transmission.

Table 2. Impact of PRRSV-SS isolate on farrowing performance and vertical transmission.

	Mock	SS-P5
Healthy piglet count	13/11/15/14	0/0/0/0
Weak piglet count	2/3/1/1	0/0/0/3

Aborted fetus count	0/0/0/0	16/0/14/0
Mummified fetus count	0/0/0/0	5/0/2/9
Total piglet count	15/14/16/15	16/0/14/12
Positive for vertical transmission	0/0/0/0	16/0/14/12

3.6 Pathogenicity of the PRRSV-SS Isolate in Immunized Pregnant Sows

After inoculation with the SS isolate, pregnant sows experienced a transient rise in rectal temperature, which normalized around one week postchallenge. In contrast, the control group remained afebrile throughout the experimental period (Figure 9A). Correspondingly, SS-inoculated sows also showed a temporary reduction in appetite, recovering within a week (Figure 9B). Serological tests measuring N-protein antibodies in cranial vena cava blood samples collected at 0, 3, 7, 14, 21, and 35 dpi revealed a distinct kinetic pattern: antibody levels in the SS group temporarily declined at 3 dpi, likely reflecting the consumption of pre-existing antibodies during early viral replication. This was quickly followed by a sharp increase, peaking at 7 dpi, then gradually declining over the remaining period (Figure 9C).

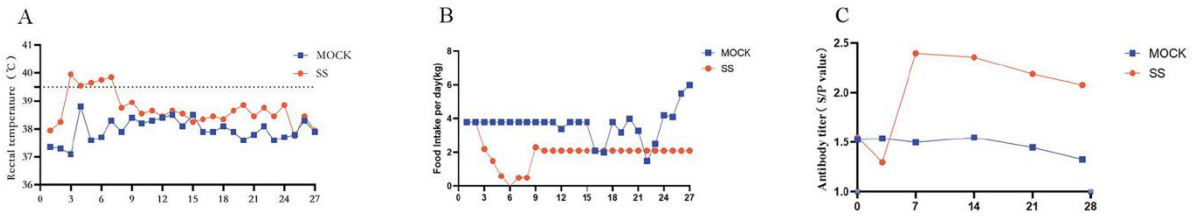


Figure 9. Clinical and immunological responses in pregnant sows following SS isolate inoculation. (A) Thermogram of rectal temperatures. (B) Mean daily feed intake. (C) Serological responses.

The farrowing performance of each experimental group was analyzed to determine the SS isolate's effect. In the control group, four sows gave birth to 14, 15, 13, and 12 piglets, with 11, 3, 12, and 11 healthy survivors, respectively. In contrast, the SS-challenged group had farrowing results of 13, 0, 0, and 12 piglets per sow, with 9, 0, 0, and 8 survivors, respectively. Notably, two sows in this group

experienced complete reproductive failure, with 0, 14, 12, and 0 aborted fetuses recorded. Additionally, mummified fetuses were found in the litters of the challenged group, with 3, 0, 0, and 2 mummies per litter (Table 3). These findings indicate that even with high pre-existing immunity, the SS isolate retains potent reproductive toxicity, leading to high abortion and fetal mummification rates.

Table 3. Reproductive performance and farrowing outcomes of pregnant sows inoculated with the SS isolate.

	Live-born piglets	Healthy piglets	Stillborn piglets	Mummified fetuses	Aborted fetuses	Total born
SS	9/0/0/8	9/0/0/8	1/0/0/2	3/0/0/2	0/14/12/0	13/0/0/12
Mock	11/13/12/11	11/13/12/11	0/0/0/0	0/0/0/0	0/0/0/0	14/15/13/12

4 Discussion

The high prevalence of PRRSV continues to cause substantial economic damage to the global swine industry. Akin to other RNA viruses, PRRSV undergoes continuous genetic and antigenic drift (Murtaugh et al., 2010). Since the initial emergence of HP-PRRSV, represented by strain JXA1, in China in 2006, strains with a characteristic 30-aa deletion in the NSP2 region have become predominant (Zhou and Yang, 2010; Zhou et al., 2014). The discovery of QYYZ-like (Lu et al., 2015) and NADC30-like (Zhou et al., 2015b) strains in 2010 and 2013, respectively, has added to viral diversity and complicated field control efforts. Current challenges are exacerbated by the limited cross-protection provided by widely used MLV vaccines, which also risk recombination, mutation, and antibody-dependent enhancement (ADE), thereby driving viral evolution under immune selection pressure (Trevisan et al., 2023; Zhou et al., 2018; Li et al., 2023a). Consequently, existing control measures and vaccines do not fully protect against circulating NADC30-like strains (Bai et al., 2016). Between late 2023 and early 2024, an abnormal surge in PRRSV positivity rates and widespread

reproductive failure were reported in Shaanxi and Anhui Provinces. In the present study, we isolated and characterized the SS strain, which belongs to the NADC30 lineage (Lineage 1.8) and exhibits the typical “111 + 1 + 19 aa” deletion pattern in NSP2. Recombination analysis revealed that the SS isolate underwent substantial genetic exchange with HP-PRRSV within the NSP5–NSP9 genomic region. Although recombination is a key factor in the high pathogenicity and genetic diversity of Chinese NADC30-like strains (Wang et al.; 2018), its underlying mechanisms remain unclear. Our pathogenicity experiments in piglets and sows demonstrated that the SS isolate causes severe symptoms, including coughing, anorexia, and retarded weight gain, along with interstitial pneumonia, lymphopenia, and notable pulmonary lesions. Moreover, some SS-inoculated piglets died, indicating virulence that exceeds most reported NADC30-like strains (Zhou et al., 2015c; Li et al., 2016). The SS strain also severely compromised maternal reproduction, resulting in stillbirths, mummified fetuses, abortions, and sow mortality, clinical features similar to those in HP-PRRSV infection (Wu et al., 2009; Zhou et al., 2008).

This study highlights the remarkable ability of the SS isolate to cause severe reproductive failure in sows, even after administering four MLV vaccinations. Although commercial MLV vaccines are expected to provide heterologous protection and mitigate clinical symptoms (Chai et al., 2020), the SS strain induced a 50% total litter abortion rate, despite high pre-existing immunity. This immune evasion suggests a strong capacity to bypass immune defenses. The observed transient decline in antibody levels followed by a rapid rebound postchallenge suggests a robust anamnestic response (Pandey et al., 2023), and we hypothesize that ADE is involved in promoting viral replication (Li et al. 2023b). Further analysis identified a recombination breakpoint between NSP3 (nt 5380) and NSP9 (nt 9492) involving HP-PRRSV JXA1. Given that nonstructural proteins are vital for viral virulence and innate immune evasion (Beura et al., 2010), and that NSP9 and NSP10 are major virulence factors for Chinese HP-PRRSV (Li et al., 2014a; Li et al., 2014b; Kwon et al., 2008), the acquisition of core HP-PRRSV genomic fragments via recombination may provide the genetic foundation for the

enhanced virulence and evolution of strain SS. These insights deepen our understanding of NADC30-like PRRSV virulence evolution and underscore the need for more cautious vaccine selection and use.

5 Conclusion

In this study, we isolated and characterized the PRRSV-SS strain, a natural recombinant derived from NADC30-like and HP-PRRSV lineages. Pathogenicity tests in piglets demonstrated that the SS isolate causes mortality and is more virulent than typical NADC30-like strains. Additionally, experiments in pregnant sows revealed that the SS strain causes abortions and results in a 25% mortality rate among infected pigs, markedly affecting reproductive success. Using a challenge model in immunized sows, we confirmed that the NADC30-like SS strain has strong immune evasion and reproductive toxicity. Even with high pre-existing immunity, the strain caused pyrexia and a 50% litter abortion rate; the antibody levels exhibited a "decline-then-rebound" pattern, indicating rapid viral replication under selective immune pressure. These findings highlight the urgent need for improved vaccines and control strategies to manage the continuously evolving PRRSV landscape. Overall, this research provides essential epidemiological insights and practical guidance for PRRSV prevention and control in the field.

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Ethics approval and consent to participate

Our animal experiments were approved by the Animal Ethics Committee of South China Agricultural University and conducted under the guidance of the South China Agricultural University Institutional Animal Care and Use Committee (SCAU-AEC-2023C043).

Declaration of competing interests

There are no conflicts of interest both in all authors.

CRediT authorship contribution statement

Yu Wu and Quihui Li conceived and designed the study. Xiaoming He and Junhua Lan devised the experimental methods. Ruying Liu and Guifa Xiao curated the data. Limiao Lin, Haishen Zhao and Yaoxin Shen provided resources and performed the experiments. Xiaoming He prepared the original manuscript draft. Yu Wu and Qunhui Li reviewed the manuscript and edited it. All authors read and approved the final manuscript.

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