Recombinant Bivalent Live Vectored Vaccine Against Classical Swine Fever and HP-PRRS Revealed Adequate Heterogeneous Protection Against NADC30-Like Strain

Liwei Li1,2†, Jinxia Chen1†, Zhengda Cao1, Yunlei Cao1, Ziqiang Guo1, Wu Tong1,2, Yanjun Zhou1,2, Guoxin Li1,2, Yifeng Jiang1,2, Changlong Liu1,2, Lingxue Yu1,2, Sina Qiao1, Jiachen Liu1, Guangzhi Tong1,2* and Fei Gao1,2*

1 Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Shanghai, China, 2 Jiangsu Co-innovation Center for the Prevention and Control of Important Animal Infectious Disease and Zoonoses, Yangzhou University, Yangzhou, China

The recombinant bivalent live vectored vaccine rPRRSV-E2 has been proved to be a favorable genetic engineering vaccine against classical swine fever (CSF) and highly pathogenic porcine reproductive and respiratory syndrome (HP-PRRS). NADC30-like strains have recently emerged in China and caused severe disease, and it is necessary to evaluate the vaccine candidate for the currently circulating viruses. This study established a good challenge model to evaluate the candidate rPRRSV-E2 vaccine in preventing infection with a representative NADC30-like strain (ZJqz21). It was shown that the challenge control piglets displayed clinical signs typical of PRRSV, including a persistent fever, dyspnea, moderate interstitial pneumonia, lymph node congestion, and viremia. In contrast, the rPRRSV-E2 vaccination significantly alleviated the clinical signs, yielded a high level of antibodies, provided adequate protection against challenge with ZJqz21, and inhibited viral shedding and the viral load in target tissues. Our results demonstrated that the recombinant bivalent live vectored vaccine strain rPRRSV-E2 can provide efficient protection against the challenge of heterologous circulating NADC30-like strain and could be a promising vaccine candidate for the swine industry.

Keywords: rPRRSV-E2, live vectored vaccine, NADC30-like strain, heterologous protection, challenge model

INTRODUCTION

Porcine reproductive and respiratory syndrome virus (PRRSV) is the causal agent of the porcine reproductive and respiratory syndrome (PRRS) and is one of the most economically infectious illnesses of the swine industry worldwide. PRRSV belongs to the order Nidovirales, family Arteriviridae and can be classified into PRRSV-1 and PRRSV-2 with Lelystad and VR-2323 as representative strains (Cavanagh, 1997; Lunney et al., 2016). These two genotypes share approximately 60% nucleotide identity (Darwich et al., 2011).

PRRSV was first reported in 1996 in China. Over the past 20 years, PRRSV has been the cause of major diseases in the swine industry, and PRRSV-2 strains are highly predominant within the field...
Daily observations were performed until obvious cytopathic effects were detected by PRRSV ORF5-specific primers, and the results were subjected to three freeze-thaw cycles in RPMI-1640 medium containing 10% fetal bovine serum (FBS; Gibco). The disease materials were obtained from a pig farm in Zhejiang province, China, in 2020. Three sick piglets displayed high clinical symptoms and viral infection NADC30-like strain in piglets. According to the global PRRSV classification system, the abundance of PRRSV-2 strains in China can be classified into four lineages: NADC30-like (lineage 1), QYYZ-like (lineage 3), VR-2332-like (lineage 5), and JXA1-like/CH-1a-like (lineage 8) lineages (Shi et al., 2010a,b). Extensive genetic variations exist between the strains in each viral subtype, and genetically distinct PRRSV variants display different clinical characteristics and virulence (Guo et al., 2018). rPRRSV-E2 was a recombinant bivalent live vectored vaccine and capable of expressing the classical swine fever virus (CSFV) E2 protein in the backbone of a commercial modified live virus (MLV) vaccine strain, HuN4-F112, which was classified into lineage 8 (Gao et al., 2018). rPRRSV-E2 could provide 100% protection against HP-PRRSV (lineage 8), but it remained to be answered about the protective effect of rPRRSV-E2 for resisting challenge with NADC30-like (lineage 1) strain.

NADC30-like PRRSVs are not as pathogenic as HP-RRRSV but can be distinguished by a greater probability of recombination with other PRRSV strains, leading to different virulence (Zhang et al., 2016). Outbreaks of NADC30-like PRRSV in vaccinated swine herds indicate that the current commercial PRRSV vaccines cannot completely protect against infection with NADC30-like strains (Tian, 2017). This study evaluated the current vaccine candidate rPRRSV-E2 against infection with a heterologous strain and demonstrated that rPRRSV-E2 vaccination exerted superior efficiency at decreasing the clinical signs and viral infection NADC30-like strain in piglets.

**MATERIALS AND METHODS**

**Virus Isolation and Viral Characteristics Analyses**

The disease materials were obtained from a pig farm in Zhejiang province, China, in 2020. Three sick piglets displayed high fever, dyspnea, and tachypnea. Lung samples were ground and subjected to three freeze-thaw cycles in RPMI-1640 medium (Gibco, Thermo Fisher Scientific, Waltham, MA). Samples were detected by PRRSV ORF5-specific primers, and the results appeared to be positive (data not shown). (PAMs) were infected with the supernatants for 1 h and subsequently cultured in RPMI-1640 medium containing 10% fetal bovine serum (FBS; Gibco). Daily observations were performed until obvious cytopathic effects (CPEs) appeared. Specific monoclonal antibodies termed SDOW17 (Rural Technologies) were used for an indirect immune fluorescent assay (IFA) to confirm the presence of the PRRSV N protein.

**Complete Genome Sequencing and Phylogenetic Analysis of ZJqz21**

The total RNA was extracted from ZJqz21-infected PAMs using TRIzol (Invitrogen, Thermo Fisher Scientific, Waltham, MA). A PrimeScript™ 1st Strand cDNA Synthesis Kit (TaKaRa, Dalian, China) was used for reverse transcription. The complete ZJqz21 genome was amplified by seven overlapping primers using the cDNA template. The sequences of all primers used for genome amplification will be made available upon request. Purified PCR products were sequenced by Personalbio (Shanghai, China). A total of 44 representative PRRSV isolates were collected from the GenBank database (Table 1), and multiple sequence alignments were performed using Clustal X. Phylogenetic trees of the complete genome and ORF5 sequences were constructed by MEGA 6.0 using the neighbor-joining method. Bootstrap confidence values from 1,000 replicates were used to analyze the evolutionary relationship of ZJqz21.

**Viruses Used for Vaccination and Challenge**

PRRSV-permissive MARC-145 cells were cultured and maintained in Minimum Essential Medium Eagle (MEM; Sigma-Aldrich Corporation, CA, United States) with 10% FBS or 2% FBS at 37°C under humidity of 5% CO2. The vaccination strain, rPRRSV-E2, and challenge virus strain, ZJqz21, were propagated and obtained when approximately 80% CPE of the MARC-145 cells appeared. The two viruses were titrated and used for vaccination and challenge, respectively. The doses used in the experiments are shown in Table 2.

**Evaluation of rPRRSV-E2 Immune Efficacy Against ZJqz21 in Piglets**

A professional veterinary pathologist was invited to guide and perform professional animal experimental operations. A total of 15, 30-day-old landrace PRRSV- and CSFV-free piglets were selected for immune efficacy experiments to assess the effect of rPRRSV-E2 against ZJqz21 and divided into three groups. Each group contained five piglets fed separately, as shown in Table 2. A 2 ml dose of rPRRSV-E2 (dosage: 10^5.0 TCID50) was used to vaccinate each piglet in the vaccinated and challenge control piglets (BC groups; PRRSV S/P < 0.4; n = 5) and 2 ml of ZJqz21 (dosage: 10^5.0 TCID50) was inoculated at 28 days post-vaccination (dpv). A cervical intramuscular injection was used for vaccination and challenge. Each piglet was injected with 2 ml DMEM in the mock group. General health status and clinical symptoms were monitored daily, and the rectal temperatures were measured daily during the experimental period. PRRS clinical symptoms were recorded and scored as described previously (Halbur et al., 1995; Thanawongnuwech et al., 2000; Li et al., 2014). All surviving piglets were euthanized and autopsied 21 days post-infection (dpi). The sera were collected every 7 days after rPRRSV-E2 vaccination. After ZJqz21 inoculation, sera were collected every 2 or 3 days. Real-time RT-qPCR evaluated the presence and amount of ZJqz21 in the sera by 90% efficiency.
TABLE 1 | Reference strains in this study.

| NO | Isolate            | Country   | Year | Accession no. |
|----|--------------------|-----------|------|---------------|
| 1  | Lelystad virus     | Europe    | 1991 | M96262.2      |
| 2  | VR-2332            | United States | 1992 | AY150564.1   |
| 3  | RespPRRS MLV       | United States | 1994 | AF0618153.4  |
| 4  | CH-1a              | China     | 1996 | AY032626.1   |
| 5  | CH-1R              | China     | 2008 | EU907840.1   |
| 6  | NB/04              | China     | 2004 | FJ536165.1   |
| 7  | JX1A               | China     | 2006 | EF112445.1   |
| 8  | HUN4               | China     | 2007 | EF635006.1   |
| 9  | NADCo              | United States | 2012 | MH500776.1   |
| 10 | WJH4               | China     | 2012 | JQ326271.1   |
| 11 | HB/1-3.9           | China     | 2002 | AY150313.2   |
| 12 | HB(sh)-1-3.9       | China     | 2016 | KX621003.1   |
| 13 | GDSg               | China     | 2011 | JN387271.1   |
| 14 | GDQY1              | China     | 2010 | JX308978.1   |
| 15 | rJXwn06            | China     | 2009 | MF187966.1   |
| 16 | GM2                | China     | 2012 | JN662424.1   |
| 17 | HN2007             | China     | 2009 | JG880437.2   |
| 18 | SH/H               | China     | 2007 | EU106888.1   |
| 19 | CH-YY              | China     | 2019 | MK450365.1   |
| 20 | TP                 | China     | 2008 | EU864233.1   |
| 21 | TJ                 | China     | 2008 | EU860248.1   |
| 22 | GD                 | China     | 2008 | EU985724.1   |
| 23 | IA/2014/NADCo34    | United States | 2017 | MF326985.1  |
| 24 | CH/2018/NCV-Anheal-1 | China     | 2018 | MH370474.1   |
| 25 | FJ0908             | China     | 2018 | MK202794.1   |
| 26 | PRRSV-ZDXY-China-2018-2 | China | 2019 | MK450350.1   |
| 27 | HLJZSD30-1902      | China     | 2019 | MN848055.1   |
| 28 | RFLP 1-4-4 lineage 1C variant | United States | 2021 | MW897665.1  |
| 29 | A2MC2              | United States | 2011 | JQ087873.1   |
| 30 | FJU72017           | China     | 2017 | MG301178.1   |
| 31 | 15LN3              | China     | 2016 | KX815425.1   |
| 32 | SDYG1606           | China     | 2016 | KY053458.1   |
| 33 | HH08               | China     | 2012 | JX679179.1   |
| 34 | GD-KP              | China     | 2016 | JU978619.1   |
| 35 | FJFS               | China     | 2015 | KP998476.1   |
| 36 | SD110-1608         | China     | 2019 | MK790825.1   |
| 37 | SD0d1501           | China     | 2019 | MN400299.1   |
| 38 | PRRSV 2            | United States | 1995 | U87392.3     |
| 39 | SD1612-1           | China     | 2019 | MN19304.1    |
| 40 | GZ2106             | China     | 2014 | KJ541663.1   |
| 41 | CH2004             | China     | 2009 | EU880439.2   |
| 42 | CH2002             | China     | 2009 | EU880438.2   |
| 43 | HK13               | China     | 2013 | KF287140.1   |

level of viral RNA replication in the serum samples containing PRRSV viremia and virus shedding and viral load in the tissues (Jiang et al., 2015; Gao et al., 2018, 2020b). An evaluation of lung lesions in each of the three groups was performed as previously described (Jiang et al., 2015; Gao et al., 2018, 2020a). For pathological observations, a pathological section analysis and detection of the viral load in the tissues of piglets infected with the ZJqz21 strain was performed. The heart, lung, inguinal lymph nodes, mesenteric lymph nodes, mandibular lymph nodes, spleen, kidneys, and tonsils were collected from each piglet.

Detection of Specific Humoral Immunity After Vaccination and Challenge

The PRRSV- and CSFV-specific antibody titers in the serum samples collected at the specified time points were tested with the commercial ELISA kits (IDEXX Laboratories, Westbrook, No. 06-40959-04 and No. 06-43230-06) as previously described (Gao et al., 2018). Based on these values, the level of humoral immunity was displayed by drawing curves with GraphPad Prism 6.0.

Determination of the Viral Load in Tissues

Tissue samples from the heart, lung, inguinal lymph nodes, mesenteric lymph nodes, mandibular lymph nodes, spleen, kidneys, and tonsils were collected by multi-point sample collection. A certain amount of each tissue sample was gathered, 1 ml of sterile PBS was added for tissue homogenates, and grinding treated tissue homogenates were centrifuged at 4°C (9,500 × g for 10 min), and the supernatant was cryopreserved in a 1.5 ml centrifuge tube. The RNA of 200 µL-treated homogenate supernatant was extracted using the QIAcube HT nucleic acid autotractor according to the QIAcube HT Plasticware kit instructions. RT-qPCR was performed, and Shanghai Sunny Biotechnology Co., Ltd., synthesized the primer pairs and probes.

Statistical Analysis of Experimental Data

A Student’s t-test was used for statistical differences analysis in the study. SPSS 14.0 and GraphPad Prism software (version 6.0) was used to analyze and chart the clinical symptom scores, lung lesion scores, viral shedding, and viral load values. A P-value < 0.05 was considered to be statistically significant.

RESULTS

ZJqz21 Was Identified as a Heterologous Strain to rPRRSV-E2

To evaluate the immune efficacy of rPRRSV-E2 against the heterologous strain, we first identified a NADC30 epidemic strain. In 2020, ZJqz21 was successfully isolated from PAMs from a RespPRRS MLV-vaccinated piglet in China. PRRSV-specific CPE was observed in the P2 passage,
and the existence of PRRSV was further identified by IFA (Figure 1A). As shown in Figure 1A, ZJqz21 could cause severe CPE. An IFA analysis was carried out on MARC-145 cells, or PAMs infected with ZJqz21 (Figure 1A). The viral growth characteristics of ZJqz21 are presented by multi-step growth curves and a plaque morphology analysis (Figures 1B–D).

The complete sequences of the ZJqz21 genome have been uploaded into GenBank (accession no. OK274266). The complete ZJqz21 genome is 15,042 nt in length, including poly(A) tails. The ZJqz21 genome shared 86.9, 87.0, 84.4, 86.8, and 90.1% homology in the nucleotide sequence with CH-1a, JXA1, GM2, VR2332, and NADC30, respectively (Table 3). Each region of the ZJqz21 genome was further compared with those representative strains, and the results showed that ZJqz21 shared the highest identity in each region with the NADC30 strain. Amino acid sequence alignment results revealed that Nsp2 of ZJqz21 has a discontinuous 131-aa (111 + 1 + 19-aa) deletion compared with VR-2332 (data not shown), which are the typical characteristics of the NADC30-like PRRSV. The ORF5 of ZJqz21 displayed higher nucleotide and amino acid identity (94.7%, 93.0%) with NADC30 (Table 3).

Phylogenetic trees were constructed according to the complete genome and ZJqz21 ORF5 sequences. The results demonstrated that PRRSV-2 strains could be divided into the following five lineages: RFLP 1-4-4 (lineage 1.5), NADC30-like (lineage 1.8), VR-2332-like (lineage 5), GM2-like (lineage 3), and JXA1-like/CH-1a-like (lineage 8) based on the complete genome sequence. ZJqz21 was classified into lineage 1.8, represented by NADC30 based on full nucleotide genome and ORF5 sequences (Figures 1E,F). These findings revealed that ZJqz21 belonged to lineage 1.8 and had characteristics of the NADC30 strain, which can be used as the heterologous challenge strain to rPRRSV-E2.
rPRRSV-E2 Protected the Piglets From the Challenge of the Heterologous NADC30-Like Strain ZJqz21

Next, the efficacy of rPRRSV-E2 against the heterologous strain ZJqz21 in piglets was tested. The BC group and vaccinated piglets (vaccinated group) were challenged with the ZJqz21 strain. In the BC group, the body temperature rose to 3 days post-challenge (dpc) and reached the peak temperature at about 11 dpc. Among them, three of the pigs reached 41.2°C. The rectal temperatures of the piglets in the mock group were below 40.5°C throughout the experimental period. In the vaccinated group, one piglet developed symptoms of fever, which lasted from 8 to 12 dpc, before returning to normal. The rectal temperature of the other piglets in this group was regular (Figure 2A). The clinical sign scores in the BC group from 8 to 21 dpc were significantly higher than those in the inoculation and mock groups (P < 0.001, Figure 2B). Of note, all ZJqz21-infected piglets survived throughout the study. The ZJqz21 strain was found to be a moderately virulent isolate. Compared with piglets in the BC group, the piglets vaccinated with rPRRSV-E2 were challenged with the ZJqz21 strain, and there were almost no signs of a high fever or other abnormal clinical symptoms. Only one pig in the vaccinated group developed symptoms of clinical fever, which lasted for 5 days. The rectal temperature was approximately 41°C; however, the mental state was normal and did not show serious clinical symptoms (Figure 2A).

The antibody reaction induced by rPRRSV-E2 and the subsequent challenge by ZJqz21 were examined to determine the protective efficacy of rPRRSV-E2 against the heterologous strain ZJqz21. Based on the serum collected at 0, 7, 14, 21, 28, 35, 42, and 49 dpi, the immune response to PRRSV and CSFV was analyzed. The group inoculated with rPRRSV-E2 produced PRRSV-specific immune effects. All of the piglets vaccinated with rPRRSV-E2 showed seroconversion by 14 dpc, and the average S/P showed a peak of 1.75 ± 0.39 at 28 dpc (Figure 2C), demonstrating that rPRRSV-E2 vaccination-induced high-level PRRSV-specific antibodies. At the same time, the level of CSFV-specific antibodies in the piglets inoculated with rPRRSV-E2 began to increase at 7 dpv and rapidly climbed to the average peak titer at 35 dpv. At about 2 weeks after vaccination with the rPRRSV-E2 vaccine, CSFV-specific antibodies in piglets were seroconverted in the vaccination group and reached 82.6 ± 2.1% after 28 dpv (Figure 2D). Following the viral challenge, the S/P values indicated that the PRRSV-specific antibodies in the BC group increased from 10 dpv and were higher than those of the mock group (Figure 2C). During the study period, PRRSV-specific and CSFV-specific antibodies in the piglets of the mock group remained negative (Figures 2C,D).

rPRRSV-E2 Vaccination Alleviated the Clinical Signs Typical of PRRSV Caused by ZJqz21

All piglets in rPRRSV-E2 vaccinated, and mock groups were generally normal and did not show obvious pathological damage typical of PRRS disease. Piglets inoculated with the ZJqz21 strain (BC group) showed severe pathological changes, including tonsil necrosis and hemorrhage, congestion, and swelling of the lymph nodes, bladder, and petechial kidney hemorrhage. Table 4 describes the overall pathological changes in the three groups. Typical pathological signs of PRRSV were observed in the BC group, such as lung lesions, enlarged bleeding of the mesenteric and inguinal lymph nodes, hemorrhage and necrosis of the spleen, and hemorrhaging in the throat and kidney, and tonsil (Table 4). An analysis of the macroscopic score in the lungs showed that infected piglets in the BC group showed severe pathological changes in the lung, significantly higher than those in the inoculation and mock groups (Table 5). The BC group displayed typical interstitial pneumonia and obvious microscopic lung lesions, including exfoliated epithelial cells in the bronchioles and collapsed alveoli infiltrating numerous inflammatory cells in alveolar spaces (Figure 3). The lymph nodes of piglets inoculated with ZJqz21 showed lower proliferation of cortical lymphocytes and enlargement and hyperplasia of the lymphatic nodules (Figure 3). During the entire study period, neither macroscopic nor histological changes in the lung and lymph node lesions were observed in the vaccinated piglets and negative control piglets (Figure 3), indicating that rPRRSV-E2 vaccination significantly alleviated the clinical signs caused by ZJqz21.

rPRRSV-E2 Vaccination Decreased Viral Replication and Virus Shedding of ZJqz21 in Piglets

RT-qPCR was used to monitor the viral titer in the sera and excreta (including nasal and anal swab samples) to monitor and evaluate the viral load in tissues and organs. Viremia was detected in all three groups. In the BC group, all piglets inoculated with the ZJqz21 strain showed viremia, which started from 3 dpc and continued to 21 dpc. One of five vaccinated piglets in the vaccinated group exhibited PRRSV-viremia, which started from 3 dpc and lasted for 5 days. Viremia was lower than the negative

| Regions | CH-1a | JXA1 | GM2 | VR-2332 | NADC30 |
|---------|-------|------|-----|---------|--------|
| Genome | 86.9 | 87.0 | 84.4 | 86.8 | 90.1 |
| 5'UTR | 95.3 | 97.9 | 93.2 | 90.0 | 93.2 |
| ORF1a | 80.8/84.0 | 79.5/85.5 | 75.5/85.0 | 80.3/84.6 | 88.1/88.5 |
| ORF1b | 89.9/95.0 | 90.1/95.8 | 88.4/95.1 | 88.9/94.9 | 89.1/95.3 |
| ORF2a | 85.7/88.7 | 86.3/86.4 | 86.8/90.3 | 88.3/90.3 | 96.1/94.9 |
| ORF2b | 91.0/90.0 | 89.6/93.2 | 92.3/90.5 | 91.0/89.2 | 95.9/93.2 |
| ORF3 | 83.7/81.2 | 83.7/80.8 | 82.7/82.4 | 84.1/82.4 | 96.2/95.7 |
| ORF4 | 87.7/88.3 | 86.6/88.3 | 86.8/87.2 | 88.3/88.3 | 97.4/97.8 |
| ORF5 | 87.7/87.6 | 86.1/86.1 | 84.7/84.6 | 86.1/83.6 | 94.7/93.0 |
| ORF6 | 88.0/92.0 | 89.0/93.1 | 90.5/92.6 | 90.1/92.6 | 98.3/99.4 |
| ORF7 | 92.2/92.7 | 91.1/91.1 | 87.1/87.9 | 93.3/94.4 | 96.8/98.4 |
| 3'UTR | 89.4 | 89.4 | 88.7 | 94.0 | 97.4 |
line at 10 dpc and decreased to an undetectable level until the end of the experiment (Figure 4A), indicating that rPRRSV-E2 vaccination mitigated the viremia caused by ZJqz21. During the entire experiment, it was determined that the Ct value related to virus shedding was close to the detection limit for the nasal and anal samples from the vaccinated group. The level of virus shedding identified in both the nasal and anal swabs of the BC group was high (Figures 4B,C). The numerical values in the viral load of the tissues and organs exhibited similar trends with the detection of virus shedding (Figure 4D). The viral load was extremely high in the mesenteric, inguinal, mandibular lymph nodes, and tonsils of the BC group, indicating that rPRRSV-E2 vaccination also inhibited virus shedding of ZJqz21.

DISCUSSION

PRRS is an important swine disease rapidly becoming prevalent worldwide and has caused huge economic losses to the pig industry since the 1990s (Lunney et al., 2010). In 2006, there was an HP-PRRSV outbreak in China, posing a serious threat to the Chinese pig industry (Tian et al., 2007, 2009; Zhou et al., 2008). Subsequently, HP-PRRSV with a 30-aa discontinuous deletion in the Nsp2-coding region gradually emerged as the major epidemic strain in China (Zhou et al., 2009, 2014). The emergence of the new lineage 3 (QYYZ-like) and lineage 1 (NADC30-like) viruses in mainland China in 2010 and 2013, respectively, complicated the epidemic situation of PRRS (Lu et al., 2015; Zhao et al., 2015; Zhou et al., 2015). The diversity of PRRSV strains poses a substantial challenge to protecting existing live vaccines. The NADC30-like strain, which is easy to recombine with other PRRSV strains, has become more complex and challenging on pig farms since it became popular in China but shows moderate or relatively low clinical virulence. Since its first outbreak, researchers in China have paid close attention to NADC30-like PRRSV.

Seven commercial MLV vaccines are currently used to control and prevent PRRSV infection in China with their corresponding virus strains, including Ingelvac PRRS MLV/RespPRRS MLV (VR-2332), CH-1R (parental strain CH-1a), HuN4-F112 (HuN4), JX11-P80 (JX11), R98 (R98), TJM-F92 (TJ), and GDr180 (GD). These PRRS MLV vaccines are obtained from the classical or HP-PRRSV strains, which can provide adequate protection against genetically homologous strains (Wang et al., 2021); however, the protective effect of MLV vaccines for resisting challenge with heterologous strains is far from conclusive. Zhou et al. (2017) found that the cross-protective efficacy of three different attenuated vaccine strains (including an HP-PRRSV attenuated vaccine strain) against the NADC30-like strain was extremely limited against infection with the NADC30-like virus. Among these strains, Ingelvac PRRS MLV was a commercial vaccine based on the classical PRRSV-2 strain that appeared to exert
### TABLE 4 | Gross evaluation and pathological examination of piglets in three groups.

| Designation | Number | Macroscopic score (lung) | Mean ± SD | Pathological changes |
|-------------|--------|--------------------------|-----------|----------------------|
| Gross evaluation and pathological examination of piglets infected with ZJqz21 | | | | |
| Lung Hemorrhage | 1/5 | 5/5 | 1/5 | |
| Mesenteric lymph node Swelling | 0/5 | 4/5 | 5/5 | |
| Spleen Hemorrhage | 0/5 | 3/5 | 0/5 | |
| Kidney Blood spots | 0/5 | 1/5 | 0/5 | |
| Tonsil Hemorrhage | 1/5 | 4/5 | 0/5 | |
| Brain Hemorrhage | 0/5 | 2/5 | 0/5 | |
| Throats Hemorrhage | 1/5 | 4/5 | 1/5 | |
| Thoracic adhesion Pleural effusion | 0/5 | 3/5 | 0/5 | |
| Peritoneal adhesion | 0/5 | 1/5 | 0/5 | |

Mean values ± SD of macroscopic lung scores ≥ 50 were defined as serious lesions.

### TABLE 5 | Mean value ± SD of macroscopic scores of the gross lung lesion in three groups.

| Designation | Number | Macroscopic score (lung) | Mean ± SD | Pathological changes |
|-------------|--------|--------------------------|-----------|----------------------|
| Vaccinated | 5 | 26.30 ± 6.01 | 4 | 1 | 0 |
| BC group | 5 | 68.20 ± 8.98 | 0 | 0 | 5 |
| Mock | 5 | 19.00 ± 3.20 | 5 | 0 | 0 |

*Macroscopic scores ≤ 30.

**Macroscopic scores > 30 and ≤ 50.

***Macroscopic scores > 50.

In dealing with complex epidemic situations, there is an urgent need to establish a good challenge model to evaluate the current vaccine candidates in preventing infection with a heterologous strain. In this study, we isolated a novel PRRSV strain, ZJqz21, and identified its genomic characterization, pathogenicity, and application as a challenge model for the heterologous protection of rPRRSV-E2. ZJqz21 was identified and clustered into the NADC30-like lineage (Figure 1). High genetic diversity is a significant characteristic of PRRSV. A previous study constructed the global classification system of PRRSV based on a comprehensive analysis of the complete ORF5 gene sequence. PRRSV-1 was divided into three subtypes (subtypes 1-3) according to the classification system, and PRRSV-2 was classified into 9 lineages with several sublineages in each lineage (Shi et al., 2010b; Zhou et al., 2014). ZJqz21 had a discontinuous 131-aa (111 + 1 + 19-aa) deletion in Nsp2 compared to VR-2332, which was identical to NADC30. A phylogenetic analysis of the full genome and ORF5 sequences showed that ZJqz21 was classified as lineage 1.8 with genetic features from NADC30 (Figure 1). ZJqz21 and NADC30 also belonged to lineage 1.8. The structural proteins only presented 5–8% differences between each other in the amino acid sequence (Table 3). NADC30-like PRRSVs have been recognized as moderately pathogenic isolate strains (Tian, 2017). The pathogenicity of ZJqz21 was evaluated to explore whether it could be applied as the challenge model for the heterologous protection of PRRSV candidate vaccines. Our results showed that none of the piglets died upon challenge with ZJqz21; however, all piglets exhibited typical PRRS clinical symptoms, including fever, cough, inappetence, dyspnea, and tachypnea (Tables 4, 5). Microscopic lung lesions and interstitial pneumonia were observed in ZJqz21-infected piglets (Figure 3). The above results demonstrated that the novel ZJqz21 strain exhibited moderate virulence in piglets and could be used as a challenge model to evaluate the current vaccine candidates in preventing infection with NADC30-like strain. Nevertheless, in this experiment, rPRRSV-E2 could not perfectly prevent the symptoms of clinical fever after a challenge with the ZJqz21 strain. One out of five pigs in the vaccinated group exhibited clinical fever from day 5 dpc, which lasted for 5 days, and then returned to normal. However, the rectal temperature was not particularly high at around 41°C. Although one piglet in the rPRRSV-E2 vaccinated group had fever symptoms, there were no other more serious clinical symptoms, and there were no significant differences in the clinical symptoms and lung lesions between the vaccinated and mock group (Table 4). There were no typical pathological PRRSV-specific changes in histopathologic slides' results (Figure 3).

Moreover, there were no significant differences in viremia, viral shedding, or viral load between this pig and other pigs in the same group and those in the mock group (Figure 4). This finding may be related to the infection of piglets with other pathogens. Future studies should confirm that our vaccine candidate strain rPRRSV-E2 displays a good cross-protection effect against heterologous strains and is a good choice for clinical prevention and control of NADC30-like strains.

In conclusion, the current vaccine candidate rPRRSV-E2 can provide good protection against the attack of the
FIGURE 3 | Histopathology analysis by hematoxylin-eosin staining in three groups. Each group’s microscopic figures of the lung, liver, spleen, mesenteric lymph nodes, and mandibular lymph nodes are shown and observed at a magnification of \( \times 200 \). The infiltration of inflammatory cells around the alveolar septa and alveolar spaces was shown clearly by black arrows.

FIGURE 4 | Detection of viremia, virus shedding, and viral load in the tissues by RT-qPCR. (A) Viremia detection in experiments assessing the immune efficacy of rPRRSV-E2 against ZJqz21. (B) Identification of viral RNA in nasal swabs to assess viral shedding. (C) Viral RNA detection in anal swabs to assess viral shedding. (D) Detection of the viral load in the lungs, heart, spleen, liver, kidneys, mesenteric lymph nodes, inguinal lymph nodes, mandibular lymph nodes, and tonsils.

moderately pathogenic heterologous PRRSV strain ZJqz21 in piglets. rPRRSV-E2 vaccination alleviated the clinical signs, viral replication, and virus shedding of ZJqz21 in piglets. Our research emphasizes the importance of developing a broad-spectrum vaccine to prevent and control further complicated PRRS epidemics in China.

DATA AVAILABILITY STATEMENT
The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.
ETHICS STATEMENT

All experimental programs involving pigs were carried out in accordance with the Guidelines for the Nursing and Use of Experimental Animals, and approved by the Ethics Committee of Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences (number SV-20201021-Y02).

AUTHOR CONTRIBUTIONS

FG and GT conceived and designed the study. LL and JC wrote the main manuscript text and prepared the figures. ZC, YC, ZG, SQ, and JL performed the experiments. WT, YZ, GL, YJ, CL, and LY prepared the manuscript. All authors reviewed the manuscript and participated in the experiments.

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