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# Lineage 1 porcine reproductive and respiratory syndrome virus attenuated live vaccine provides broad cross-protection against homologous and heterologous NADC30-like virus challenge in piglets

Zhijun Tian[1], HongLiang Zhang[1], Lirun Xiang[1], Hu Xu[1], Chao Li[1], Yandong Tang[1], Bangjun Gong[1], Wenli Zhang[1], Jing Zhao[1], Shuaijie Song[1], Jinmei Peng[1], Qian Wang[1], Tongqing An[1], and Xuehui Cai[1]

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February 22, 2024

**Abstract**

Porcine reproductive and respiratory syndrome virus (PRRSV) is an important pathogen that endangers the swine industry worldwide. Recently, lineage 1 PRRSVs, especially NADC30-like PRRSV, have become the major endemic strains in many pig-breeding countries. Since 2016, NADC30-like PRRSV has become the predominant strain in China. Unfortunately, current commercial vaccines cannot provide sufficient protection against this strain. Here, an attenuated lineage 1 PRRSV strain, named SD-R, was obtained by passaging NADC30-like PRRSV strain SD in Marc-145 cells for 125 passages. Four-week-old PRRSV-free piglets were vaccinated intramuscularly with $10^{5.0}$TCID $_{50}$ SD-R and then challenged intramuscularly (2 ml) and intranasally (2 ml) with homologous NADC30-like PRRSV SD ($1\times10^{5.0}$TCID $_{50}$/ml) and heterologous NADC30-like PRRSV HLJWK108-1711 ($1\times10^{5.0}$TCID $_{50}$/ml). The results showed that 5 of 5 immunized piglets had a perceptible humoral immune response to vaccination and did not develop fever or clinical diseases after NADC30-like PRRSV challenge. Additionally, compared to challenge control piglets, vaccinated piglets gained significantly more weight and showed much milder pathological lesions. Furthermore, the viral replication levels of the immunized group were significantly lower than those of the challenge control group. These results demonstrate, for the first time, that lineage 1 PRRSV SD-R is a good candidate for an efficacious vaccine against NADC30-like PRRSVs.

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**Abstract**

Porcine reproductive and respiratory syndrome virus (PRRSV) is an important pathogen that endangers the swine industry worldwide. Recently, lineage 1 PRRSVs, especially NADC30-like PRRSV, have become the major endemic strains in many pig-breeding countries. Since 2016, NADC30-like PRRSV has become the predominant strain in China. Unfortunately, current commercial vaccines cannot provide sufficient protection against this strain. Here, an attenuated lineage 1 PRRSV strain, named SD-R, was obtained by passaging NADC30-like PRRSV strain SD in Marc-145 cells for 125 passages. Four-week-old PRRSV-free piglets were vaccinated intramuscularly with $10^{5.0}$TCID$_{50}$ SD-R and then challenged intramuscularly (2 ml) and intranasally (2 ml) with homologous NADC30-like PRRSV SD ($1\times10^{5.0}$TCID$_{50}$/ml) and heterologous NADC30-like PRRSV HLJWK108-1711 ($1\times10^{5.0}$TCID$_{50}$/ml). The results showed that 5 of 5 immunized piglets had a perceptible humoral immune response to vaccination and did not develop fever or clinical diseases after NADC30-like PRRSV challenge. Additionally, compared to challenge control piglets, vaccinated piglets gained significantly more weight and showed much milder pathological lesions. Furthermore, the viral replication levels of the immunized group were significantly lower than those of the challenge control group. These results demonstrate, for the first time, that lineage 1 PRRSV SD-R is a good candidate for an efficacious vaccine against NADC30-like PRRSVs.

**Keywords:**

PRRSV, Lineage 1, NADC30-like, SD-R, Pathogenicity, cross-protection efficacy

**Introduction**

Porcine reproductive and respiratory syndrome virus (PRRSV), an enveloped, positive-sense, single-stranded RNA virus of the family*Arteriviridae* and the genus *Porarterivirus* , is the aetiological agent of porcine reproductive and respiratory syndrome (PRRS), which causes enormous economic losses to the global swine industry (Nathues et al., 2017). PRRSVs can be divided into two distinct species, *Betaarterivirus suid 1* (PRRSV-1) and*Betaarterivirus suid 2* (PRRSV-2) (ICTV2021). PRRSV-1 is mainly prevalent in Europe, and PRRSV-2 is prevalent in America and Asia; partial subtypes of both PRRSVs can be found across North America, Europe, and Asia (Shi et al., 2010a; Stadejek et al., 2013). In 2010, a phylogenetic lineage-based PRRSV typing method was proposed. This classification system grouped PRRSV-1 strains into four subtypes (subtype I (Global), subtype I (Russia), subtype II and III) and PRRSV-2 strains into nine lineages (lineage 1-lineage 9) based on phylogenetic relationships in the ORF5 region (Shi et al., 2010a; Shi et al., 2010b). Although subtype I (Global) of PRRSV-1 has been reported in Asia and America, the other subtypes have not been reported in regions other than Europe (Chen et al., 2011; Dewey et al., 2000; Fang et al., 2007; Lee et al., 2010; Ropp et al., 2004; Thanawongnuwech et al., 2004). PRRSV-2 has a high degree of genetic diversity, and the 9 lineages can be further divided into several sublineages (Shi et al., 2010b). The earliest reported lineage was lineage 5, which appeared in the United States and is mainly distributed in the United States, southern Canada and parts of China (Shi et al., 2010a). Then, lineages 8 and 9 were discovered throughout the United States (Shi et al., 2010b). However, it is puzzling that sublineage 8.7 (HP-PRRSV), which was first reported in China in 2006 with the characteristics of causing high temperatures in infected pigs and having a high incidence and high mortality, is only found in Asian countries (Shi et al., 2010b). Lineages 3, 4, 6 and 7 have been identified in only a small number of areas: lineage 3 mainly in southern China (including the Taiwan region and Hong Kong) (Chueh et al., 1998; Deng et al., 2015; Shi et al., 2010a; Shi et al., 2010b; Zhang et al., 2019b), lineage 4 mainly in Japan (Shi et al., 2010b), and lineages 6 and 7 in the United States(Shi et al., 2010a). Undoubtedly, lineage 1 has become the most prevalent and diverse lineage within the American and Asian swine industries(Paploski et al., 2021; Sun et al., 2020). Through 2021, lineage 1 (NADC30-like and NADC34-like) continued to be the most prevalent and diverse lineage within the U.S. swine industry (Makau et al., 2021a; Makau et al., 2021b; Paploski et al., 2021; Yu et al., 2020). In Peru, 75% of the strains detected were associated with PRRSV 1-7-4 strains (sublineage 1.5; NADC34-like) during 2015-2017 (Ramirez et al., 2019). In South Korea and Japan, lineage 1 (sublineage 1.8; NADC30-like) comprised the second-largest population of PRRSVs (Fukunaga et al., 2021; Kim et al., 2021). According to the latest report, lineage 1 (NADC30-like and NADC34-like) strains accounted for 64%

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of positive samples in China, much higher than the proportions of other lineages (Xu et al., 2022). Unlike PRRSV-1 and other lineage strains of PRRSV-2, which circulate on only one continent, lineage 1 strains have a global pandemic trend. Furthermore, lineage 1C variants also threaten the global swine industry (Trevisan et al., 2021).

Due to the large genetic diversity and complex recombination of NADC30 strains, the pathogenicity of NADC30-like strains varies greatly (Chen et al., 2018; Li et al., 2021b; Zhang et al., 2019a). As prototypes of NADC30-like strains, MN184 and NADC30 have moderate pathogenicity (Brockmeier et al., 2012). The NADC30-like strains in Korea show mild-to-moderate pathogenicity in challenged pigs (Jeong et al., 2018; Kwon et al., 2019; Oh et al., 2019). Additionally, the Japanese strain Jpn5-37 induces moderate symptoms in animals (Iseki et al., 2016). Some NADC30-like strains in China show high pathogenicity (JL580, SD17-38, 14LY01-FJ, 14LY02-FJ, 15LY01-FJ, 15LY02-FJ, FJXS15, HBap4-2018, JS18-3) (Chen et al., 2018; Han et al., 2020; Liu et al., 2017a; Liu et al., 2017b; Zhao et al., 2015); however, most strains show moderate pathogenicity (HNjz15, CHsx1401, SD53-1603, SC-d, TJnh1501, SCN17, HB17A, SCya18, HN201605, FJZ03, FJWQ16, ZJqz21, v2016/ZJ/09-03, FJ1402) (Bian et al., 2017; Sui et al., 2018; Sun et al., 2016; Wang et al., 2018; Wei et al., 2019; Zhang et al., 2019a; Zhang et al., 2018; Zhang et al., 2016; Zhou et al., 2018; Zhou et al., 2019; Zhou et al., 2017). Based on cumulative data, recombination may be responsible for the pathogenicity variance of NADC30-like PRRSVs in China, and the pathogenicity tends to be intermediate between those of the parental strains (Yu et al., 2021).

Prevention and control of PRRSV with vaccines has a long history. As early as 1994, a PRRSV-2 modified-live virus (MLV) vaccine was first commercialized in North America (Chae, 2021). In China, there are currently two types of PRRS vaccines: MLV and killed virus (KV) vaccines (Li et al., 2022). Nine commercial vaccines are currently used to control and prevent PRRSV infection in China, including Ingelvac PRRS MLV/RespPRRS MLV, CH-1R, HuN4-F112, JXA1-P80, R98, TJM-F92, GDr180, PC and CH-1a (KV) (Li et al., 2021a). Of these, RespPRRS MLV and R98 are of lineage 5, and the others belong to lineage 8 (Li et al., 2022). KV vaccines have poor protection against homologous and heterologous strains (Renukaradhya et al., 2015), and MLV vaccines can provide adequate protection against genetically homologous strains (Wang et al., 2021). Unfortunately, existing MLV vaccines offer only limited protection against NADC30-like strains, which are the main circulating strains in the country (Bai et al., 2016; Li et al., 2022; Sun et al., 2018; Wei et al., 2019; Zhang et al., 2016; Zhou et al., 2017). This limitation may be responsible for the rapid spread of NADC30-like PRRSVs in China. Therefore, it is necessary to develop a new vaccine against NADC30-like PRRSVs. In addition, the new vaccine must be evaluated for its cross-protection effect because of the highly variable genome sequences among NADC30-like PRRSVs caused by recombination and rapid mutation. In the present study, we developed an attenuated lineage 1 PRRSV vaccine, SD-R ($125^{\text{th}}$ passage of strain SD in Marc-145 cells), and evaluated its homologous and heterologous protection effects. SD-R provides safe and effective protection against homologous NADC30-like PRRSV SD and heterologous NADC30-like PRRSV HLJWK108-1711 challenge, and therefore can serve as an adequate vaccine against PRRSV infection in herds. To the best of our knowledge, the lineage 1 PRRSV vaccine SD-R is the first developed and evaluated attenuated NADC30-like PRRSV candidate vaccine strain in the world.

## Materials and Methods

### Ethics statements

This study was approved by the Animal Ethics Committee of the School of Harbin Veterinary Research Institute of the Chinese Academy of Agricultural Sciences and was performed in accordance with animal ethics guidelines and approved protocols. The Animal Ethics Committee Approval Number was SYXK (Hei) 2011022.

### Cells and viruses

The Marc-145 cell line was used for propagation and titration of wild-type strains and adapted strains. The NADC30-like PRRSV strains SD and HLJWK108-1711 were isolated and maintained in our laboratory.

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**Phylogenetic and genomic recombination analysis**

Multiple sequence alignments were generated using ClustalW in Lasergene (Version 7.1, DNASTAR Inc., Madison, WI, USA). Phylogenetic trees based on the whole genome were constructed in MEGA 6.0 using the neighbor-joining method with 1000 bootstrap replicates. Recombination analysis used RDP4 software with seven different algorithms (RDP, GeneConv, BootScan, MaxChi, Chimera, SiScan, and 3Seq) and SimPlot (version 3.5.1) by advancing a 500-bp sliding window along the genome alignments with a 20-bp step size.

**Viral culturing and attenuation**

Lineage 1 PRRSV strain SD was continuously passaged in Marc-145 cells using Dulbecco's modified Eagle's medium (DMEM) supplemented with 2% foetal bovine serum and incubated at 37degC with 5% $CO_2$. The virus was harvested once the virus-infected Marc-145 cells showed ˜80% cytopathic effect (CPE). The titres of the wild-type strains SD and HLJWK108-1711 and NADC30-like PRRSV SD at different passages were measured by seeding Marc-145 cells into 96-well cell culture plates 2 d before infection. The 50% tissue culture infective dose ($TCID_{50}$) was calculated according to the Reed-Muench method. The $125^{th}$ passage of PRRSV strain SD was harvested and designated lineage 1 PRRSV strain SD-R, which was characterized and evaluated in the present study.

**Whole-genome sequencing of lineage 1 PRRSV SD at different passages**

RNA was extracted from different SD passages: F5, F6, F8, F10, F20, F30, F40, F60, F80, F100, F105, F110, F125 (SD-R), F135 and F150. Reverse transcription PCR (RT–PCR), whole-genome sequencing, genome assembly and sequence alignments were performed as previously described (Zhang et al., 2019a). Detailed information on the whole-genome amplification primers is shown in a previous article (Zhang et al., 2019a).

**Evaluation of immunoprotection of SD-R against homologous and heterologous strains**

Twenty-six 28-day-old PRRSV-free piglets were obtained from two PRRS-free farms in Harbin. Thirteen PRRSV-free piglets used to assess homologous protection were randomly divided into three groups (A1-C1). Other PRRSV-free piglets used to test heterologous protection were randomly divided into three groups (A2-C2). Five piglets for each group were used for immunization and inoculation (A1, B1, A2 and B2), except for three piglets in negative control groups C1 and C2. Piglets in groups B1 and B2 were inoculated intramuscularly with $10^{5.0}TCID_{50}$ SD-R. After 28 days post-vaccination (dpv), piglets in groups A1, B1 and A2, B2 were infected with $5^{th}$-passage SD ($4x10^{5.0}TCID_{50}$ per pig) and $5^{th}$-passage HLJWK108-1711 ($4x10^{5.0}TCID_{50}$ per pig) intramuscularly (2 ml) and intranasally (2 ml), respectively. The animals were maintained in individual biosafety rooms. Clinical signs and rectal temperatures were recorded daily. The body weights of the piglets were measured weekly. Blood samples were periodically collected from individual piglets and tested for viremia. All of the piglets were euthanized at 21 dpi. Ten tissue samples were obtained from the hearts, livers, spleens, lungs, kidneys, lymph nodes, tonsils, small intestines, bladders, and stomachs for viral detection by TaqMan$^{(r)}$-based real-time fluorescence quantitative RT–PCR (65).

**Serological examination**

Serum samples were collected at dpv 0, 7, 14, 21, and 28 and dpi 3, 5, 7, 10, 14, and 21. PRRSV-specific antibodies were measured using a commercial ELISA kit (IDEXX, Inc., Westbrook, ME, USA) according to the manufacturer's instructions. The PRRSV-specific antibody titre was reported as the S/P ratio, and the serum samples were considered positive if the S/P ratio was [?]0.4.

**Viremia and viral loads in tissue assessment**

To determine the duration of viremia and viral loads in different tissues after treatment with the SD-R vaccine strain, serum samples collected at dpv 0, 7, 14, 21, and 28 and dpi 3, 5, 7, 10, 14, and 21 and ten tissues of all the piglets were used to detect the RNA copy number of PRRSV by TaqMan$^{(r)}$-based real-time fluorescence quantitative RT–PCR (Wei et al., 2008).

**Histological examination**

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At necropsy, the lungs and lymph nodes were harvested and examined for histopathology following haematoxylin and eosin (H&E) staining as previously described (Zhang et al., 2019a).

**Statistical analysis**

Significant differences between two groups were determined using a$t$ test in GraphPad 5.0 (San Diego, CA, USA). The level of significance was set at $p$ <0.05.

**Results**

**Genomic characteristics of NADC30-like PRRSV SD and HLJWK108-1711**

SD and HLJWK108-1711 were isolated from two diseased pig farms in Shandong (2016) and Heilongjiang Province (2017), respectively. Phylogenetic analysis showed that Chinese NADC30-like PRRSV formed a relatively independent branch and was closely related to NADC30 and XW018 (both strains isolated in the United States) based on a total of 344 whole genomes of lineage 1 (Fig. 1A). Both SD and HLJWK108-1711 were classified into branches of NADC30-like PRRSV (L1.8/L1C) (Fig. 1A). The Nsp2 proteins of SD and HLJWK108-1711 had a discontinuous 131-amino acid (aa) deletion (111 aa at position 323-433, 1 aa at position 483 and 19 aa at position 504-522) (Fig. 1B). Recombination analysis revealed that both NADC30-like PRRSVs were recombinant viruses (SD: parental virus NADC30 and minor virus ATCC VR2332-like PRRSV; HLJWK108-1711: parental virus NADC30 and minor virus JXA1-like PRRSV) (Fig. 1C). The recombination breakpoints of SD were observed at positions 7365, 7661, 12305, and 12773 (ATCC-VR2332 positions 7762, 8058, 12702 and 13170). The breakpoints separated the HLJWK108-1711 genome into ten regions, where the positions located at 521, 631, 1065, 1310, 1810, 5183, 6367, 7488, 8443(JXA1 positions 524, 634, 1068, 1313, 1813, 5490, 6674, 7795 and 8750) (Fig. 1C). However, the genomic nucleotide similarity between SD-R and HLJWK108-1711 was 89.9%, and the nucleotide similarity of the skeleton section of NADC30 was only 91.4% (Table 1). The nucleotide and amino acid similarity among different genes between SD-R and HLJWK108-1711 were 82.7-97.7% and 80.2-100%, respectively (Table 1).

**Nucleotide and amino acid mutations of different SD passages**

To develop a live attenuated lineage 1 PRRSV vaccine, we first isolated an SD strain and passaged it in Marc-145 cells. Compared to the parental virus SD, there were 75 nucleotide changes at the $125^{\text{th}}$ passage (Table 2). Among these mutations were two nucleotide changes (at position 29 [C-T] and 36 [C-T]) in the 5'-UTR and two (at position 22 [T-C] and 69 [T-C]) in the 3'-UTR (Table 2). Other nucleotide mutations were observed in Nsps and structural proteins, 31 of which were missense mutations, causing a change in 31 amino acids in Nsp2-5, 9-12, GP2a-5, M, N and ORF5a (Table 2). The major changes in amino acids were located on Nsp2 and minor structural proteins GP2a-4 (Table 2). No nucleotide amino acid changes were observed from passages 125 through 150 (Table 2).

**Clinical reactions after immunization and challenge**

After immunization, none of the piglets in groups A1 (SD-R vaccine-treated and SD-challenge group) and A2 (SD-R vaccine-treated and HLJWK108-1711-challenge group) showed any clinical signs of PRRS compared to the negative control group and challenge control group. After challenge, none of the piglets in groups A1 (Fig. 2A, Fig. 3A) and A2 (Fig. 2B, Fig. 3B) showed significant changes in body temperature or weight loss. However, the piglets in group B1 (nonvaccinated and SD-challenge group) had various disease manifestations at 5 dpi, including fever persisting for 6-13 d ([?]40.5) (Fig. 2A), and the piglets in group B2 (nonvaccinated and HLJWK108-1711-challenge group) had various disease manifestations as early as 1 dpi, including intermittent fever for 5-10 d ([?]40.5) (Fig. 2B). In addition, the piglets in groups B1 and B2 had various levels of anorexia and emaciation. Compared to the negative control piglets in groups C1 and C2, the piglets in groups B1 and B2 gained less body weight ($p$ <0.05) during 8-14 dpi and 15-21 dpi (Fig. 3A, Fig. 3B). In the course of the study, the piglets in groups C1 and C2 had no clinical signs of disease.

**Antibody responses in immunized or challenged piglets**

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The antibody response in the ELISA showed that all immunized piglets in groups A1 and A2 were seroconverted by 14 dpi (Fig. 4A, Fig. 4B). A total of 3 of 5 piglets in group B1 were seroconverted by 7 dpi, and the remaining piglets seroconverted by 10 dpi (Fig. 4A). Two of five piglets in group B2 were seroconverted by 5 dpi, and the remaining piglets seroconverted by 7 dpi (Fig. 4B). No PRRSV-specific antibodies were detected in the control piglets prior to challenge (Fig. 4A, Fig. 4B). Antibody responses of piglets in groups C1 and C2 were negative throughout the study (Fig. 4A, Fig. 4B).

**Viremia and viral tissue distribution between the immunized-challenge group and the challenge group**

To further evaluate the difference in viremia and distribution in ten tissues among different groups, serum samples from dpv 0, 7, 14, 21 and 28, dpi 3, 5, 7, 10, 14 and 21 and ten organ tissues were evaluated using real-time PCR. The RNA copy numbers of the serum samples in groups B1 and B2 reached their highest levels at 5 dpi and then gradually declined in these two groups (Fig. 5A, Fig. 5C). The viremia levels at every time point in groups A1 and A2 were significantly lower than those in groups B1 and B2, respectively (Fig. 5A, Fig. 5C). Furthermore, the viremia levels and the number of pigs with viremia in the homologous protection group (groups A1 and B1) were significantly lower than those in the heterologous protection group (groups A2 and B2) (Fig. 5A, Fig. 5C). The viral loads of ten tissues in groups A1 (except liver, stomach, intestine, brain and tonsil) and A2 (except tonsil and lymph nodes) were significantly lower than those in groups B1 and B2, respectively (Fig. 5A, Fig. 5C).

**Gross pathological and histopathological changes**

Compared with the piglets in the immunized-challenge groups (A1 and A2) (Fig. 6A b, h and Fig. 6B n, t) and negative control groups (C1 and C2) (Fig. 6A c, i and Fig. 6B o, u), the piglets in the challenge groups (B1 and B2) showed lesions typical of PRRS, such as consolidation in the lungs and haemorrhaging in the lymph nodes (Fig. 6A a, g and Fig. 6B m, s). Histopathology revealed a large amount of inflammatory cell infiltration, epithelial cell proliferation and significant alveolar diaphragm widening in the lungs (Fig. 6A d, p) and decreased lymphocyte and medullary bleeding in the lymph nodes (Fig. 6A j, v) in the challenge groups (B1 and B2) compared with the negative control groups (Fig. 6A f, l and Fig. 6B r, x). Notably, there was almost no pathological damage to the lungs (Fig. 6A e and Fig. 6B q) and only mildly decreased levels of lymphocytes in the immunized-challenge groups (A1 and A2) (Fig. 6A k and Fig. 6B w).

## DISCUSSION

Lineage 1, especially NADC30-like PRRSV, has become the most prevalent PPRSV lineage in North America and Asia (de Avellar and Markus, 1993; Kawabori et al., 1983; Savary and Ferron, 1982; Sun et al., 2020). In 2013, a new PRRSV strain called NADC30-like PRRSV, which has a unique 131-aa deletion within its NSP2 protein, was isolated from diseased piglets in China (Zhao et al., 2015; Zhou et al., 2015). This PRRSV originated in the United States and has become one of the major endemic strains in China since 2016 (Guo et al., 2019). The pathogenicity of NADC30-like PRRSVs ranges from moderate (Sun et al., 2016; Wang et al., 2018; Zhang et al., 2019a) to high (Chen et al., 2021; Liu et al., 2017b; Zhao et al., 2015), and most of them are moderately pathogenic. However, the current commercial vaccines, Ingelvac PRRS MLV/RespPRRS MLV(Sun et al., 2018; Wei et al., 2019), CH-1a(Li et al., 2022), HuN4-F112(Bai et al., 2016), JXA1-P80(Sun et al., 2018), R98(Zhang et al., 2016), TJM-F92(Bai et al., 2016) and GDr180(Bai et al., 2016; Zhang et al., 2019a), do not provide completely effective protection against NADC30-like PRRSVs. Here, we selected the moderately pathogenic NADC30-like PRRSV SD strain and described a newly developed lineage 1 PRRSV vaccine candidate, SD-R, which is efficacious in the prevention of clinical infection caused by NADC30-like PRRSVs.

Unlike CH-1a or HP-PRRSV, NADC30-like PRRSVs have lower levels of whole-genome similarity (Guo et al., 2019; Zhang et al., 2019a) and a wider variety of recombination patterns (Yu et al., 2020; Zhang et al., 2019a). Almost all NADC30-like PRRSVs are recombinant viruses (Yu et al., 2020; Zhang et al., 2019a). Although recombination breakpoints are relatively random, statistical analysis reveals that recombination hotspots range from nucleotide positions of approximately 7,900 to 8,100 and 12,400 to 13,500 (Yu et al.,

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2020). We speculated that NADC30-like PRRSVs with two recombination regions may be more stable and important for viral survival. Therefore, we selected an NADC30-like PRRSV SD with the above two recombination regions (7365-7661 in the NSP9 region and 12305-12773 in the GP2a-GP3 region) for passage in Marc-145 cells.

In the present study, SD-R, a genetically stable attenuated viral strain, was obtained by serial passaging in Marc-145 cells with the lineage 1 PRRSV SD strain. All amino acid mutations related to SD were observed before the $125^{th}$ passage. No nucleotide and amino acid mutations were observed between the $125^{th}$and $150^{th}$ passages. This indicated that the SD strain at the $125^{th}$ passage had adapted to the Marc-145 cells and was subsequently stably passaged. The HP-PRRSV vaccines JXA1-R, TJM, HuN4-F112 and GDr180 were obtained through passaging in Marc-145 cells for 80, 92, 112 and 180 passages, respectively (Leng et al., 2012; Liu et al., 2015; Tian et al., 2009; Yu et al., 2015). Because the pathogenicity of SD is far lower than that of HP-PRRSV, we believe that SD-R (the $125^{th}$ passage in Marc-145 cells) is safer for piglets than commercial HP-PRRSV vaccines. Furthermore, both high-dose and repeated-dose tests based on SD-R were safe for all piglets (data not shown).

In this study, piglets immunized with SD-R developed a rapid and effective humoral response and were effectively protected against NADC30-like PRRSV challenge. Indeed, PRRSV vaccines have poor cross-protection effects (Bai et al., 2016; Yu et al., 2021). However, surprisingly, SD-R could provide better cross-protection, even though the genomic nucleotide similarity of SD and HLJWK108-1711 was only 89.9%. All the piglets immunized with SD-R and then challenged with SD or HLJWK108-1711 survived without any major clinical signs at any point in the experimental period. Piglets in the immunized and challenge groups were healthier than those in the challenge control group based on clinical signs, body temperature, body weight, viremia and viral loads in tissues. Altogether, these results suggested that the SD-R candidate vaccine is effective against infections caused by different NADC30-like PRRSVs. However, the detailed molecular basis of cross-protection induced by the SD-R vaccine and its attenuation mechanism remain unclear. Furthermore, the cross-protection against other types of PRRSVs, such as NADC34-like PRRSV, QYYZ-like PRRSV and HP-PRRSV, should be studied further.

In conclusion, we developed the first attenuated lineage 1 PRRSV candidate vaccine strain, SD-R. Furthermore, SD-R was sufficiently attenuated and antigenic enough to confer protection against homologous and heterologous NADC30-like PRRSV challenge.

**Compliance with ethical standards**

The authors declare that they have no conflicts of interest.

**Acknowledgements**

This study was supported by grants from the National Natural Science Foundation of China (Grant nos. 32002315 and 32172890) and the China Postdoctoral Fund (Grant no. 2020M680788).

**References**

Bai, X., Wang, Y., Xu, X., Sun, Z., Xiao, Y., Ji, G., Li, Y., Tan, F., Li, X., Tian, K., 2016. Commercial vaccines provide limited protection to NADC30-like PRRSV infection. Vaccine 34, 5540-5545.

Bian, T., Sun, Y., Hao, M., Zhou, L., Ge, X., Guo, X., Han, J., Yang, H., 2017. A recombinant type 2 porcine reproductive and respiratory syndrome virus between NADC30-like and a MLV-like: Genetic characterization and pathogenicity for piglets. Infect Genet Evol 54, 279-286.

Brockmeier, S.L., Loving, C.L., Vorwald, A.C., Kehrli, M.E., Jr., Baker, R.B., Nicholson, T.L., Lager, K.M., Miller, L.C., Faaberg, K.S., 2012. Genomic sequence and virulence comparison of four Type 2 porcine reproductive and respiratory syndrome virus strains. Virus Res 169, 212-221.

Chae, C., 2021. Commercial PRRS Modified-Live Virus Vaccines. Vaccines (Basel) 9.

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Chen, N., Cao, Z., Yu, X., Deng, X., Zhao, T., Wang, L., Liu, Q., Li, X., Tian, K., 2011. Emergence of novel European genotype porcine reproductive and respiratory syndrome virus in mainland China. J Gen Virol 92, 880-892.

Chen, N., Ye, M., Li, S., Huang, Y., Zhou, R., Yu, X., Tian, K., Zhu, J., 2018. Emergence of a novel highly pathogenic recombinant virus from three lineages of porcine reproductive and respiratory syndrome virus 2 in China 2017. Transbound Emerg Dis 65, 1775-1785.

Chen, P., Tan, X., Lao, M., Wu, X., Zhao, X., Zhou, S., Yu, J., Zhu, J., Yu, L., Tong, W., Gao, F., Yu, H., Liu, C., Jiang, Y., Tong, G., Zhou, Y., 2021. The Novel PRRSV Strain HBap4-2018 with a Unique Recombinant Pattern Is Highly Pathogenic to Piglets. Virol Sin 36, 1611-1625.

Chueh, L.L., Lee, K.H., Wang, F.I., Pang, V.F., Weng, C.N., 1998. Sequence analysis of the nucleocapsid protein gene of the porcine reproductive and respiratory syndrome virus Taiwan MD-001 strain. Adv Exp Med Biol 440, 795-799.

de Avellar, M.C., Markus, R.P., 1993. Age-related changes in norepinephrine release and its modulation by presynaptic alpha-2 adrenoceptors in the rat vas deferens. J Pharmacol Exp Ther 267, 38-44.

Deng, M.C., Chang, C.Y., Huang, T.S., Tsai, H.J., Chang, C., Wang, F.I., Huang, Y.L., 2015. Molecular epidemiology of porcine reproductive and respiratory syndrome viruses isolated from 1991 to 2013 in Taiwan. Arch Virol 160, 2709-2718.

Dewey, C., Charbonneau, G., Carman, S., Hamel, A., Nayar, G., Friendship, R., Eernisse, K., Swenson, S., 2000. Lelystad-like strain of porcine reproductive and respiratory syndrome virus (PRRSV) identified in Canadian swine. Can Vet J 41, 493-494.

Fang, Y., Schneider, P., Zhang, W.P., Faaberg, K.S., Nelson, E.A., Rowland, R.R., 2007. Diversity and evolution of a newly emerged North American Type 1 porcine arterivirus: analysis of isolates collected between 1999 and 2004. Arch Virol 152, 1009-1017.

Fukunaga, W., Hayakawa-Sugaya, Y., Koike, F., Van Diep, N., Kojima, I., Yoshida, Y., Suda, Y., Masatani, T., Ozawa, M., 2021. Newly-designed primer pairs for the detection of type 2 porcine reproductive and respiratory syndrome virus genes. J Virol Methods 291, 114071.

Guo, Z., Chen, X.X., Li, X., Qiao, S., Deng, R., Zhang, G., 2019. Prevalence and genetic characteristics of porcine reproductive and respiratory syndrome virus in central China during 2016-2017: NADC30-like PRRSVs are predominant. Microb Pathog 135, 103657.

Han, G., Lei, K., Xu, H., He, F., 2020. Genetic characterization of a novel recombinant PRRSV2 from lineage 8, 1 and 3 in China with significant variation in replication efficiency and cytopathic effects. Transbound Emerg Dis 67, 1574-1584.

Iseki, H., Morozumi, T., Takagi, M., Kawashima, K., Shibahara, T., Uenishi, H., Tsunemitsu, H., 2016. Genomic sequence and virulence evaluation of MN184A-like porcine reproductive and respiratory syndrome virus in Japan. Microbiol Immunol 60, 824-834.

Jeong, J., Kim, S., Park, C., Park, K.H., Kang, I., Park, S.J., Chae, C., 2018. Commercial porcine reproductive and respiratory syndrome virus (PRRSV)-2 modified live virus vaccine against heterologous single and dual Korean PRRSV-1 and PRRSV-2 challenge. Vet Rec 182, 485.

Kawabori, S., Okuda, M., Unno, T., 1983. Mast cells in allergic nasal epithelium and lamina propria before and after provocation. An electron microscopic study. Clin Allergy 13, 181-189.

Kim, S.C., Jeong, C.G., Park, G.S., Park, J.Y., Jeoung, H.Y., Shin, G.E., Ko, M.K., Kim, S.H., Lee, K.K., Kim, W.I., 2021. Temporal lineage dynamics of the ORF5 gene of porcine reproductive and respiratory syndrome virus in Korea in 2014-2019. Arch Virol 166, 2803-2815.

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Kwon, T., Yoo, S.J., Park, J.W., Kang, S.C., Park, C.K., Lyoo, Y.S., 2019. Genomic characteristics and pathogenicity of natural recombinant porcine reproductive and respiratory syndrome virus 2 harboring genes of a Korean field strain and VR-2332-like strain. Virology 530, 89-98.

Lee, C., Kim, H., Kang, B., Yeom, M., Han, S., Moon, H., Park, S., Kim, H., Song, D., Park, B., 2010. Prevalence and phylogenetic analysis of the isolated type I porcine reproductive and respiratory syndrome virus from 2007 to 2008 in Korea. Virus Genes 40, 225-230.

Leng, X., Li, Z., Xia, M., He, Y., Wu, H., 2012. Evaluation of the efficacy of an attenuated live vaccine against highly pathogenic porcine reproductive and respiratory syndrome virus in young pigs. Clin Vaccine Immunol 19, 1199-1206.

Li, C., Liu, Z., Chen, K., Qian, J., Hu, Y., Fang, S., Sun, Z., Zhang, C., Huang, L., Zhang, J., Huang, N., 2022. Efficacy of the Synergy Between Live-Attenuated and Inactivated PRRSV Vaccines Against a NADC30-Like Strain of Porcine Reproductive and Respiratory Syndrome Virus in 4-Week Piglets. Front Vet Sci 9, 812040.

Li, L., Chen, J., Cao, Z., Cao, Y., Guo, Z., Tong, W., Zhou, Y., Li, G., Jiang, Y., Liu, C., Yu, L., Qiao, S., Liu, J., Tong, G., Gao, F., 2021a. Recombinant Bivalent Live Vectored Vaccine Against Classical Swine Fever and HP-PRRS Revealed Adequate Heterogeneous Protection Against NADC30-Like Strain. Front Microbiol 12, 822749.

Li, Y., Xu, G., Du, X., Xu, L., Ma, Z., Li, Z., Feng, Y., Jiao, D., Guo, W., Xiao, S., 2021b. Genomic characteristics and pathogenicity of a new recombinant strain of porcine reproductive and respiratory syndrome virus. Arch Virol 166, 389-402.

Liu, C., Zhang, W., Gong, W., Zhang, D., She, R., Xu, B., Ning, Y., 2015. Comparative Respiratory Pathogenicity and Dynamic Tissue Distribution of Chinese Highly Pathogenic Porcine Reproductive and Respiratory Syndrome Virus and its Attenuated Strain in Piglets. J Comp Pathol 153, 38-49.

Liu, J., Zhou, X., Zhai, J., Wei, C., Dai, A., Yang, X., Luo, M., 2017a. Recombination in JXA1-R vaccine and NADC30-like strain of porcine reproductive and respiratory syndrome viruses. Vet Microbiol 204, 110-120.

Liu, J.K., Zhou, X., Zhai, J.Q., Li, B., Wei, C.H., Dai, A.L., Yang, X.Y., Luo, M.L., 2017b. Emergence of a novel highly pathogenic porcine reproductive and respiratory syndrome virus in China. Transbound Emerg Dis 64, 2059-2074.

Makau, D.N., Alkhamis, M.A., Paploski, I.A.D., Corzo, C.A., Lycett, S., VanderWaal, K., 2021a. Integrating animal movements with phylogeography to model the spread of PRRSV in the USA. Virus Evol 7, veab060.

Makau, D.N., Paploski, I.A.D., Corzo, C.A., VanderWaal, K., 2021b. Dynamic network connectivity influences the spread of a sub-lineage of porcine reproductive and respiratory syndrome virus. Transbound Emerg Dis.

Nathues, H., Alarcon, P., Rushton, J., Jolie, R., Fiebig, K., Jimenez, M., Geurts, V., Nathues, C., 2017. Cost of porcine reproductive and respiratory syndrome virus at individual farm level - An economic disease model. Prev Vet Med 142, 16-29.

Oh, T., Kim, H., Park, K.H., Jeong, J., Kang, I., Yang, S., Chae, C., 2019. Effectiveness of a commercial porcine reproductive and respiratory syndrome virus (PRRSV) subunit vaccine against heterologous PRRSV-1 and PRRSV-2 challenge in late-term pregnant gilts. Can J Vet Res 83, 248-254.

Paploski, I.A.D., Pamornchainavakul, N., Makau, D.N., Rovira, A., Corzo, C.A., Schroeder, D.C., Cheeran, M.C., Doeschl-Wilson, A., Kao, R.R., Lycett, S., VanderWaal, K., 2021. Phylogenetic Structure and Sequential Dominance of Sub-Lineages of PRRSV Type-2 Lineage 1 in the United States. Vaccines (Basel) 9.

Posted on 31 Mar 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — https://doi.org/10.22541/au.164873626.61635141/v1 — This is a preprint and has not been peer-reviewed. Data may be preliminary.

Ramirez, M., Bauermann, F.V., Navarro, D., Rojas, M., Manchego, A., Nelson, E.A., Diel, D.G., Rivera, H., 2019. Detection of porcine reproductive and respiratory syndrome virus (PRRSV) 1-7-4-type strains in Peru. Transbound Emerg Dis 66, 1107-1113.

Renukaradhya, G.J., Meng, X.J., Calvert, J.G., Roof, M., Lager, K.M., 2015. Inactivated and subunit vaccines against porcine reproductive and respiratory syndrome: Current status and future direction. Vaccine 33, 3065-3072.

Ropp, S.L., Wees, C.E., Fang, Y., Nelson, E.A., Rossow, K.D., Bien, M., Arndt, B., Preszler, S., Steen, P., Christopher-Hennings, J., Collins, J.E., Benfield, D.A., Faaberg, K.S., 2004. Characterization of emerging European-like porcine reproductive and respiratory syndrome virus isolates in the United States. J Virol 78, 3684-3703.

Savary, P., Ferron, P., 1982. Screening of hearing disorders in schoolchildren. Am J Otolaryngol 3, 388-391.

Shi, M., Lam, T.T., Hon, C.C., Hui, R.K., Faaberg, K.S., Wennblom, T., Murtaugh, M.P., Stadejek, T., Leung, F.C., 2010a. Molecular epidemiology of PRRSV: a phylogenetic perspective. Virus Res 154, 7-17.

Shi, M., Lam, T.T., Hon, C.C., Murtaugh, M.P., Davies, P.R., Hui, R.K., Li, J., Wong, L.T., Yip, C.W., Jiang, J.W., Leung, F.C., 2010b. Phylogeny-based evolutionary, demographical, and geographical dissection of North American type 2 porcine reproductive and respiratory syndrome viruses. J Virol 84, 8700-8711.

Stadejek, T., Stankevicius, A., Murtaugh, M.P., Oleksiewicz, M.B., 2013. Molecular evolution of PRRSV in Europe: current state of play. Vet Microbiol 165, 21-28.

Sui, X., Guo, X., Jia, H., Wang, X., Lin, W., Li, M., Gao, X., Wu, J., Jiang, Y., Willems, L., Zhu, H., Xin, T., Hou, S., 2018. Genomic sequence and virulence of a novel NADC30-like porcine reproductive and respiratory syndrome virus isolate from the Hebei province of China. Microb Pathog 125, 349-360.

Sun, Y.F., Zhou, L., Bian, T., Tian, X.X., Ren, W.K., Lu, C., Zhang, L., Li, X.L., Cui, M.S., Yang, H.C., Yu, H., 2018. Efficacy evaluation of two commercial modified-live virus vaccines against a novel recombinant type 2 porcine reproductive and respiratory syndrome virus. Vet Microbiol 216, 176-182.

Sun, Y.K., Chen, Y.J., Cai, Y., Li, Q., Xie, J.X., Liang, G., Gao, Q., Yu, Z.Q., Lu, G., Huang, L.Z., Ma, C.Q., Gong, L., Wang, H., Shi, M., Zhang, G.H., 2020. Insights into the evolutionary history and epidemiological characteristics of the emerging lineage 1 porcine reproductive and respiratory syndrome viruses in China. Transbound Emerg Dis 67, 2630-2641.

Sun, Z., Wang, J., Bai, X., Ji, G., Yan, H., Li, Y., Wang, Y., Tan, F., Xiao, Y., Li, X., Tian, K., 2016. Pathogenicity comparison between highly pathogenic and NADC30-like porcine reproductive and respiratory syndrome virus. Arch Virol 161, 2257-2261.

Thanawongnuwech, R., Amonsin, A., Tatsanakit, A., Damrongwatanapokin, S., 2004. Genetics and geographical variation of porcine reproductive and respiratory syndrome virus (PRRSV) in Thailand. Vet Microbiol 101, 9-21.

Tian, Z.J., An, T.Q., Zhou, Y.J., Peng, J.M., Hu, S.P., Wei, T.C., Jiang, Y.F., Xiao, Y., Tong, G.Z., 2009. An attenuated live vaccine based on highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) protects piglets against HP-PRRS. Vet Microbiol 138, 34-40.

Trevisan, G., Li, G., Moura, C.A.A., Coleman, K., Thomas, P., Zhang, J., Gauger, P., Zeller, M., Linhares, D., 2021. Complete Coding Genome Sequence of a Novel Porcine Reproductive and Respiratory Syndrome Virus 2 Restriction Fragment Length Polymorphism 1-4-4 Lineage 1C Variant Identified in Iowa, USA. Microbiol Resour Announc 10, e0044821.

Wang, H., Xu, Y., Feng, W., 2021. Porcine Reproductive and Respiratory Syndrome Virus: Immune Escape and Application of Reverse Genetics in Attenuated Live Vaccine Development. Vaccines (Basel) 9.

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Wang, H.M., Liu, Y.G., Tang, Y.D., Liu, T.X., Zheng, L.L., Wang, T.Y., Liu, S.G., Wang, G., Cai, X.H., 2018. A natural recombinant PRRSV between HP-PRRSV JXA1-like and NADC30-like strains. Transbound Emerg Dis 65, 1078-1086.

Wei, C., Dai, A., Fan, J., Li, Y., Chen, A., Zhou, X., Luo, M., Yang, X., Liu, J., 2019. Efficacy of Type 2 PRRSV vaccine against challenge with the Chinese lineage 1 (NADC30-like) PRRSVs in pigs. Sci Rep 9, 10781.

Wei T, Tian Z, An T, Zhou Y, Xiao Y, Jiang Y, Hao X, Zhang S, Peng J, Qiu H, Tong G (2008) Development and application of Taq Man-MGB fluorescence quantitative RT-PCR assay for detection of porcine reproductive and respiratory syndrome virus. Chin J Prev Vet Med 30:5

Xu, H., Li, C., Li, W., Zhao, J., Gong, B., Sun, Q., Tang, Y.D., Xiang, L., Leng, C., Peng, J., Wang, Q., Meng, F., Yu, Y., An, T., Cai, X., Tian, Z.J., Zhang, H., 2022. Novel characteristics of Chinese NADC34-like PRRSV during 2020-2021. Transbound Emerg Dis.

Yu, F., Yan, Y., Shi, M., Liu, H.Z., Zhang, H.L., Yang, Y.B., Huang, X.Y., Gauger, P.C., Zhang, J., Zhang, Y.H., Tong, G.Z., Tian, Z.J., Chen, J.J., Cai, X.H., Liu, D., Li, G., An, T.Q., 2020. Phylogenetics, Genomic Recombination, and NSP2 Polymorphic Patterns of Porcine Reproductive and Respiratory Syndrome Virus in China and the United States in 2014-2018. J Virol 94.

Yu, X., Zhou, Z., Cao, Z., Wu, J., Zhang, Z., Xu, B., Wang, C., Hu, D., Deng, X., Han, W., Gu, X., Zhang, S., Li, X., Wang, B., Zhai, X., Tian, K., 2015. Assessment of the safety and efficacy of an attenuated live vaccine based on highly pathogenic porcine reproductive and respiratory syndrome virus. Clin Vaccine Immunol 22, 493-502.

Yu, Y., Zhang, Q., Cao, Z., Tang, Y.D., Xia, D., Wang, G., Shan, H., 2021. Recent Advances in Porcine Reproductive and Respiratory Syndrome Virus NADC30-Like Research in China: Molecular Characterization, Pathogenicity, and Control. Front Microbiol 12, 791313.

Zhang, H., Leng, C., Ding, Y., Zhai, H., Li, Z., Xiang, L., Zhang, W., Liu, C., Li, M., Chen, J., Bai, Y., Kan, Y., Yao, L., Peng, J., Wang, Q., Tang, Y.D., An, T., Cai, X., Tian, Z., Tong, G., 2019a. Characterization of newly emerged NADC30-like strains of porcine reproductive and respiratory syndrome virus in China. Arch Virol 164, 401-411.

Zhang, H., Xia, M., Wang, W., Ju, D., Cao, L., Wu, B., Wang, X., Wu, Y., Song, N., Hu, J., Tian, C., Zhang, S., Wu, H., 2018. An Attenuated Highly Pathogenic Chinese PRRS Viral Vaccine Confers Cross Protection to Pigs against Challenge with the Emerging PRRSV NADC30-Like Strain. Virol Sin 33, 153-161.

Zhang, Q., Jiang, P., Song, Z., Lv, L., Li, L., Bai, J., 2016. Pathogenicity and antigenicity of a novel NADC30-like strain of porcine reproductive and respiratory syndrome virus emerged in China. Vet Microbiol 197, 93-101.

Zhang, W.L., Zhang, H.L., Xu, H., Tang, Y.D., Leng, C.L., Peng, J.M., Wang, Q., An, T.Q., Cai, X.H., Fan, J.H., Tian, Z.J., 2019b. Two novel recombinant porcine reproductive and respiratory syndrome viruses belong to sublineage 3.5 originating from sublineage 3.2. Transbound Emerg Dis 66, 2592-2600.

Zhao, K., Ye, C., Chang, X.B., Jiang, C.G., Wang, S.J., Cai, X.H., Tong, G.Z., Tian, Z.J., Shi, M., An, T.Q., 2015. Importation and Recombination Are Responsible for the Latest Emergence of Highly Pathogenic Porcine Reproductive and Respiratory Syndrome Virus in China. J Virol 89, 10712-10716.

Zhou, L., Kang, R., Yu, J., Xie, B., Chen, C., Li, X., Xie, J., Ye, Y., Xiao, L., Zhang, J., Yang, X., Wang, H., 2018. Genetic Characterization and Pathogenicity of a Novel Recombined Porcine Reproductive and Respiratory Syndrome Virus 2 among Nadc30-Like, Jxa1-Like, and Mlv-Like Strains. Viruses 10.

Zhou, L., Kang, R., Zhang, Y., Yu, J., Xie, B., Chen, C., Li, X., Chen, B., Liang, L., Zhu, J., Tian, Y., Yang, X., Wang, H., 2019. Emergence of two novel recombinant porcine reproductive and respiratory syndrome viruses 2 (lineage 3) in Southwestern China. Vet Microbiol 232, 30-41.

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Zhou, L., Wang, Z., Ding, Y., Ge, X., Guo, X., Yang, H., 2015. NADC30-like Strain of Porcine Reproductive and Respiratory Syndrome Virus, China. Emerg Infect Dis 21, 2256-2257.

Zhou, L., Yang, B., Xu, L., Jin, H., Ge, X., Guo, X., Han, J., Yang, H., 2017. Efficacy evaluation of three modified-live virus vaccines against a strain of porcine reproductive and respiratory syndrome virus NADC30-like. Vet Microbiol 207, 108-116.

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