



Comparison of Protective Efficacy between a Live Attenuated and an Inactivated Porcine Reproductive and Respiratory Syndrome Vaccine

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Abstract

In the study the protective efficacy of a live attenuated and an inactivated porcine reproductive and respiratory syndrome vaccine was comparatively evaluated. A total of 55 healthy piglets, which were negative to PRRSV antigen and antibody, were randomly divided into 4 groups. In group 1, 20 piglets were immunized with PRRS live vaccine TJM-F92 strain (Live vaccine TJM-F92). In group 2, 20 piglets were inoculated with commercial PRRS inactivated vaccine (Inactivated vaccine). Piglets of group 3 (n = 10) were inoculated with PBS (Negative control). 5 piglets remained as a not-immunized and not-challenged in control group 4 (Mock group). At 49 days post immunization (dpi), 10 piglets from groups 1 and 2 were selected and challenged with HP-PRRSV TJ strain and PRRSV NADC30-like strain respectively. The results showed the level of antibodies arose from live vaccine was significantly higher and faster than that from inactivated vaccine after immunization. In live vaccine immunized group, neither high fever nor signs of clinical disease were observed. While piglets in inactivated vaccine group exhibited serious clinical symptoms, pathological lesions, and viremia load after HP-PRRSV TJ strain challenge. There were no severe clinical signs, gross pathology lesions and viremia load in live vaccine group, however, severe clinical syndromes, pathology change of lungs and high viremia load were recorded in piglets of inactivated vaccine immunized group after NADC30-like strain challenge. Those results suggested that PRRS live vaccine (TJM-F92 strain) could confer effective protection against the challenge of HP-PRRSV TJ strain and virulent NADC30-like strain. The PRRS inactivated vaccine could not effectively induced immune system and provide strong immune cross-protection. Therefore, it is essential to choose suitable, widely application and effective PRRS vaccine for PRRS control according to the situation of pig farms.

Keywords: PRRSV; Live Vaccine; Inactivated Vaccine; Protective Efficacy

Introduction

Porcine reproductive and respiratory syndrome (PRRS), popularly termed "blue ear disease," is induced by the PRRS virus (PRRSV). This virus primarily impairs macrophages, which eventually leads to reproductive dysfunction in sows and respiratory dyspnea in fattening pigs and piglets. Additionally, in the case of secondary infection with bacteria, the mortality rate significantly increases in infected pigs [1,2]. In China, the first case of PRRS was

reported in 1995 in Beijing's pig farms. Subsequently, successful PRRSV isolation was accomplished from these farms [3]. Gene sequence alignment studies in conjunction with antigenicity assessment have confirmed this virus to be of the North American category [4]. Since 2006, a highly pathogenic PRRSV variant, displaying both high morbidity and mortality across all kinds of pigs, has been severely affecting the Chinese pork industry [5]. It has been found that all highly pathogenic PRRSV (HP-PRRSV) strains display an

identical intermittent 30 amino acid (aa) deletion in their nsp2 gene [6]. Such strains are more virulent than the classical PRRSVs, whose morbidity and mortality rates are 50%-100% and 20%-100%, respectively.

The initial report of PRRSV NADC30-like strains was made in 2013, wherein PRRSV was found to be significantly homologous with the NADC30 strain found in the USA and displayed intermittent 131 aa deletions in the nsp2 gene [7]. Evolutionary assessment studies indicate that PRRSV NADC30-like strains probably originated in and evolved from North America, which subsequently recombined with local strains to develop broad variations [8,9]. Such variant strains have turned out to be the most prevailing ones in China since 2014. There has also been the emergence of a multitude of plural wild-type strains, such as PRRSV NADC34-like, European, and other recombinant strains, which has made PRRS prevention and management even more challenging [10,11]. Presently, vaccination is one of the most cost-effective measures for preventing and managing PRRS. Commercially available PRRS vaccines include modified live, inactivated, and genetically engineered types, among others. The selection of an appropriate vaccine is one of the most important considerations for swine raisers.

In the present work, commercially available live and inactivated vaccines against PRRS were inoculated into experimental piglets. The two vaccine types were then comparatively assessed for their protective efficacy following challenge against HP-PRRSV and NADC30-like virulent strains. This study provides guidance for selecting an appropriate PRRS vaccine and strives to enable them to prevent and manage PRRS on a scientific basis.

Materials and Methods

Cells, virus, vaccines, and reagents

Marc-145 cells were kept in 10% fetal bovine serum (FBS)-containing minimum essential medium (MEM) at 37 °C and 5% CO₂. Thereafter, viral propagation and titration were carried out in 2% FBS-containing MEM as per a previously reported procedure [12]. The isolation and sustenance of PRRSV strain TJ (GenBank accession no. EU860248) were conducted by following a previously reported protocol [13]. The NADC30-like HN strain was isolated from a PRRS-affected dead piglet obtained in Henan, China, in 2018. This strain was further culturally passaged on MARC-145 cells. The live PRRS vaccine (strain TJM-F92) (Lot: 20200505008) used herein

was supplied by Sinovet company (Taizhou, China). The inactivated PRRS vaccine (Lot: 200104) used in these studies was obtained from an animal biological company (Hangzhou, China). The PRRSV antibody ELISA kit was procured from the IDEXX Laboratories (ME, USA).

Experimental design and animal studies

Fifty-five healthy PRRSV antigen- and antibody-free piglets aged 21 d were randomized into 4 groups. In group 1, named Live vaccine TJM-F92 (n = 20), every piglet was given one dose of live PRRS vaccine (TJM-F92 strain) inoculation. In group 2, named inactivated vaccine (n = 20), every piglet was given 2.0 mL of inactivated PRRSV vaccine inoculation along with an identical dose of a booster for a 21d duration. In group 3, named the negative control (n = 10), piglets were given phosphate-buffered saline (PBS, pH 7.2). Group 4, named Mock (n = 5), consisted of nonimmunized and nonchallenged control piglets. Following vaccination, the presence of anti-PRRSV antibodies in blood sampled at 7 d intervals was determined by ELISA. At 49 d postvaccination, 10 piglets were randomly chosen from groups 1 and 2 each along with 5 piglets from group 3 and challenged intranasally (i.e.) with 10^{4.5} 50% tissue culture infective doses (TCID₅₀) of PRRSV TJ strain F3 at 2.0 mL per piglet. Every remaining piglet in groups 1-3 was given an i.e., inoculation of 2.0 mL PRRSV NADC30-like HN strain F5 (10^{6.5} TCID₅₀). The entire experimental protocol conformed to the regulatory criteria and guidelines approved by the Taizhou Local Committee on Animal Care and Use, China.

Postimmunization side effects evaluation and serological examination

Daily surveillance was conducted to monitor side effects after immunization. Clinical signs such as appetite, stress response, psychological state, and injection site inflammatory status were also monitored on a daily basis. Sera were gathered at 0, 7, 14, 21, 28-, 35-, 42-, and 49-days post infection (dpi), and the levels of PRRSV-specific antibodies were determined as per the protocol of the commercially procured ELISA kit. The antibody levels were documented as S/P ratios, wherein an S/P ratio of ≥ 0.4 indicated positivity of the serum samples.

Clinical assessment after challenge

Surveillance was conducted twice daily to monitor clinical signs in piglets, including inappetence, cough, despondency, conjunctivitis, abdominal respiration, and difficulty breathing. During the

| Group | Number | Immunization | | Challenge virus | Challenge dose |
|----------------------|--------|---------------|--------------|--|----------------|
| | | 0d | 21d | 49d | |
| Live vaccine TJM-F92 | 10 | 1 dose/piglet | N/A | PRRSV TJ F3 $10^{4.5}$ TCID ₅₀ /mL | Intranasally |
| Inactivated vaccine | 10 | 2.0mL/piglet | 2.0mL/piglet | | 2.0mL/piglet |
| Negative control | 5 | 2.0mL/piglet | N/A | | |
| Mock group | 5 | N/A | N/A | N/A | N/A |

Table 1: Experimental design for PRRSV TJ strain challenge.

Note: "N/A": Not available. #: Mock group was used in the PRRSV TJ strain challenge and NADC30-like HN strain challenge experiments.

| Group | Number | Immunization | | Challenge virus | Challenge dose |
|----------------------|--------|---------------|--------------|---|----------------|
| | | 0d | 21d | 49d | |
| Live vaccine TJM-F92 | 10 | 1 dose/piglet | N/A | PRRSV NADC30-like HN F5 $10^{6.5}$ TCID ₅₀ /mL | Intranasally |
| Inactivated vaccine | 10 | 2.0mL/piglet | 2.0mL/piglet | | 2.0mL/piglet |
| Negative control | 5 | 2.0mL/piglet | N/A | | |
| Mock group | 5 | N/A | N/A | N/A | N/A |

Table 2: Experimental design for PRRSV NADC30-like HN strain challenge.

whole course of the study, the rectal temperatures were also documented daily.

Viremia detection by RT-PCR

At 0, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 21 days post challenge (dpc), blood was sampled for assessment of viremia in sera via RT-PCR, wherein the forward and reverse primers (nsp2 gene) were 5'-CACCTTCCYAAAAGAGTRA-3' and 5'-CCTCATATTCMGCTTGAGGAH-3', respectively (designed using the PRRSV NADC30, GenBank No: JN654459, and TJ strain, GenBank No: EU860248 gene sequence). The amplicon lengths for the NADC30-like strain and the HP-PRSSV strain were 1,122 bp and 1,425 bp, respectively.

Gross pathology and histological evaluations of lungs

At 21 d post challenge, pulmonary samples were resected from all piglets. Gross pulmonary pathology was assessed and documented at necropsy. Following immobilization in 10% buffered formalin, the chosen samples were processed for staining with hematoxylin and eosin (H and E) according to a prior procedure [14].

Statistical Analysis

All the data are reported as the means ± SDs. The intergroup differences were evaluated through a t-test using GraphPad Prism (ver. 8.01, San Diego, CA) software. Statistical significance was assessed at P < 0.05.

Results

Side effects evaluation after immunization

Following immunization, the piglets from group 1 exhibited clinically normal appetite, body temperature, and psychological state with no evident side effects. In contrast, 40% of piglets from group 2 suffered from transient fever along with inappetence, which subsequently returned to normal within 1 d. Slight swelling was noted at the inoculation sites of these piglets (Table 3).

Antibody response in piglets after immunization

Group 1 exhibited an increasing trend in the PRRSV antibody level following immunization as determined by ELISA. The antibody was initially identified at 7 days post infection (dpi), followed by 100% antibody positivity at 14 dpi; the S/P average was up to 2.0 at 49 d. In contrast, in the inactivated vaccine group, initial recognition of PRRSV antibody was performed at 21 d post primary vaccination, 100% antibody positivity was attained at 7 d post booster vaccination, and the S/P average was a mere 0.73 at 49 d. In contrast to the piglets immunized with the inactivated vaccine, a significantly faster and greater increase in antibody levels was observed among those immunized with the live vaccine (P < 0.01) (Table 4 and Figure 1).

Clinical observation after PRRSV TJ strain challenge

Clinical signs were absent in groups 3 and 4. In group 2, 8 of 10 piglets displayed varying degrees of symptoms, such as inap-

| Group | High fever | Depression | Anorexia | Local side effects |
|----------------------|------------|------------|----------|--------------------|
| Live vaccine TJM-F92 | 0/20 | 0/20 | 0/20 | 0/20 |
| Inactivated vaccine | 8/20 | 5/20 | 7/20 | 3/20 |
| Negative control | 0/10 | 0/10 | 0/10 | 0/10 |

Table 3: Side effects observation after vaccination.

| Group | Days post immunization | | | | | | | |
|----------------------|------------------------|-------------|-------------|---------------|---------------|---------------|---------------|---------------|
| | 0 | 7 | 14 | 21 | 28 | 35 | 42 | 49 |
| Live vaccine TJM-F92 | - | 0.02 ± 0.01 | 1.18 ± 0.29 | 1.59 ± 0.25 | 2.01 ± 0.22 | 2.22 ± 0.20 | 2.52 ± 0.12 | 2.51 ± 0.29 |
| Inactivated vaccine | - | - | - | 0.57 ± 0.39** | 0.73 ± 0.40** | 0.71 ± 0.32** | 0.66 ± 0.27** | 0.65 ± 0.28** |
| Negative control | - | - | - | - | - | - | - | - |

Table 4: PRRSV ELISA antibody levels after immunization.

Note: PRRSV ELISA antibody was expressed as S/P, S/P ≥ 0.4 means positive for PRRSV antibody.

**Significantly different between PRRSV TJM-F92 strain group and inactivated vaccine group, *P* < 0.01.

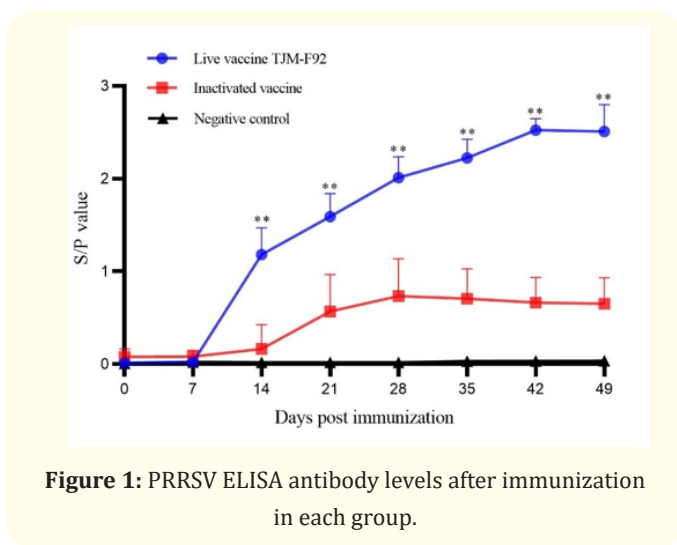


Figure 1: PRRSV ELISA antibody levels after immunization in each group.

petence, depression, and conjunctivitis. The death of 1 piglet each on the 7th, 9th, and 10th d was also observed. All of the negative control piglets displayed typical clinical symptoms of PRRS, such as severe loss of appetite, depression, chills, lameness, difficulty breathing, and dermal cyanosis, and resulted in the death of one piglet each on the 11th, 18th, and 19th days. In contrast to the negative control group, the PRRS symptoms emerged 2-4 days earlier in the inactivated vaccine group (Table 5).

Following challenge against HP-PRRSV, the live TJM-F92-immunized piglets remained healthy, and their body temperature was normal. At 3 dpi, relentlessly high fever (≥ 40.8 °C) appeared

| Group | Depression | Anorexia | Conjunctivitis | Skin cyanosis | Death |
|----------------------|------------|----------|----------------|---------------|-------|
| Live vaccine TJM-F92 | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 |
| Inactivated vaccine | 8/10 | 8/10 | 8/10 | 4/10 | 3/10 |
| Negative control | 5/5 | 5/5 | 5/5 | 3/5 | 3/5 |
| Mock group | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 |

Table 5: Clinical symptoms of piglets post challenge with PRRSV TJ strain.

among the piglets in the inactivated vaccine group, which lasted for 1-5 d. All the piglets in the negative control group experienced persistently high fever (≥ 41 °C) that lasted for more than 3 days. In contrast, the piglets in the mock group maintained a normal body temperature (Figure 2).

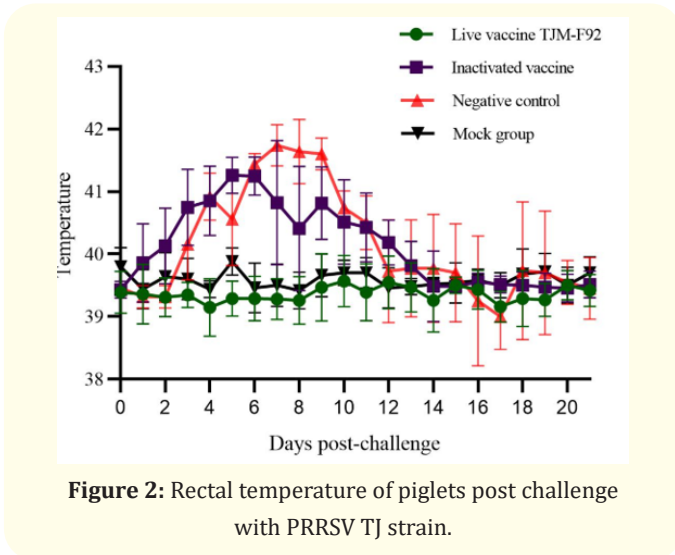


Figure 2: Rectal temperature of piglets post challenge with PRRSV TJ strain.

Viremia detection after PRRSV TJ strain challenge

After 6 dpc, PRRSV was detectable in the sera of TJM-F92-immunized piglets. The virus was shed in more than 4 piglets between 10 and 16 dpc but in fewer than 3 piglets after 16 dpc. In contrast, among the piglets immunized with the inactivated vaccine, PRRSV was detectable in the sera as early as 2 dpc. Moreover, the virus was shed in over 7 piglets at 4, 6, and 8 dpc. Viremia was detectable in the sera of all the negative control piglets since 2 dpc (Table 6).

Gross pathology evaluations of lungs after PRRSV TJ strain challenge

Macroscopic (gross) pulmonary lesions were not observed during necropsy in the live vaccine (Figure 3A) and mock (Figure 3D) groups. However, in the inactivated vaccine group, 4 out of 5 piglets exhibited severe macroscopic lesions, characterized by pulmonary tissue consolidation and bleeding (Figure 3B). In the negative control group, the entire porcine pulmonary tissues showed firmer and heavier parenchyma with bleeding and consolidation (Figure 3C).

| Group | Days post challenge (Number of viremia/animals detected) | | | | | | | | | | |
|----------------------|--|------|------|------|------|------|------|------|------|------|------|
| | 0 | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 16 | 18 | 21 |
| Live vaccine TJM-F92 | 0/10 | 0/10 | 0/10 | 2/10 | 2/10 | 4/10 | 4/10 | 5/10 | 4/10 | 3/10 | 2/10 |
| Inactivated vaccine | 0/10 | 2/10 | 8/10 | 9/10 | 7/9 | 5/7 | 5/7 | 4/7 | 3/7 | 2/7 | 2/7 |
| Negative control | 0/5 | 3/5 | 4/5 | 4/5 | 5/5 | 5/5 | 4/4 | 4/4 | 3/4 | 2/3 | 1/2 |
| Mock group | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 |

Table 6: Viremia in serum of piglets post challenge.

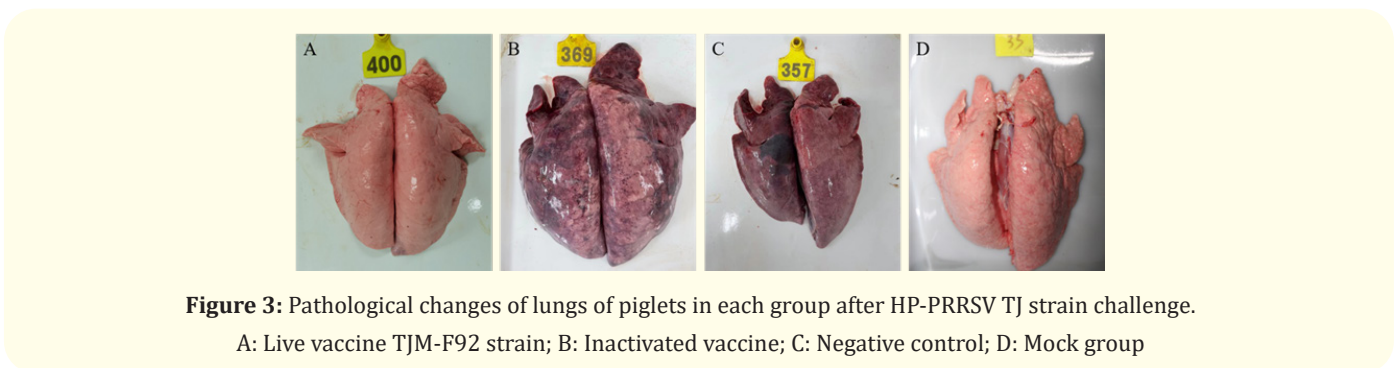


Figure 3: Pathological changes of lungs of piglets in each group after HP-PRRSV TJ strain challenge. A: Live vaccine TJM-F92 strain; B: Inactivated vaccine; C: Negative control; D: Mock group

Histopathological observation after PRRSV TJ strain challenge

According to the histological findings, there were no typical features associated with acute PRRSV infection in the lung tissues of any of the mock piglets (Figure 4D). Mild pathological changes were

observed in 2 out of 10 lungs from the piglets immunized with the live vaccine, characterized by hyperplastic alveolar epithelial cells and thickened alveolar septa, while the remaining lungs appeared normal (Figure 4A). In the inactivated vaccine-immunized piglets, 4 out of 10 lungs exhibited dilated and hemorrhagic blood capillar-

ies, necrotic tissues, and a significant infiltration of inflammatory cells. Additionally, infiltration of fibrin and exudative neutrophils was observed in the alveolar cavity. In the remaining lungs, interstitial pneumonia was mild, characterized by hyperplastic epithelial

cells and a small quantity of fibroblasts (Figure 4B). In contrast, interstitial pneumonia and bleeding were severe in 4 out of 5 negative control piglets (Figure 4C).

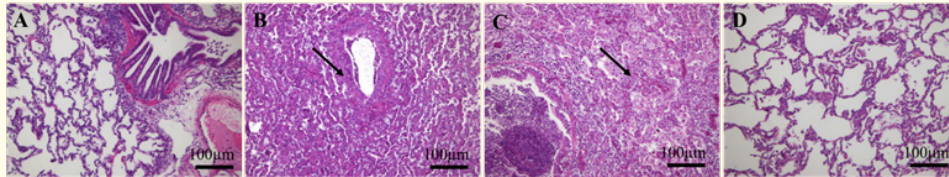


Figure 4: Histopathological examination of the lungs.

A: Live vaccine TJM-F92 strain; B: Inactivated vaccine; C: Negative control; D: Mock group

Protective rates after PRRSV TJ strain challenge

Based on the postchallenge body temperature, pulmonary pathological alterations, and clinical sign observations, all of the TJM-F92-immunized piglets demonstrated valid protection against

HP-PRRSV challenge. In contrast, none of the piglets in the inactivated vaccine group exhibited valid protection against the same challenge (Table 7).

| Group | High fever | Clinical symptoms | Lung lesions | Morbidity | Protection rate |
|----------------------|------------|-------------------|--------------|-----------|-----------------|
| Live vaccine TJM-F92 | 0/10 | 0/10 | 0/10 | 0/10 | 10/10 |
| Inactivated vaccine | 10/10 | 8/10 | 4/10 | 8/10 | 0/10 |
| Negative control | 5/5 | 5/5 | 5/5 | 5/5 | N/A |
| Mock group | 0/5 | 0/5 | 0/5 | 0/5 | N/A |

Table 7: Protection rates of piglets post challenge with PRRSV TJ strain in each group.

Note: "N/A": Not available.

Clinical observation after PRRSV NADC30-like strain challenge

Clinical signs were absent in both the mock and TJM-F92 groups. However, in the inactivated vaccine and negative control groups, each had 4 piglets that displayed symptoms such as depression,

loss of appetite, and conjunctivitis. Following challenge with the PRRSV NADC30-like strain, no piglet deaths were observed in any of the four groups (Table 8).

| Group | Depression | Anorexia | Conjunctivitis | Skin cyanosis | Death |
|----------------------|------------|----------|----------------|---------------|-------|
| Live vaccine TJM-F92 | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 |
| Inactivated vaccine | 4/10 | 4/10 | 4/10 | 0/10 | 0/10 |
| Negative control | 4/5 | 4/5 | 4/5 | 0/5 | 0/5 |
| Mock group | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 |

Table 8: Clinical symptoms of piglets post challenge with NADC30-like HN strain.

Following the challenge, the piglets in the live vaccine group remained healthy, with normal body temperatures. However, in the inactivated vaccine-immunized group, 8 out of 10 piglets developed high fever ($\geq 40.5^\circ\text{C}$), and among them, 6 experienced persistently high fever ($\geq 41^\circ\text{C}$) for a duration of 2 to 6 days. Additionally, 3 out

of 5 piglets in the negative control group suffered from persistent high body temperatures ($\geq 41^\circ\text{C}$) for over 2 days. In contrast, the mock piglets maintained normal body temperatures (Figure 5).

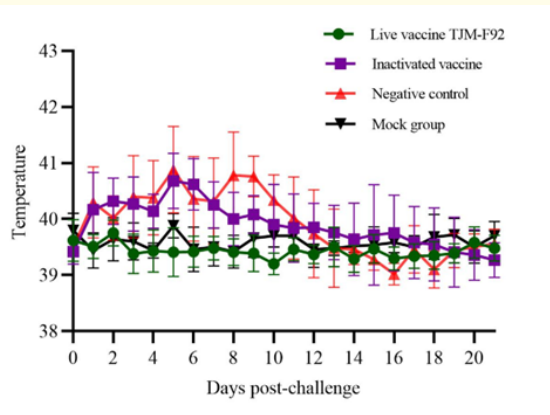


Figure 5: Daily monitoring of rectal temperature of all the pigs post challenged with NADC30-like strain.

Viremia detection after PRRSV NADC30-like strain challenge

Regarding viremia detection after the PRRSV NADC30-like strain challenge, based on RT-PCR findings, in the live vaccine TJM-F92 group, the PRRSV NADC30-like virus was detectable in the serum of only 1 piglet at 10 dpc and 12 dpc, and no other piglets showed virus shedding after the challenge. Conversely, in the inactivated vaccine group, PRRSV was identifiable in the serum as early as 2 dpc. The virus was shed by 5 piglets at 6 dpc but in less than 2 piglets after 8 dpc. Serum detection of viremia was possible in 4 out of 5 negative control piglets since 2 dpc. However, the mock piglets did not show any virus detection (Table 9).

| Group | Days post challenge (Number of viremia/Animals with detected) | | | | | | | | | | | |
|----------------------|---|------|------|------|------|------|------|------|------|------|------|--|
| | 0 | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 16 | 18 | 21 | |
| Live vaccine TJM-F92 | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 | 1/10 | 1/10 | 0/10 | 0/10 | 0/10 | 0/10 | |
| Inactivated vaccine | 0/10 | 3/10 | 2/10 | 5/10 | 3/9 | 2/10 | 2/10 | 2/10 | 2/10 | 1/10 | 0/10 | |
| Negative control | 0/5 | 2/5 | 3/5 | 3/5 | 4/5 | 3/5 | 2/5 | 1/5 | 1/5 | 0/5 | 0/5 | |
| Mock group | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | |

Table 9: Viremia in serum of piglets post challenge.

Gross pathology evaluations of lungs after PRRSV NADC30-like strain challenge

No noticeable macroscopic pulmonary pathological changes were observed during necropsy in the live vaccine (Figure 6A) or mock (Figure 6D) groups. However, significant macroscopic le-

sions were observed in 2 out of 5 piglets immunized with the inactivated vaccine, characterized by pulmonary tissue consolidation and bleeding (Figure 6B). In 3 out of 5 negative control piglets, the parenchyma was denser and firmer, accompanied by bleeding and consolidation (Figure 6C).

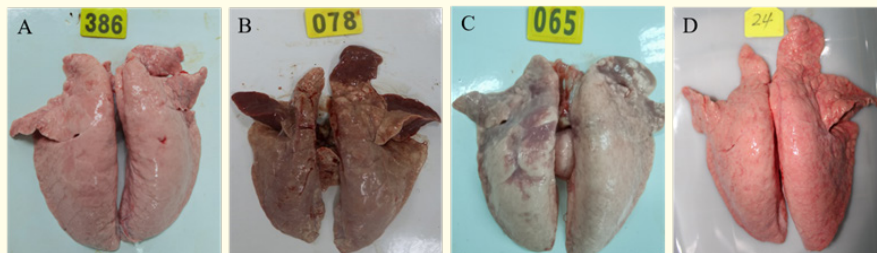


Figure 6: Pathological changes of lung in piglets post challenged NADC30-like HN strain. A: Live vaccine TJM-F92 strain; B: Inactivated vaccine; C: Negative control; D: Mock group

Histopathological observation after PRRSV NADC30-like strain challenge

After conducting histological assessments following the PRRSV NADC30-like strain challenge, no typical features related to acute PRRSV infection were observed in the lungs of piglets from the live vaccine group (Figure 7A). Among the piglets immunized with the inactivated vaccine, 2 out of 10 lungs displayed interstitial pneumonia, characterized by hyperplastic epithelial cells, infiltrative in-

flammatory cells, and thickened alveolar septa. In 3 out of 10 piglet lungs, interstitial pneumonia was mild, with hyperplastic epithelial cells (Figure 7B). In the negative control piglets, 3 out of 5 lungs exhibited severe interstitial pneumonia with bleeding, while 1 out of 5 lungs displayed mild interstitial pneumonia (Figure 7C). The mock piglets did not show any signs of interstitial pneumonia in their lungs (Figure 7D).

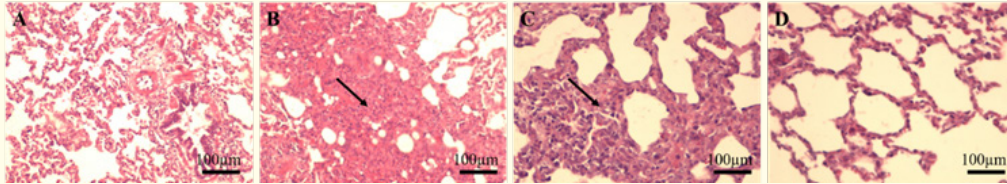


Figure 7: Histopathological examination of the lungs.

A: Live vaccine TJM-F92 strain; B: Inactivated vaccine; C: Negative control; D: Mock group.

Protective rates after PRRSV NADC30-like strain challenge

When assessing protective rates after the PRRSV NADC30-like strain challenge, based on postchallenge body temperature, pulmonary pathological changes, and clinical signs, all of the live

vaccine-immunized piglets demonstrated valid protection against the challenge. In contrast, only 2 out of 10 piglets in the inactivated vaccine group were considered to have valid protection against the challenge (Table 10).

| Group | High fever | Clinical symptoms | Lung lesions | Morbidity | Protection rate |
|----------------------|------------|-------------------|--------------|-----------|-----------------|
| Live vaccine TJM-F92 | 0/10 | 0/10 | 0/10 | 0/10 | 10/10 |
| Inactivated vaccine | 8/10 | 4/10 | 2/10 | 4/10 | 2/10 |
| Negative control | 4/5 | 4/5 | 3/5 | 4/5 | N/A |
| Mock group | 0/5 | 0/5 | 0/5 | 0/5 | N/A |

Table 10: Protection rates of piglets post challenge with NADC30-like HN strain in each group.

Note: "N/A": Not available.

Discussion

PRRSV is a single-stranded, positive-sense, enveloped RNA virus that is susceptible to various genetic variations introduced during replication, such as insertions, deletions, gene recombination, and point mutations. The presence of diversified variations in different isolates has led to the emergence of immune escape mechanisms, making PRRS prevention and management quite challenging for the swine industry. Although stringent production governance and biosafety regulations have led to a rise in PRRSV antigen- and antibody-free porcine herds in several Chinese porcine breeding firms [15], a vast majority of Chinese porcine breeding companies are still unable to fulfill the requirements of various biosafety regulations, some of which include strong managerial awareness, establishment of sound facilities, and extensive capital investment.

Hence, vaccination is an essential measure for the prevention of PRRS.

In the present work, commercially available live and inactivated PRRS vaccines were compared via assessment of their immunological effect and protective efficacy. It was found that the level of antibody induction by the live vaccine TJM-F92 was much faster and better than that of the inactivated vaccine. Furthermore, TJM-F92 strain immunization was found to result in desirable protection against the NADC30-like and HP-PRRSV TJ strains in piglets. Inactivated vaccine immunization failed to offer valid protection, with some piglets even exhibiting antibody-dependent enhancement (ADE) effects, as well as representative clinical PRRS signs more severely and in advance. During the period from 7 to 14 days after

PRRSV infection or vaccination, no neutralizing anti-N protein antibodies were detectable. The ADE effect primarily occurs through Fcγ receptor mediation. The virus binds to the Fc receptor on PAM cells through the Fc domain of low-level antibodies, facilitating virus adsorption to the target PAM cell, enhancing internalization capability, and promoting viral replication in the cells, ultimately triggering the ADE effect [16].

Despite the elicitation of a greater number of nonneutralizing anti-N protein antibodies by the live PRRS vaccine, the role played by the cellular immune response induced by the live vaccine prevails during the defense against PRRSV infection. According to field observations, emergency inoculation with live PRRS vaccine could improve production efficiency and reduce the incidence of wild-type PRRSV infection in porcine herds. This phenomenon may be associated with the competitive replication of the vaccine strain against the wild virus, but it requires further verification through additional research. On the other hand, inactivated PRRS vaccine-induced neutralizing antibodies have been shown to have effective anti-PRRSV infection activity in piglets. In comparison to the control group, no differences were noted in the duration of viremia and the virus titer levels post infection [17].

The superiority of live PRRS vaccines is demonstrated by the presence of a better immunological effect at a lower immunizing dose [18]. In contrast to the inactivated vaccine, prominently higher levels of anti-PRRSV antibody were induced by the PRRS-modified live vaccines (both domestic and imported) [19]. As demonstrated by comparing the immunological effects of the live HP-PRRS vaccine (JXA1-R strain) to the inactivated vaccine (NVDC-JXA1 strain), inoculation with the live vaccine resulted in a significantly faster and higher induction of PRRSV antibodies than inoculation with the inactivated vaccine. While classic inactivated PRRS vaccines are generally considered safe, their effectiveness remains a subject of debate. These vaccines primarily stimulate humoral immunity, leading to a slow production of neutralizing antibodies and limited elimination of infected macrophages [20]. Due to the *in vivo* replicative nature of vaccine viruses, extant live modified vaccines evoke robust cellular and humoral immunoreactions. After immunizing 4-week-old piglets with the modified live PRRS vaccine (CH-1R strain) and the inactivated HP-PRRS vaccine, they were subsequently challenged with the HP-PRRSV HuN-4 strain. The results showed that the piglets receiving the inactivated vaccine exhibited more severe viral distribution and pathological lesions compared to the live vaccine receivers [21]. According to a comparative study conducted in China, which investigated the application and immunological effects of inactivated PRRS vaccines (JXA-1 and CH-1a strains) versus traditional live attenuated vaccines (V2322 and CH-1R strains), live attenuated vaccines showed superiority in terms of

their immunological effects [22]. This collective evidence suggests that inactivated PRRSV vaccines may not provide a robust immune response or comprehensive protection against PRRSV infection.

In China, further research has been undertaken to address the prevention and management of the currently prevailing PRRSV NADC30-like strain. A novel recombinant PRRSV strain was isolated from piglets exhibiting clinical symptoms in Fujian, which resulted from a combination of the PRRSV NADC30-like and HP-PRRSV strains. Commercially available modified live PRRS vaccines such as TJM-F92 and R98 have been shown to provide partial cross-protective efficacy for piglets when challenged with this recombinant PRRSV strain [23]. Assessment of TJM-F92's cross-protective efficacy for piglets against challenge with HN201605, a PRRSV NADC30-like strain, demonstrated that the vaccine offered a valid shield against NADC30-like strain infection [24]. The PRRS-modified live vaccine (MLV) has been verified to offer considerable cross-protection against NADC30-like virus. However, it is worth noting that in piglets infected with the NADC30-like virus, the inactivated PRRS vaccine provided minimal additional protection when used as a booster, as indicated by [25].

Despite the commercial availability of various modified attenuated (live) and inactivated (killed) PRRS vaccines, MLV vaccine safety and inactivated vaccine efficacy are still the subject of investigation [26]. Although live vaccines fail to achieve thorough prevention of wild-type viral infections and pose the risk of viral spread, their administration leads to observable amelioration of clinical symptoms and reduction in viral shedding by over 90%. This ultimately enhances production efficiency and decreases morbidity and mortality. It is possible that PRRSV-negative swine farms with outstanding biosafety strategies potentially implement PRRSV control by using inactivated PRRSV vaccines that are much safer. Nonetheless, as proven by innumerable data, the inactivated vaccines failed to deliver a valid shield against PRRSV. The present research has verified that the live vaccine TJM-F92 can deliver valid cross-protection against NADC30-like and HP-PRRSV strains, while immunization with a commercially available inactivated PRRS vaccine is prone to induce the ADE effect, thereby exacerbating viral infection. Addressing challenges associated with inactivated PRRS vaccines is crucial. These challenges include finding solutions to prevent the exacerbation of ADE effect-triggered infections, overcoming weak cellular immune responses, and expediting the generation of neutralizing antibodies. Porcine farms are in need of safer, more efficient, and stable PRRS vaccines, making their development a top priority.

Competing Interests

The authors declare that they have no competing interests.

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Authors Contribution

YH and HW conceived and designed the study. YH and ZL wrote the manuscript. WS, DY, CZ, and FC executed the experiment and detected the clinical samples. XY and GZ analyzed the data. All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

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