Veterinary Microbiology 229 (2019) 117–123

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Efficacy evaluation of three modified-live PRRS vaccines against a local strain of highly pathogenic porcine reproductive and respiratory syndrome virus

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ARTICLE INFO

Keywords:
HP-PRRSV
MLV vaccine
Efficacy evaluation

ABSTRACT

Highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) is characterized by high fever and high mortality in pigs of all ages and has severely affected the pork industry of China in the last decades. This study evaluated the differences in protection conferred by of three MLV PRRS vaccines derived from classical PRRSV (C-PRRSV, VR2332) and HP-PRRSV (TJM-F92 and JXA1-R) against the field challenge of HP-PRRSV TP strain (JXA1-R like). Compared to the experiment pigs in control group which were vaccinated with normal saline, the MLV PRRS vaccinated pigs had milder clinical symptoms, fewer pathological changes in the lung, and higher body weight gain at the end of the study. However, piglets vaccinated with VR2332 had higher body temperature, higher viral loads and lower body weight gain when compared with piglets vaccinated with TJM-F92 or JXA1-R vaccines at the end of the study. The results demonstrated that VR2332 vaccine provided a limited cross-protection against the HP-PRRSV TP strain infection, while in contrast the TJM-F92 and JXA1-R vaccines provided more efficacious protection. The findings of this study could serve as a valuable reference guide for the pig producers and veterinarians when considering the choice of which type of MLV PRRS vaccines to protect their pig herds against field challenge by HP-PRRSV TP strain.

1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) is a viral disease that infects pigs at all ages and has major economic impact to the global swine industry. The disease is characterized by reproductive problems in sows and respiratory distress in growing pigs. PRRS virus (PRRSV), the causal pathogen of PRRS was first identified in the United States in 1987;and Europe in 1990 (Benfield et al., 1992; Christopher-Hennings et al., 1998; Terpstra et al., 1991; Wensvoort et al., 1991). PRRSV is classified into the genus *Arterivirus* of family *Arteriviridae* in the order *Nidovirales* (Cavanagh, 1997), and its genome is a single-stranded, positive-sense RNA that contains at least 12 overlapping open reading frames (ORFs) (Conzelmann et al., 1993; Li et al., 2015; Lunney et al., 2016). ORF1a and ORF1b encode viral replicate polyproteins that are immediately translated upon viral entry and then proteolytically processed by virus encoded proteinases into 12 mature nonstructural proteins (NSP1-NSP12) (Snijder and Meulenberg, 1998). ORF2a, ORF2b, and ORFs 3–7 encode the viral structural proteins GP2, E, GP3, GP4, GP5, M, and N, respectively (Benfield et al., 1992; Stadejek et al., 2002). To date, two genotypes of PRRSV have been recognized, namely European type (type 1) and North American type(type 2) (Meng et al., 1995).

The genetically, antigenically and biologically heterologous strains of PRRSV cause extreme diversity in clinical phenotypes and severities of the affected pigs (Karniychuk et al., 2010; Meng, 2000; Zhou et al., 2009). Moreover, the extensively genetic variation and recombination among field strains always lead to the emergence of novel and more virulent strains of PRRSV worldwide (Han et al., 2006; Karniychuk et al., 2010; Murtaugh et al., 2010). In 2006, an unparalleled large-scale outbreak of an originally unknown, but so-called “high fever” disease with clinical symptoms of PRRS occurred in China, affected over 2,000,000 pigs with about 400,000 fatal cases. This atypical PRRS pandemic was initially classified as a hog cholera-like disease manifesting neurological symptoms, high fever (40–42°C), anorexia and reddening of the skin and ears. Necropsies combined with immunological analyses showed clearly that multiple organs were infected

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https://doi.org/10.1016/j.vetmic.2018.12.016
Received 21 October 2018; Received in revised form 15 December 2018; Accepted 15 December 2018
0378-1135/

by highly pathogenic PRRSV isolates associated with severe pathological changes (Tian et al., 2007).

Since the first HP-PRRS outbreak in China in 2006, PRRSV vaccines are widely used to control PRRS in pig farms. It is widely perceived that killed vaccines are less efficacious than modified live virus (MLV) vaccines. In China, the commercially available MLV PRRS vaccines against classical PRRSV are the CH-1R and VR-2332 (Ingelvac PRRS MLV) vaccines, developed from the North American strains CH-1a and VR-2332, respectively (Charerntantanakul, 2012). For the protection pf pigs against HP-PRRSV, the commercially available vaccines are JXA1-R, HuN4-F112, GDr180 and TJM-92, developed from the HP-PRRSV strains JXA1, HuN4, GD and TJM respectively (Gao et al., 2015; Tian et al., 2009).

In the absence of a new generation of vaccines, the currently available modified live vaccines for PRRSV should be used rationally to protect the commercial pig production herds. As the field PRRSV evolves, new studies to evaluate the safety and efficacy of the currently available modified live vaccines against new emergent strains of HP-PRRSV are required (Meng, 2000; Ren et al., 2012). Therefore, the aim of this study was to evaluate the efficacy of three MLV PRRS vaccines VR2332, TJM-F92 and JXA1-R against HP-PRRSV TP strain JXA1-R like infection by assessing their clinical symptoms, rectal temperature, body weight gain, viral loads and lung microscopic lesion post-challenge.

2. Materials and methods

2.1. Virus and MLV PRRS vaccines

The PRRSV challenge used in this study was HP-PRRSV TP strain (GenBank no. EU864233, Chinese isolate). Three commercially available modified live PRRS vaccines, namely: VR-2332 (Boehringer-Ingelheim), JXA1 (Guangdong Wens Dahuanong Biotechnology Co., Ltd.), and TJM-F92 (Zoetis) strains were used in this study. VR-2332 belongs to classical PRRSV MLV vaccines. The JXA1-R vaccine was obtained by passing HP-PRRSV JXA1 through Marc-145 cells (82nd passage), and TJM-F92 vaccine was obtained by passaging HP-PRRSV strain TJ on MARC-145 cells for 92 passages.

2.2. Animals and experimental design

Thirty-five 21-day-old healthy crossbred piglets which were laboratory confirmed free of PRRSV, PRV and CSFV were randomly divided into 5 groups including non-vaccinated/non-challenged group (T1), non-vaccinated/ challenged group (T2), VR2332 vaccinated/ challenged group (T3), TJM-F92 vaccinated/ challenged group (T4), JXA1-R vaccinated/ challenged group (T5). As the control groups, pigs in T1 and T2 were vaccinated with normal saline. Each group of 7 pigs was separately raised in different isolation rooms. Each pig in T2-T5 groups was challenged at 28 day post vaccination (DPV) with 2 ml inoculation of $1 \times 10^{4.0}TCID_{50}$/mL of HP-PRRSV TP strain, and each pig in T1 group was inoculated with 2 mL of DMEM medium. The half of challenge dose was administered intramuscularly (i.m.), in a 1-ml volume, and another half was administered intranasally (i.n.), in a 1-ml volume. All the surviving experimental animals were euthanized and subjected to necropsy at 21 day post challenge (DPC). All experimental procedures and animal care strictly followed the recommended guidelines by the Ethics Committee of South China Agricultural University.

2.3. Clinical examination

Rectal temperatures and clinical signs of each individual pig were daily observed and recorded daily throughout the study. A scoring system was deployed to monitor and score the clinical symptoms as previously described (Li et al., 2014). Briefly, the clinical scoring included gross clinical score (GCS), respiratory clinical score (RCS) and nervous signs score (NSS). Total scores for each piglet represented the sum of GCS, RCS and NSS. Each piglet was scored from 0 to 20, and the mean values of 1–3 DPI, 4–6 DPI, 7–9 DPI, 10–12 DPI, 13–15 DPI, 16–18 DPI and 19–21 DPI were calculated. In addition, animals were weighed on the days of vaccination (day 0), challenge (day 28), and necropsy (day 49) for the assessment of average daily weight gain (ADWG).

2.4. Serum examination for PRRSV viremia and antibodies

Serum samples were collected at 0, 3, 7, 14, 21, 28, 31, 35, 42 and 49 DPV during the experiment. The samples were subjected to determine the viral loads by using Real-time PCR. Total RNA was extracted from serum samples by using a MagMAX-96 Viral RNA Isolation Kit (Thermo, USA) according to the manufacturer's instructions. After RNA isolation, 7 uL of RNA were used to perform a real-time PCR by using VetMAX PRRSV NA & EU Reagents (Thermo, USA) according to the manufacturer's instructions. The viral load of each sample was represented by CT value. IDEXX HerdChek PRRSX3 ELISA kit was employed to detect antibodies specific for N protein of PRRSV.

2.5. Lung microscopic lesion examination

At necropsy, sections of lungs were collected and fixed with 4% paraformaldehyde solution at room temperature for 48 h and then processed by routine histopathological procedures. The section was stained with hematoxylin and eosin (H&E) for the observation of pathological changes, and the H&E staining was operated automatically by Leica fully automatic dyeing machine according to standard procedures.

2.6. Statistical analysis

A nonparametric test was used to analyze the significant difference in clinical sign and lung microscopic lesion scores. The difference analyses on the ADWG and virus titers were performed by using two-way RM ANOVA of variance at each time point. Differences were analyzed by GraphPad Prism software (version 5.0) and were considered statistically significant at $P < 0.05$.

3. Results

3.1. Clinical assessment of pigs pre-challenge

The rectal temperature of all animals in T3 – T5 between 0–7 days and the average daily weight gain in each group between 0–28 days post vaccination were examined to assess the vaccines' safety. Except T5 group, the rectal temperatures of the other groups were normal. The T5 group presented elevated rectal temperature with a peak of 40 °C at 5 days after vaccination (Fig. 1A). And piglets in T5 group gained weight less than piglets in the other groups ($P < 0.05$) between 0–28 days post vaccination (Fig. 1B). These data indicated that the safety of JXA1-R vaccine should be considered when it is used in the field.

3.2. Clinical assessment of pigs post challenge

To evaluate the efficacy of these three MLV PRRS vaccines against challenge by HP-PRRSV TP strain, the vaccinated pigs were challenged at 28 DPV. Rectal temperatures, clinical signs and ADWG of pigs were examined post-challenge. Throughout the whole experiment, none of the piglets in T1 group showed any clinical signs of PRRS, and all piglets survived by the end of the study (Fig. 2A–C). In contrast after the challenge, the piglets in T2 group exhibited typical clinical signs of HP-PRRS infection, such as persistently high fever (40.5 °C–42 °C), depression, anorexia, cough, asthma, lameness and shivering (Fig. 2A& B). Five of the seven piglets in T2 group died between days 9 and 21 post challenge (Fig. 2C). Piglets in T3 group also exhibited typical clinical

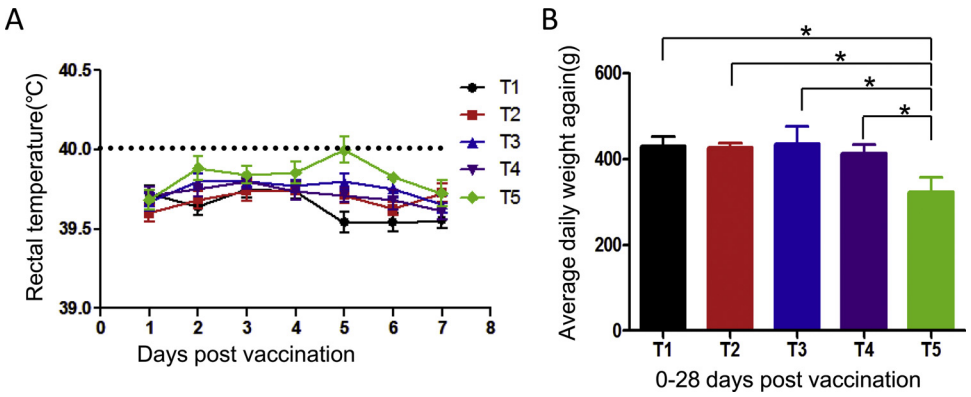

Fig. 1. Rectal temperature and average daily weight gain measurements post vaccination.
(A) Rectal temperatures shown are means ± standard error (error bars) (n = 7).
(B) The average daily weight gain (ADWG) (n = 7). Asterisk indicates significant differences between different vaccinated groups and control group (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

signs of HP-PRRS, but the high fever period was shorter than T2 group (Fig. 2B), and two of seven piglets in T3 group died during this study (Fig. 2C). Piglets in T4 group and T5 group showed shorter duration of the fever (about 3 days) and all piglets survived at the end of the study (Fig. 2B, & C). The clinical sign scores of the T2 group and T3 group were significantly higher than other groups ($P < 0.001$) after the challenge (Fig. 2A). Of all the groups, the average daily weight gain of the T2 group was the lowest, followed by T3 group which was also significantly lower than T1, T4 and T5 groups ($P < 0.05$) (Fig. 2D).These data indicated that although these three MLV PRRS vaccinations could reduce clinical signs of pigs challenged with the HP-PRRSV TP strain, but their efficacy or protection rate are different.

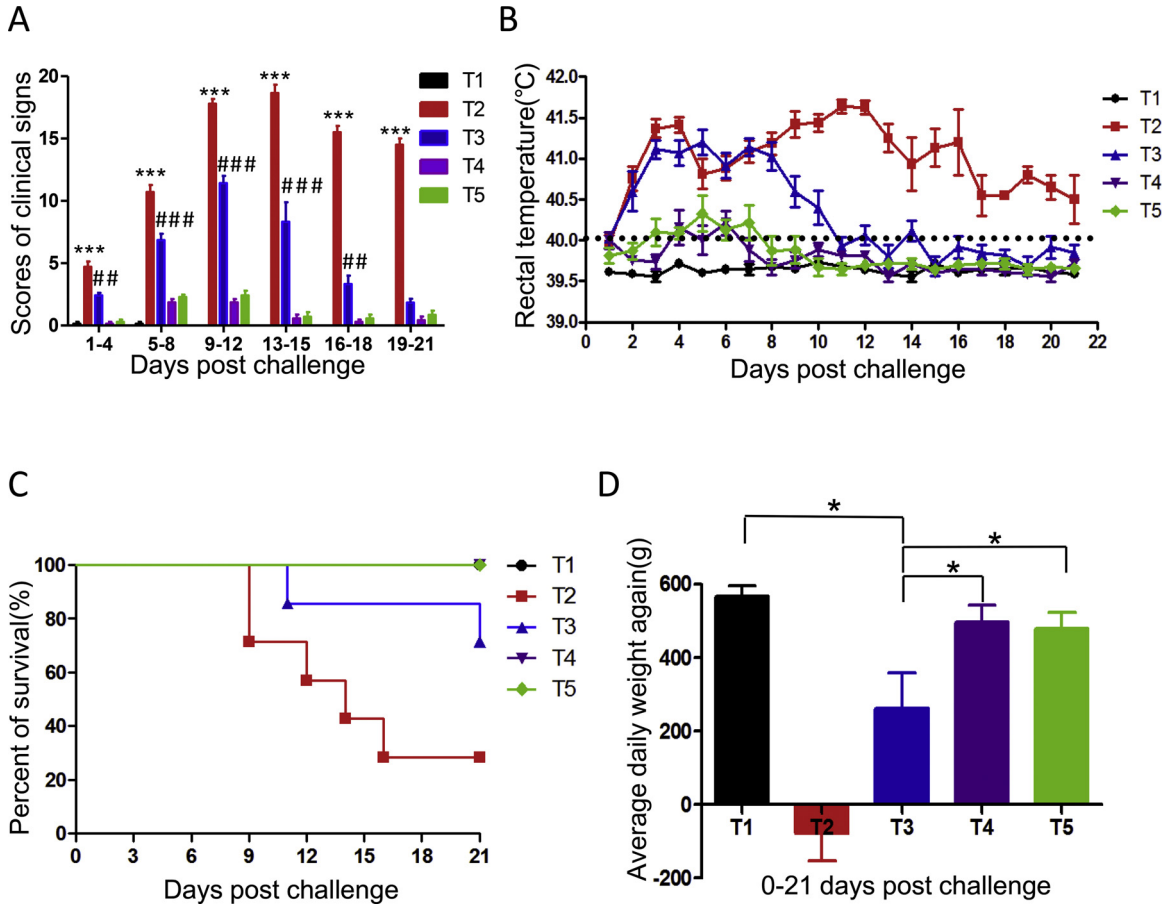

Fig. 2. Clinical sign scores and rectal temperature, and survival curve, and average daily weight gain measurements of challenged piglets.
(A) The clinical scoring included gross clinical score (GCS), respiratory clinical score (RCS) and nervous signs score (NSS). Total scores for each piglet represented the sum of GCS, RCS and NSS. Each piglet was scored from 0 to 20, and the mean values of 1–3 DPI, 4–6 DPI, 7–9 DPI, 10–12 DPI, 13–15 DPI, 16–18 DPI and 19–21 DPI were calculated. Rectal temperatures shown are means ± standard error (error bars). Asterisk indicates significant differences between T2 groups and T4 group, or between T2 group and T5group (*$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). Pound (#) indicates a significant difference between T3 group and T4 group, or between T3 group and T5 group. (#$P < 0.05$; ## $P < 0.001$, ### $P < 0.001$).
(B). Rectal temperatures shown are means ± standard error (error bars).
(C). Survival curves of piglets infected with TP in each group are shown.
(D).The average daily weight gain (ADWG). Asterisk indicates significant differences between TP-inoculated group and control group (*$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

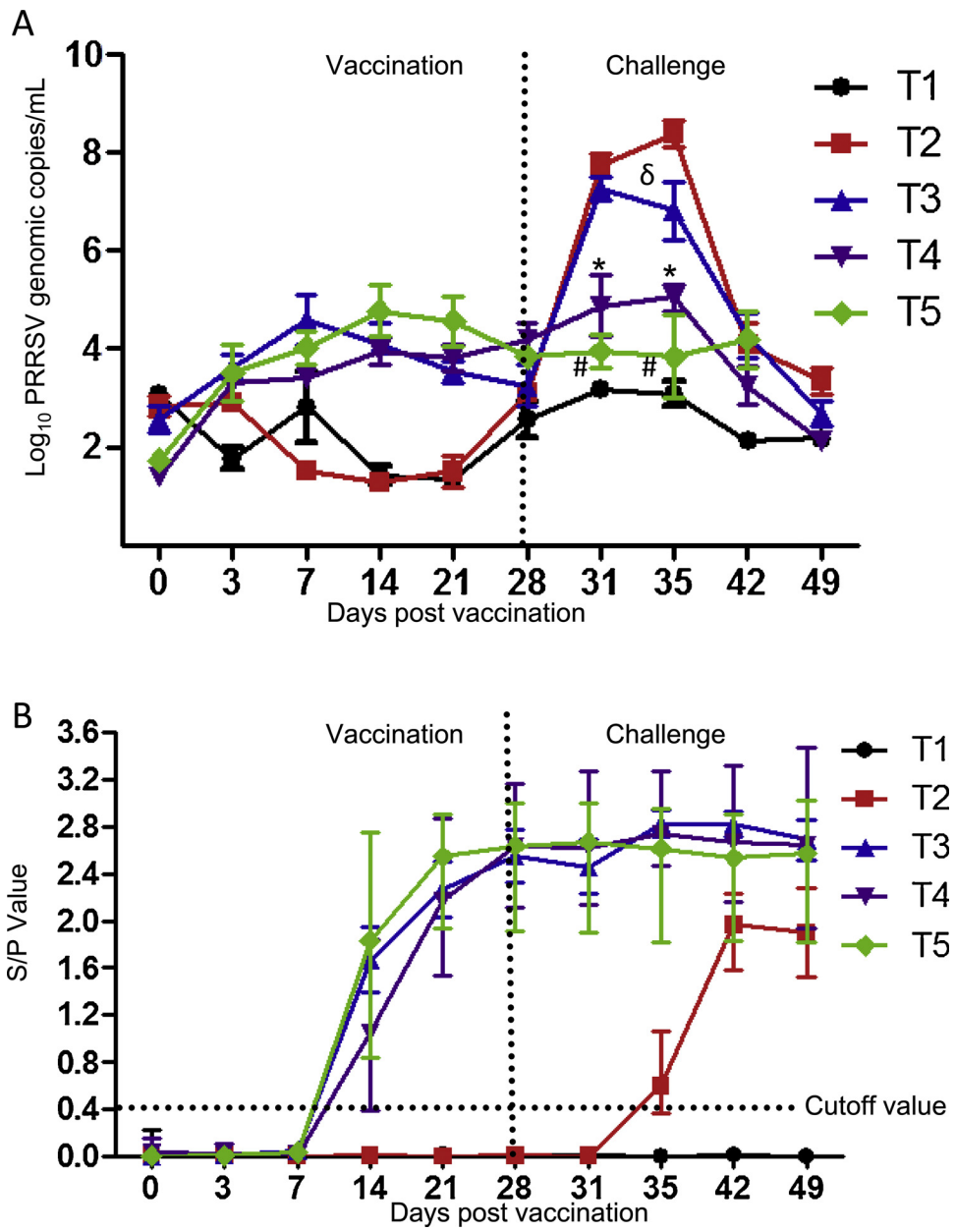

Fig. 3. Mean values of the genomic copy number of PRRSV RNA and antibody response in sera of piglets.
(A) The genomic copy number of PRRSV RNA post vaccination by Real-time PCR.
Data are shown as means ± standard error (error bars). Asterisk indicates significant differences between T4 groups and T2 group, or between T4 group and T3group (*$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). Pound (#) indicates a significant difference between T5 group and T2 group, or between T5 group and T3 group. ((# $P < 0.05$;
$P < 0.001$). Delta (δ) indicates a significant difference between T3 group and T2 group (δ $P < 0.05$; δδ $P < 0.01$). (B) The antibody response of the vaccinated and challenged piglets by using an IDEXX HerdChek PRRS X3 ELISA kit.

3.3. Viremia test and serological detection

Virus RNA copy numbers and antibody levels of PRRSV N protein in sera of the challenged pigs were examined. The results showed that the virus RNA copy numbers of piglets in T4 and T5 groups were significantly lower than those of T2and T3 groups at 3 DPC and 7 DPC ($P < 0.05$) (Fig. 3A). There was no significant difference of the virus RNA copy numbers between T2 and T3 groups at 3 DPC, but the virus RNA copy numbers of piglets in T3 groups were significantly lower than those of T2 groups at 7 DPC ($P < 0.05$) (Fig. 3A). All pigs in T1 group were negative for PRRSV throughout the entire experiment period. The levels of antibody in sera of pigs against PRRSV N protein were monitored by IDEXX HerdChek PRRS X3 ELISA kits post- vaccination and following the virus challenge. The data showed that these MLV PRRS vaccines were able to induce the antibody response against PRRSV N protein in similar level (Fig. 3B). All pigs in the T3, T4 and T5 groups were seroconverted at 14 DPV. After challenge, the S/P values of all T3, T4 and T5 groups were higher than those of T2 group (Fig. 3B).These results indicated TJM-F92 and JXA1-R MLV PRRS vaccines were very efficacious in reducing PRRS virus RNA copies in sera of the challenged pigs.

3.4. Lung microscopic lesion examination

At necropsy, no lung gross lesions were observed in T1 group, while pigs in the T2 group exhibited severe gross lesions with consolidation, firmer and dense parenchyma in the lung tissues with hemorrhage. Two of five pigs in T3 group have also exhibited mild gross lesions consistent with interstitial pneumonia. No obvious gross lesions were observed in the lungs of T4 and T5 piglets (data not shown). The lungs of piglets in T2 group exhibited the most severe histopathological changes showing infiltration with inflammatory cells, necrotic debris and exfoliated epithelial cells in the bronchiole with hemorrhage as well as infiltration of inflammatory cells within alveolar septa and spaces (Fig. 4). Pigs in T3 and T5 groups also showed moderate histopathological lesions characterized by inflammatory cells infiltration and exfoliated epithelial cells in the bronchiole, while no microscopic lesions were observed in T1 and T4 groups (Fig. 4). These data indicated that the three MLV

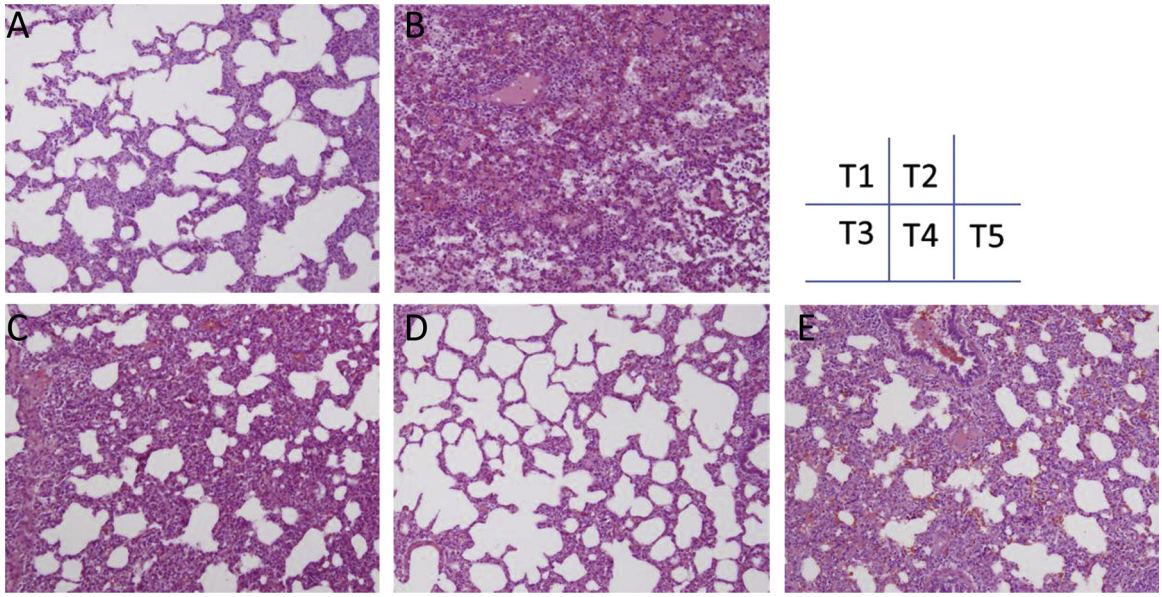

Fig. 4. Examination of histological lesions.
(A to E), Representative section views of the lung from one piglet in the T1 to T5 group, respectively.

PRRS vaccines have varied capability in reducing lung gross and microscopic lesions of vaccinated pigs challenged with the HP-PRRSV TP strain.

4. Discussion

In 2006, highly pathogenic PRRS broke out in China and brought enormous economic loss to China pig industry. A year later a novel HP-PRRSV JXA1 strain, which has a unique 30-amino-acid deletion within its Nsp2 coding region, was isolated from diseased piglets (Tian et al., 2007). Since then, HP-PRRSV has become the major epidemic viral strain in China. HP-PRRSV has 89.4% to 97.2% nucleotide identities with North American(NA) PRRSV strains VR-2332, BJ-4, CH-1a, HB-1(sh)/2002, andHB-2(sh)/2002 (Zhou et al., 2011). HP-PRRSVs share 98.2% to 100% nucleotide identity among themselves (Zhou et al., 2011). These results indicate significant genetic diversity across the HP-PRRSV and the NA-type PRRSV strains. Studies have shown that currently commercial available PRRS vaccines only protect pigs against homologous or very closely related strains of PRRSV (Charoenchanikran et al., 2016; Du et al., 2012; Duy et al., 2018; Sirisereewan et al., 2018). Therefore, better understanding the immune responses to homologous and heterologous challenge with HP-PRRSV is important for developing effective vaccines and optimal vaccination strategies. In this study we characterized the clinical course of disease and the immune response in piglets vaccinated with MLV PRRS vaccines developed from either a C-PRRSV or HP-PRRSV strain followed by a challenge with HP-PRRSV TP strain.

Vaccination with MLV PRRS vaccines is the most common strategy used to contain the spread of PRRSV. However, there is still some doubt about the safety and efficacy of MLV PRRS vaccines as some reported MLV PRRS vaccinated pigs have developed PRRS-like disease and atypical PRRS, characterized by abortion and high mortality in pregnant gilts (Botner et al., 1997). In our study, we found that after vaccinated with PRRS JXA1-R strain vaccine, the rectal temperature of pigs was higher than the other groups and the ADWG was lower than the other groups (Fig. 1). These results indicate that the safety of the PRRS JXA1-R strain vaccine should be carefully considered when it is used in the field.

In this study, we found that VR2332 provided a mediocre level of protection against a heterologous challenge, while the TJM-F92 and JXA1-R vaccines were effective against homologous challenge. After challenge, the unvaccinated/challenged piglets in T2 group exhibited severe clinical symptoms including persistently high fever (> 41°C), cyanosis of the ears, red coloration of the body, conjunctivitis, dyspnea, and severe diffuse pulmonary lesions that were consolidated, consistent with those reported in a previous study and five of seven piglets died by the end of the study (Zhou et al., 2008). All piglets in T4 and T5 groups were protected, and no clinical symptom was observed throughout the entire experimental period and all pigs were survived (Fig. 2). Piglets in T3 group also exhibited typical clinical signs of PRRS and two of seven piglets died during the study (Fig. 2). These data indicated that some level of cross-protective immunity between C-PRRSV and HP-PRRSV may exist. These findings are consistent with previous reports and may be attributable to antigenic differences between the vaccine and challenge viruses (Jeong et al., 2016; Lager et al., 2014; Roca et al., 2012; Tornimbene et al., 2015; Wang et al., 2016b; Wei et al., 2013).

In this study, the duration between vaccination and challenge was 4 weeks. The humoral immune response was assessed by ELISA. All pigs in the T3, T4 and T5 groups were seroconverted at 14 DPV (Fig. 3B). Some researchers reported that the levels of neutralizing antibodies were not always correlated with protection and that animals lacking neutralizing antibodies were nonetheless resistant to reinfection (Murtaugh et al., 2010; Zuckermann et al., 2007). Another study showed that neutralizing antibodies were detected only at 91 dpv but still protected against PRRSV challenge (Park et al., 2014). In this study, we choose two time point to do neutralizing antibody test. One time point was 28 days post vaccination. As we expected, we did not detected any neutralizing antibody at this time point (data not shown). Another time point was 49 days post vaccination. At this time point, the neutralizing antibody titer of T5 was higher than the other two groups, but there were no significant difference among T3, T4 and T5 groups (data not shown). Viremia analyses indicated that VR2332 vaccines was partially efficacious in the reduction of virus loads in sera of the challenged pigs a week later post-challenge, while the TJM-F92 and JXA1-R vaccines were very efficacious in the reduction of virus loads at 3 DPC and 7 DPC (Fig. 3A). Consistent with the result of the viremia, pathological analyses revealed that piglets in T3 group exhibited slightly lung gross and microscopic lesion, while no significant lung microscopic lesions were observed in T4 and T5 group (Fig. 4). These findings are consistent with previous reports that the CH-1R vaccine which is classical vaccine could not complete protect against HP-PRRSV infection (Wang et al., 2016a).

In conclusion, both C-PRRSV and HP-PRRSV MLV vaccines could reduce disease magnitude caused by HP-PRRSV infection. However, their varying efficacy in reducing clinical signs, viremia and lung microscopic lesion suggested there are likely be underlying differences in the immune response to the different MLV PRRS vaccines against HP-PRRSV TP strain that needs further study.

Author contributions

KWW, ZHL and CK conceived and designed the experiments; ZHL, JL and KWW performed the experiments; ZHL, KWW and BY analyzed the data; YBK, ZHL, KWW and YMH contributed eagents/materials/analysis tools; ZHL wrote the paper. YMH checked and finalized the manuscript. All authors read and approved the final manuscript.

Conflict of interest

Zhonghua Li, JunLi, Yibo Kong, Limei Yang, ChoewKong Mah, Guangzhen Liu and Kewen Wang are employees of Zoetis that manufactures a vaccine based on TJM-F92.

Ethical approval

The animal study was supervised by the Institutional Animal Care and Use Committee of SCAU and used in accordance with regulation and guidelines of this committee.

Acknowledgements

This work was supported by the National Key Research and Development Program, China (2017YFD0501901).

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