Original article

Efficacy evaluation of two live virus vaccines against an emerging pseudorabies virus variant

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Abstract

Since late 2011, porcine infections with highly virulent and antigenic variant of pseudorabies virus (PRV) cause great economic loss in the swine industry in China, and its emergence leads to variable protection efficacy of the commercially available PRV vaccine.

In the present study, the potential cross-protective efficacy of two live virus vaccines, including a commercial vaccine, and an attenuated low pathogenic PRV variant (rPRVTJ-delTK/gE/gI) against a PRV variant Tianjing (TJ) was evaluated in piglets. Vaccination of piglets with the live vaccine Bartha-K61 could not reduce the clinical signs, and was partially efficacious in the reduction of viral loads upon PRV variant TJ challenge, indicating that this live vaccine provided limited cross-protection efficacy against the PRV variant infection. Additionally, rPRVTJ-delTK/gE/gI appeared to exert some beneficial efficiency in shortening the period of clinical fever and improving the growth performance of the challenged pigs.

Our findings give a valuable guidance for the choice and use of PRV vaccines to control PRV variant infection in the field.

Key words: pseudorabies virus (PRV), variant TJ, live virus vaccines, cross-protection

Introduction

Pseudorabies virus (PRV), the causative agent of pseudorabies (PR), is a member of the family *Herpesviridae*, subfamily *Alphaherpesvirinae*, and genus *Varicellovirus* (Pomeranz et al. 2005). It is a serious pathogen that produces fatal encephalitis in newborn pigs and a milder syndrome in older animals, resulting in significant losses to swine industry worldwide (Klupp BG et al. 2004).

Extensive vaccination of pigs with the Bartha-K61 vaccine is one of the most efficient methods to control this disease. In China, this disease has been effectively controlled for 30 years with the Bartha K61 vaccine. However, since late 2011, massive PR outbreaks have been reported in many pig farms in China, where regular vaccination has been carried out. Several studies have indicated that Bartha-K61 strain vaccine provide partial or limited cross-protection against the emerging PRV variants, which exhibit high pathogenicity and unique molecular signatures (An et al. 2013, Wu et al. 2013, Luo et al. 2014). Therefore, it is necessary to evaluate the vaccine efficacy to the emerging virus strains.

Prevalence and transmission of novel PRV variants in China caused the re-emergence of clinical PR in Bartha K61-vaccinated pig herds in many pig-producing areas in recent years (Zhang et al. 2015). Some emergent PRV variants have been isolated by different research groups in China (Gu et al. 2015). Currently, limited data concerning the pathogenicity of PRV variants for pigs and protection efficiency of current live vaccines against these viruses are presented (Wang et al. 2014, Dong et al. 2017, Zhou et al. 2017). In the present study, we evaluated the potentiality of cross--protective efficacy of the commercial live vaccine (Bartha-K61) and attenuated low pathogenic PRV variant (rPRVTJ-delTK/gE/gI) against the PRV variants infection under experimental conditions.

Materials and Methods

Ethics approval and consent to participate

Animal experiments were approved by Animal Ethics Committee of Harbin Veterinary Research Institute of the Chinese Academy of Agricultural Sciences (CAAS) and performed in accordance with animal ethics guidelines and approved protocols. The Animal Ethics Committee approval number was SYXK (Hei) 2011022.

Cells, virus and live vaccines

PK-15 cells used for PRV growth and viral titration were cultured in Dulbecco's modified Eagle medium (DMEM) (Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS) (Sigma-Aldrich, St. Louis, MO, USA) at 37°C under a humid 5% CO_2 atmosphere. The PRV TJ variant was isolated and identified from the brain tissues of diseased pigs suspected of PR in Tianjing, China, in 2012. Fifth-passage culture of PRV TJ variant was used in this study. PRV SC strain, a virulent strain used to test the efficacy of the Bartha-K61 vaccine and the PRV thymidine kinase (TK)/gE/gI deleted strain (rPRVTJ-delTK/gE/gI) was kindly provided by Professor Qiu from Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences. Commercial PRV Bartha K61 strain vaccine was produced by Weike Biotech Co., Harbin, China.

Animal trials for vaccination and challenge

Four-week-old weaned pigs (n = 35) were purchased from a large-scale pig farm in Heilongjiang Province, which was free of PRV, classical swine fever virus (CSFV), and porcine circovirus 2 (PCV2) infection. After weaning at 4 weeks, pigs were housed in controlled ambient conditions (27°C, 12 h/12 h light/ /dark cycle) with unrestricted access to food and water. All pigs were randomly allocated into six groups (n = 5 in each experimental group, n = 5 for the control group) including Bartha K61 vaccinated/SC challenged group (Bartha K61/SC), Bartha K61 vaccinated /PRV TJ challenged group (Bartha K61/PRV TJ), rPRVTJ-delTK/ /gE/gI vaccinated/SC challenged group (rPRVTJ--delTK/gE/gI/SC), rPRVTJ-delTK/gE/gI vaccinated/ PRV TJ challenged group (rPRVTJ-delTK/gE/gI/PRV TJ), non-vaccinated/SC challenged group (mock/SC), non-vaccinated/PRV TJ challenged group (mock/PRV TJ) non-vaccinated/non-challenged group (control). Each pig in Bartha K61/PRV TJ, rPRVTJ-delTK/gE/gI/ /PRV TJ and mock/PRV TJ was challenged on day 14 post-immunization (dpi) with 2 ml of the virus PRV TJ containing 1.0×10^5 $TCID_{50}$, Each pig in Bartha K61/ /SC, rPRVTJ-delTK/gE/gI/SC and mock/SC was challenged on day 14 dpi with 2 ml of the virus SC containing 1.0×10^5 $TCID_{50}$, and each pig in control group was inoculated with 2 ml of DMEM. Serum samples were obtained from the challenged piglets 0, 3, 5, 7, 10 and 14 days post-challenge (dpc).

All pigs were euthanized on 15 dpi, after anesthesia (Zoletil®, Virbac, Carros, France, using 15 mg/kg), followed by bleeding and then necropsied. Post-mortem examination of lesions was carried out in each pig and tissue samples were collected and stored at -80°C for further analysis.

Clinical examination

After vaccination and challenge, clinical signs and rectal temperatures of pigs were daily observed and recorded throughout the experiment. Meanwhile, all the pigs were weekly weighed on 0, 7 and 14 dpc to calculate the body weight gain.

Serological test

All serum samples were evaluated using the seroconversion of PRV-specific gB and gE antibodies with the ELISA kits (IDEXX Laboratories, Westbrook, ME, USA) according to the manufacturer's instructions. In addition, neutralization assays were performed for detecting the presence of anti-PRV neutralizing antibodies as previously described (Gravier et al. 2007, Hong et al. 2007).

Virus isolation

Virus titers in challenged piglets was determined by inoculating PK-15 cells with respective nasal and rectal swabs collected during the vaccination and infection procedures. Briefly, tissue homogenates were
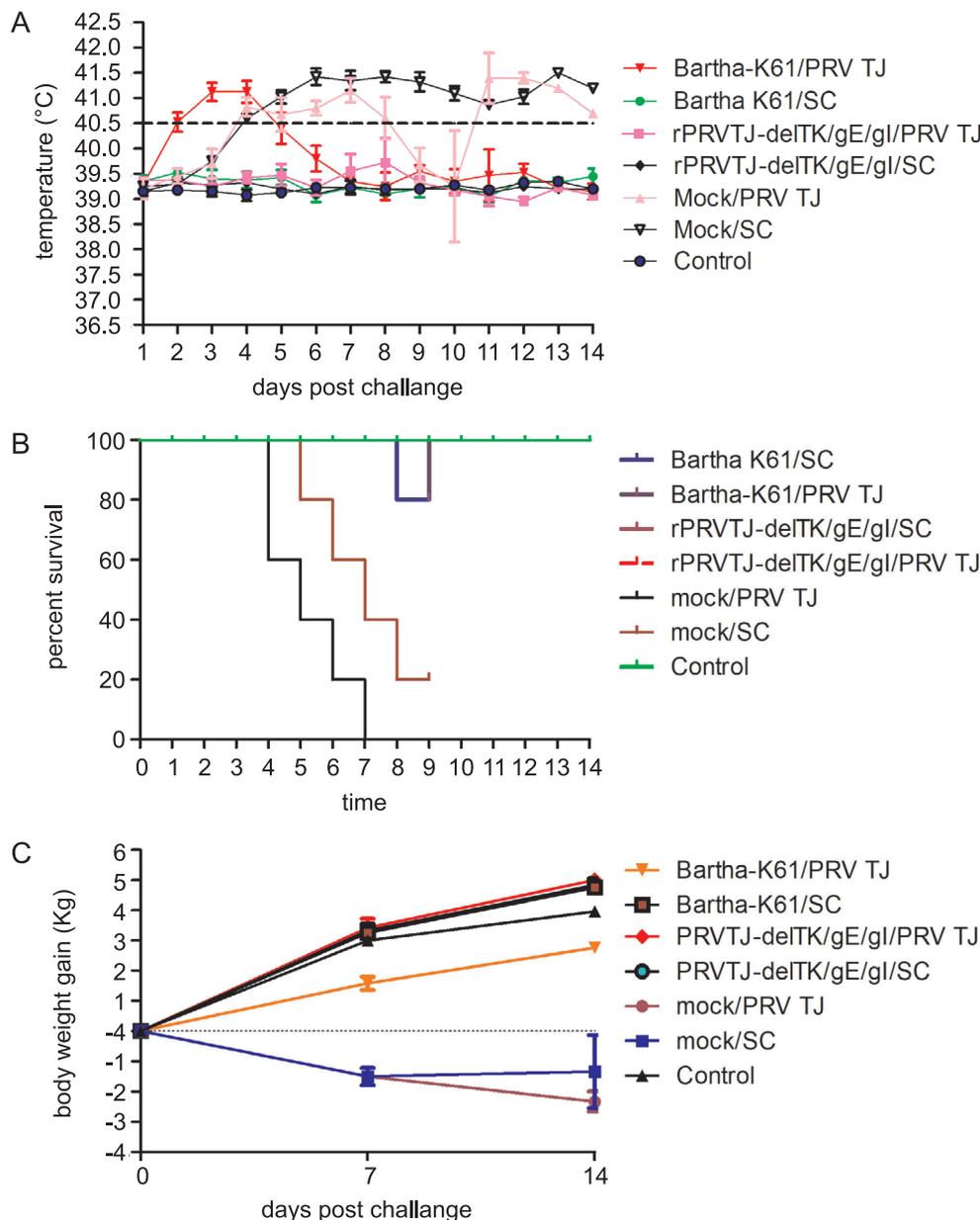
Fig. 1 Protection efficacy of Bartha-K61 vaccination against SC and PRV TJ challenge in growing pigs. (A) Daily rectal temperatures of piglets in vaccinated and unvaccinated pigs after challenge. (B) Survival rate. (C) Body weight gain at 0, 7 and 14 dpc.

prepared and centrifuged at 3000 rpm for 10 min. The supernatant was passed through a 0.45-μm filter and inoculated into PK-15 cells cultured in 96-well plates with 10-fold serially dilution. The plates were incubated for an additional 48 h, and then the virus titers were determined by an IFA using PRV-specific monoclonal antibody.

Statistical analysis

Data were analyzed using the SPSS 14.0 software (SPSS, Chicago, IL, USA). One-way ANOVA followed by Duncan's multiple range test was used to compare the parameters between the different groups.

Results

Vaccination of piglets with the Bartha-k61 vaccine could not alleviate the clinical signs upon PRV variant challenge

To evaluate the potentiality of cross-protective efficacy of live vaccines against challenge with the classical or emerging PRV strain, piglets were vaccinated with one commercial live vaccine (Bartha-k61) and an attenuated low-pathogenic PRV (rPRVTJ-delTK/ /gE/gI). The vaccinated pigs were challenged with SC and PRV TJ 14 dpi, the rectal temperatures and clinical signs, body weight gain and viral titers in blood of the challenged pigs were examined. As shown in Fig. 1A,
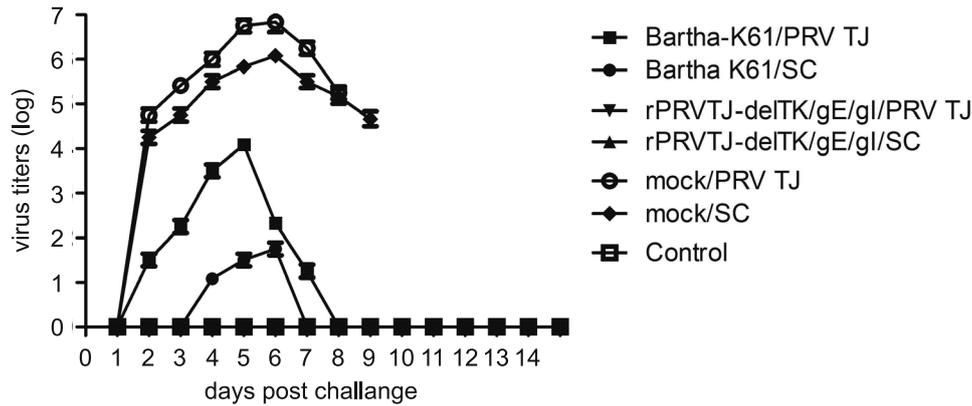
Fig. 2. Viral excretion by piglets challenged with SC and PRV TJ challenge in growing pigs.
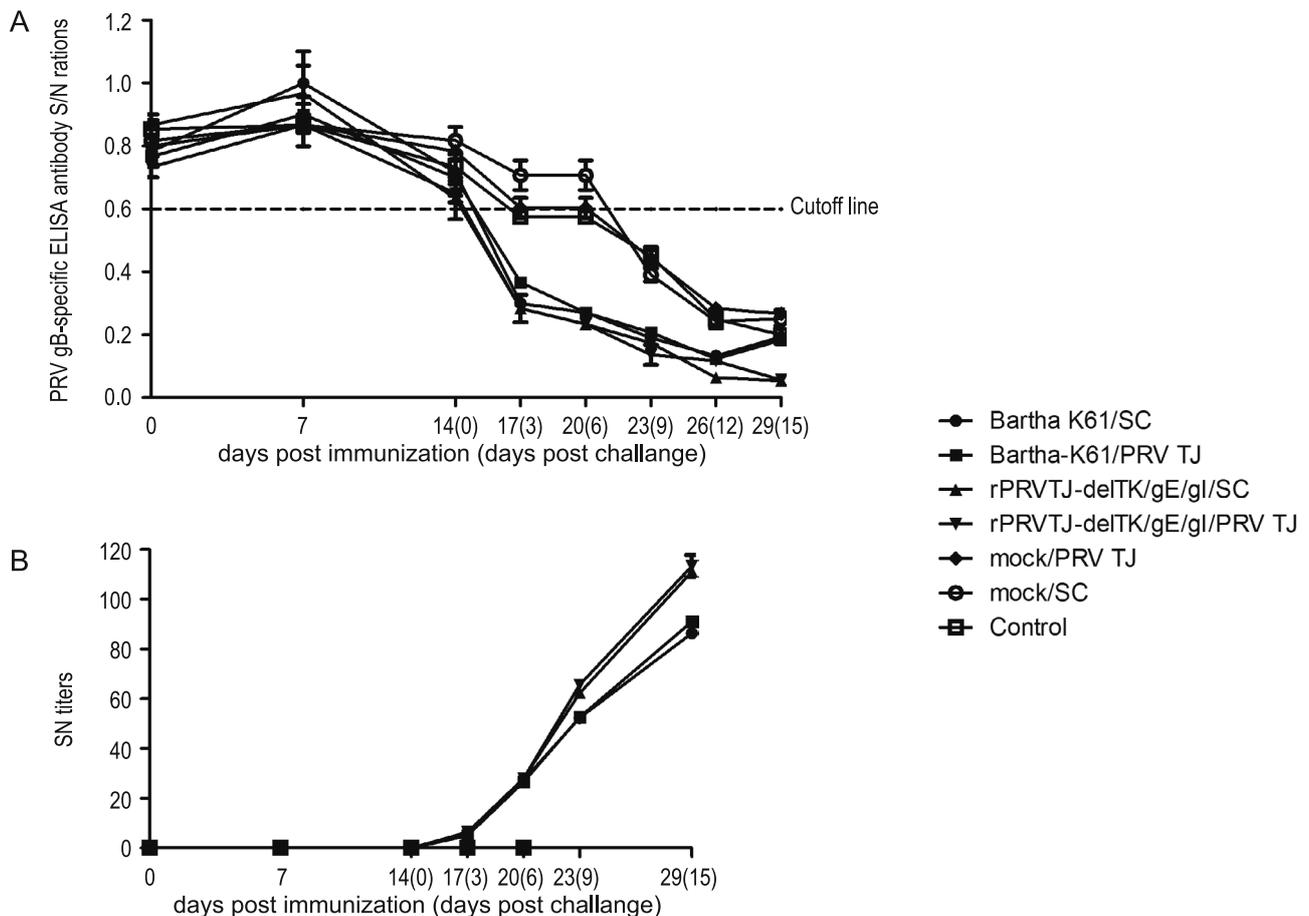

Fig. 3 Serological assay. (A) Anti-PRV seroconversion of pigs before and after challenge. (B) Virus neutralizing titers. Serum collected at 0, 3, 6, 9 and 15-day post-challenge was examined for reactivity to PRV.

the mock/SC group or mock/PRV TJ group presented elevated rectal temperatures on 2 dpc, with temperatures as high as 40.0-41.9°C, but the peak of average temperature in mock/PRV TJ group was higher than that of mock/SC group. They also displayed a steady progression of PR syndrome, evidenced by depression, respiratory distress, vomiting, trembling, and ataxia, ultimately resulting in death within 7 dpc. The non--immunized piglets challenged with PRV TJ died between 4 and 7 dpc. Four of five non-immunized piglets challenged with SC died between 6 and 8 dpc (Fig. 1B). These results suggest that PRV TJ was highly virulent in 4-week-old piglets. However, all the piglets in rPRVTJ-delTK/gE/gI/PRV TJ, rPRVTJ-delTK/gE//gI/SC and Bartha-K61/SC groups remained healthy, with no PR symptoms during the 2-week observation period, which are similar with the control group. In addition, the pigs in Bartha-K61/PRV TJ had no obvious clinical signs but exhibited high fever for 2-3 days, depression, anorexia and retarded growth from 4 dpc.
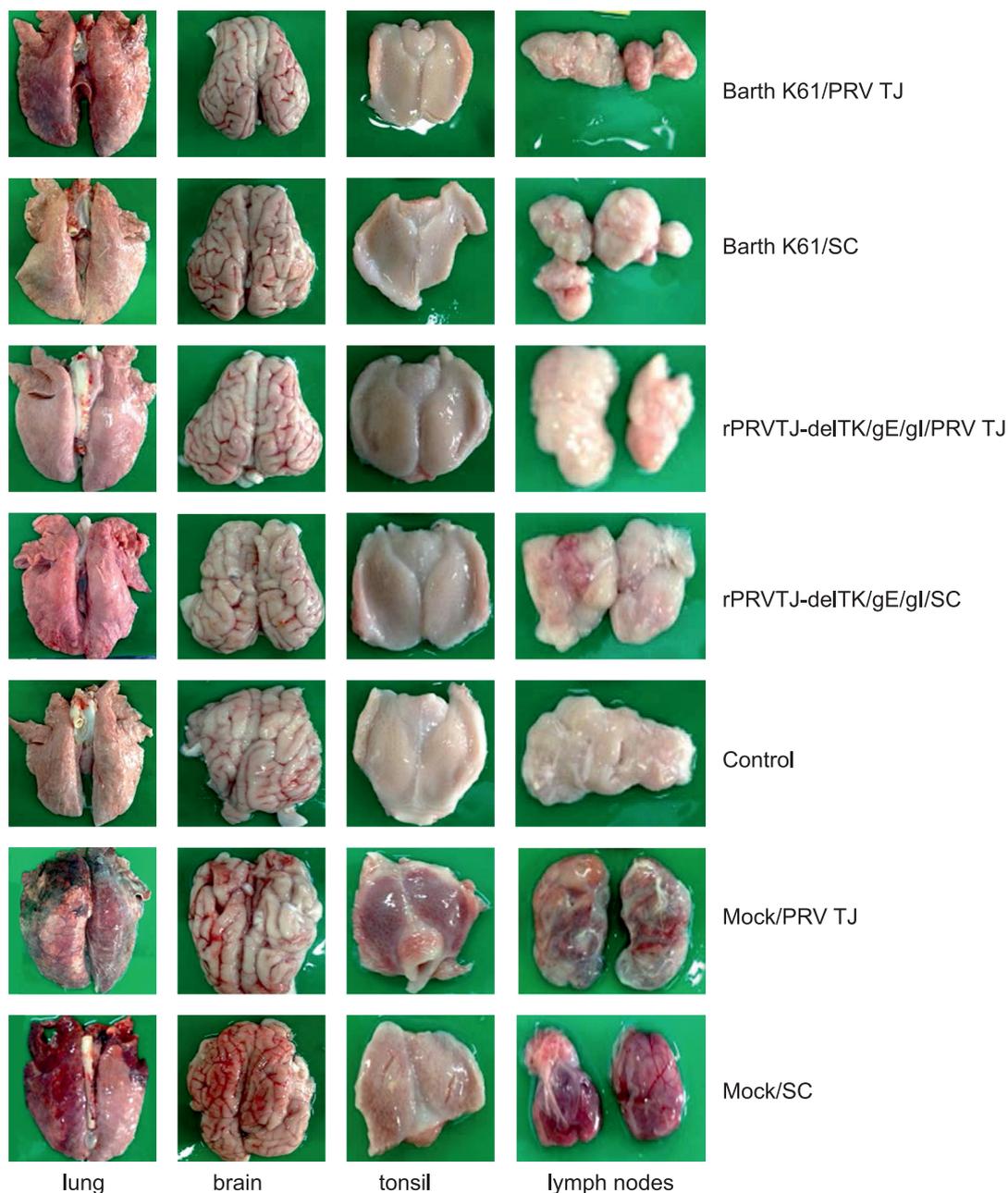
Fig. 4 Histopathology examination of the lungs, brains, lymph nodes and tonsils of piglets in different groups at 14 d post-challenge.

It is widely accepted that vaccination markedly shortened growth arrest periods and will therefore reduce financial losses due to PRV infection in the field. Thus, we assessed the effects of live vaccines on the body weight gain after SC and PRV TJ variant challenge. As shown in Fig. 1C, the body weight gains of all challenged groups were significantly lower than that of control group. Pigs in rPRVTJ-delTK/gE/gI/SC and Bartha-k61/SC groups had similar body weight gain throughout the experiment. However, pigs in rPRVTJ-delTK/gE/gI/PRV TJ group gained significantly more body weight than those in the Bartha-K61//PRV TJ group, following challenge.

Viral detection in challenged piglets

The virus excretion titration was determined by collecting nasal and rectal swabs from each group from 0 to 14 dpc. The results showed that virus shedding was found in all challenged pigs, either vaccinated or unvaccinated. In the four vaccinated groups, virus excretion began on 2 dpc and was sustained for only 5 days, which was shorter than the pigs in the control challenge group, as shown in Fig. 2. Furthermore, the excreted virus titers in rPRVTJ-delTK/gE/gI/PRV TJ group were significantly lower than those in Bartha-K61/PRV TJ group from 2 to 7 dpc. Consistent

results were observed between the rPRVTJ-delTK/gE/ /gI/SC and Bartha-K61/SC groups. Collectively, vaccination of piglets with the Bartha-K61 vaccines was partially efficacious in the reduction of virus loads upon PRV TJ challenge.

Antibody responses to PRV infections

In order to monitor PRV gB-specific antibody responses and neutralization antibodies against the PRV TJ and SC strains, the serum samples were collected after vaccination and the antibodies to PRV were detected with ELISA and neutralizing antibody assay. The results indicated that the gB-specific antibodies were detected after 3 days in piglets immunized with rPRVTJ-delTK/gE/gI or Bartha-K61, and on 15 dpc, all the infected pigs developed high-level anti-gB antibodies, and no gB-specific antibodies could be detected in mock and control groups during the 14-day immunization period (Fig. 3A). Meanwhile, the levels of neutralization antibodies against the PRV TJ or Bartha-K61 strain were similar in the four vaccinated groups at 14 days post-inoculation (Fig. 3B). These results indicate that the novel mutant virus vaccine could protect pigs against both classical and variant strain infection.

Histopathologic examination

All surviving piglets (euthanized at 15 dpc) and dead piglets were subjected to necropsy. All dead piglets in mock/PRV and mock/SC groups showed severe gross pathological lesions in the lungs, tonsils, lymph nodes, and cerebrum. One piglet in mock/SC group had hemorrhages in the right lymphoglandulae inguinales, and two pigs in the Bartha-K61/PRV TJ group showed mild lesions (such as slight hemorrhages in the brain, lymph nodes and lung), the other piglets in rPRVTJ-delTK/gE/gI/PRV TJ, rPRVTJ-delTK/gE/ /gI/SC and Bartha-K61/SC showed no gross pathological lesions (Fig. 4). These results indicated that rPRVTJ-delTK/gE/gI were able to protect piglets from lethal challenge with either classical PRV SC or PRV variant TJ.

Discussion

In the present study, we confirmed that PRV TJ is a high virulent PRV, and Bartha-K61 vaccine used in this study provide limited protection against PRV TJ infection, while rPRVTJ-delTK/gE/gI can provide complete protection against challenge with PRV TJ. Meanwhile, our present study suggests that the limitations of live vaccination should be considered for prevention and control of PRV infection.

In 2011, a novel PRV variant was identified in many

vaccinated pig farms in China that caused severe disease in pigs of all ages and resulted in great economic losses to the swine industry. To date, effective control of PRV variant infection remains a conundrum even though various commercial vaccines are available. For the measures to control PRV variant infection, Bartha-K61 vaccine has been accepted generally in many countries in terms of its protective efficacy against homologous virus. However, the homology between Bartha strains and the novel PRV variants was only 92% (Ye et al. 2015, Yu et al. 2016). For example, Wang et al. showed that Bartha K61 vaccine cannot provide full protection against the emerging variant strain infection (Wang et al. 2014). Similarly, An and Luo et al., isolated PRV variant in Bartha-K61-vaccinated pigs and analyzed the pathogenicity and genomic characterization, indicating that Bartha K61 vaccine indeed can not protect pigs against PRV variant challenge (An et al. 2013, Luo et al. 2014). Consistent with previous reports, our results also showed that vaccination of piglets with the Bartha-K61 vaccines was partially efficacious in shortening the duration of fever and improving the growth performance and reduction of virus loads upon PRV TJ challenge. However, an attenuated low pathogenic PRV variant vaccine (rPRVTJ-delTK/gE/gI) can provide complete protection against challenge with the PRV variant.

Humoral immunity plays an important role to protect from PRV infection (Tong et al. 2015). To explore the humoral immunity induced by vaccination, gB-specific ELISA antibodies and NABs were evaluated. The IDEXX ELISA antibodies were generated in the early stage of PRV vaccination/infection which was mainly induced by gB protein. The gB specific IDEXX ELISA antibodies appeared positive in all vaccinated groups during the 14-day immunization period and most of them kept increasing even after PRV challenge. However, the virus neutralizing antibodies to PRV TJ in serum of Bartha-K61-vaccinated pigs were kept at low level throughout the study, which may partially explain the weaken protection of vaccination.

In summary, our findings indicate that Bartha-K61 vaccines provide partial protection against PRV TJ infection, whereas rPRVTJ-delTK/gE/gI strain exerts full protection. Therefore, an improvement of vaccine and vaccination strategies against PRV variant is required, as current vaccines have limited efficacy.

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