Veterinary Microbiology 230 (2019) 278–282

Contents lists available at ScienceDirect

Veterinary Microbiology

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

Short communication

Evaluation and comparison of immunogenicity and cross-protective efficacy of two inactivated cell culture-derived GIIa- and GIIb-genotype porcine epidemic diarrhea virus vaccines in suckling piglets

Xinsheng Liu[a,b,*,1], Liping Zhang[a,b,1], Qiaoling Zhang[a,b], Peng Zhou[a,b], Yuzhen Fang[a,b], Donghong Zhao[a,b], Jiaxin Feng[a,b], Weiyan Li[a,b], Yongguang Zhang[a,b,*], Yonglu Wang[a,b,*]

[a] State Key Laboratory of Veterinary Etiological Biology, Key Laboratory of Animal Virology of Ministry of Agriculture, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, 730046, China
[b] Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou, 225009, China

ARTICLE INFO

Keywords:
Porcine epidemic diarrhea virus
Immunogenicity
Protective efficacy
Inactivated vaccine
Suckling piglets

ABSTRACT

Although highly virulent GII-genotype PEDV strains have become pandemic in the swine population worldwide, little is known about the differences in immunogenicity and cross-protective efficacy between the GIIa and GIIb subgenotypes. Hence, in the present study, we vaccinated suckling piglets with GIIa (CH/HBXT/2018) and GIIb (CH/HNPJ/2017) PEDV strain-based inactivated vaccine candidates and compared their immunogenicity and cross-protective efficacy. The results showed that both vaccine candidates induced high levels of PEDV-specific IgG antibodies and IFN-γ and reduced the levels of neutralizing antibodies at 21 dpv in suckling piglets. The GIIa-based inactivated vaccine protected all piglets (8/8) against virulent homologous and heterologous virus challenge, while the GIIb strain-based inactivated vaccine protected only 2/4 and 1/4 piglets against virulent homologous and heterologous virus challenge, respectively. Furthermore, antibodies against the GIIa and GIIb strains cross-reacted and cross-neutralized both strains *in vitro*. Taken together, the data presented in this study indicate that GIIa strain-based inactivated vaccine candidates are more promising than GIIb-based candidates for the development of an effective vaccine against the current highly virulent pandemic PEDV strains.

1. Introduction

Porcine epidemic diarrhea (PED), which is caused by porcine epidemic diarrhea virus (PEDV), is a highly contagious disease that leads to high mortality (up to 100%) in suckling piglets (Song and Park, 2012). PEDV was first discovered in the United Kingdom in 1971 and was later found in many European countries (Chasey and Cartwright, 1978; Jarvis et al., 2016). In October 2010, a widespread outbreak of PED caused by highly virulent PEDV variants distinct from the classic strain, CV777, occurred in China (Sun et al., 2012). Then, highly virulent and S-INDEL PEDV strains were subsequently reported in the United States beginning in April 2013 (Oka et al., 2014; Wang et al., 2014). To date, PEDV has been reported in many North American, Asian and European countries (Park et al., 2014; Crawford et al., 2016; Jarvis et al., 2016).

Phylogenetic studies have suggested that PEDV can be genetically separated into two genotypes: GI (classic) and GII (field epidemic). Each genotype can be further divided into the subgenotypes GIa and GIb and GIIa and GIIb, respectively (Lee, 2015). At present, the most prevalent PEDV strains worldwide, especially in China, are GII strains rather than GI strains (Lin et al., 2016; Wang et al., 2016). Differences in pathogenicity and cross-protection between GI and GII (Chen et al., 2016; Opriessnig et al., 2017; Sato et al., 2018) and between GIa and GIb (Song et al., 2007) have been reported in previous studies, but there is still no information on the differences between GIIa and GIIb. Therefore, in this study, we developed two inactivated cell culture-derived PEDV vaccine candidates based on newly isolated GIIa and GII2b PEDV strains, CH/HBXT/2018 and CH/HNPJ/2017, respectively, and

* Corresponding authors at: State Key Laboratory of Veterinary Etiological Biology, Key Laboratory of Animal Virology of Ministry of Agriculture, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, 730046, China.
E-mail addresses: liuxinsheng@caas.cn (X. Liu), zhanglp2319@163.com (L. Zhang), 876835192@qq.com (Q. Zhang), zhoupeng02@caas.cn (P. Zhou), fangyuzhen@caas.cn (Y. Fang), zhaodonghong123@163.com (D. Zhao), fengjiaxin0524@163.com (J. Feng), liweiyan160129@163.com (W. Li), zhangyongguang@caas.cn (Y. Zhang), wangyonglumd@hotmail.com (Y. Wang).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.vetmic.2019.02.018
Received 22 November 2018; Received in revised form 8 February 2019; Accepted 11 February 2019

evaluated and compared their immunogenicity and protective efficacy against homologous and heterogeneous PEDV challenge in suckling piglets.

2. Methods, techniques

2.1. Cells and viruses

African green monkey kidney (Vero) cells (ATCC CCL-81) were grown in Dulbecco's modified Eagle's medium (DMEM, Invitrogen, USA) containing 10% heat-inactivated fetal bovine serum (FBS; Invitrogen, Australia) and 1% antibiotics (10,000 units/mL penicillin, 10,000 μg/mL streptomycin, and 25 μg/mL Fungizone) (Gibco™, USA) and cultured at 37 °C with 5% CO_2.

A virulent Chinese GIIa PEDV strain isolated in our laboratory, CH/HBXT/2018 (GenBank accession number: MH816969), was propagated in Vero cells, as described in our previous study (Liu et al., 2018). Briefly, culture medium was removed from 100% confluent Vero cell monolayers in T-75 culture flasks (Corning, USA), and the cells were washed thrice with sterile phosphate-buffered saline (PBS; pH 7.2, Gibco™, USA). Then, 3 mL of prepared virus stock was inoculated into the flasks with 20 μg/mL trypsin (Gibco™, USA). After incubation for 1 h at 37 °C with 5% CO_2, 5 mL of virus growth medium was added, and cell culture was continued at 37 °C and 5% CO_2 with daily observation for cytopathic effects (CPEs). When more than 90% of the cells showed CPEs, the supernatant and cells were harvested, frozen and thawed three times, and stored at −80 °C for the next virus passage. Similarly, a virulent Chinese GIIb PEDV strain, CH/HNPJ/2017 (GenBank accession number: MF152604), was also isolated and propagated on Vero cells in our laboratory (Liu et al., 2018).

2.2. Preparation of the vaccines

The twentieth passages (P20) of the cell culture-adapted GIIa PEDV strains CH/HBXT/2018 ($10^{5.°}$ $TCID_{50}$/mL) and GIIb CH/HNPJ/2017 ($10^{5.2}$ $TCID_{50}$/mL) were chemically inactivated using binary ethyleneimine (BEI) according to the methods described in a previous study (Liu et al., 2018). In brief, GIIa PEDV CH/HBXT/2018-P20 and GIIb CH/HNPJ/2017-P20 were obtained from Vero cells cultured in T-75 flasks (Corning, USA). After three freeze-thaw cycles, the viral supernatant was collected through centrifugation and then inactivated by addition of 0.2 M BEI to a final concentration of 2 mM and incubation at 30 °C for 24 h. After the incubation, the remaining BEI was neutralized by addition of 20% sodium thiosulfate. The success of inactivation was verified by the absence of viral growth in Vero cell cultures and upon inoculation of the piglets. The inactivated vaccines were prepared by mixing BEI-inactivated GIIa PEDV CH/HBXT/2018-P20 and GIIb CH/HNPJ/2017-P20 with Freund's complete adjuvant (Sigma, Germany) according to the directions for use, and the vaccines were stored at 4 °C until inoculation.

2.3. Pig immunization and challenge experiment

Twenty-four PEDV-naïve newborn piglets (3–5 days old) were obtained from a commercial pig farm with no previous herd history of PED outbreak or PEDV vaccination. Serum IgG antibody levels and viral shedding in the feces of all piglets were tested with a commercial ELISA kit (Biovet, Canada) and real-time PCR (Liu et al., 2018), respectively, before vaccination. All of the piglets were raised in the laboratory animal facility at the Lanzhou Veterinary Research Institute. During the period of the experiment, all of the piglets were only fed the commercial milk (Yili, China) supplement multiple times per day. The animal use protocols were reviewed and approved by the Institutional Animal Use and Care Committee of the Lanzhou Veterinary Research Institute. All piglets used in the present study were taken good care of during the experiment and were euthanized at the onset of obvious clinical symptoms and PEDV RNA fecal shedding identified in the rectal swab samples.

The twenty-four piglets were randomly divided into three groups: a GIIa PEDV CH/HBXT/2018-P20 inactivated vaccine group (n = 8), a GIIb CH/HNPJ/2017-P20 inactivated vaccine group (n = 8) and a mock control group (n = 8). The groups were housed in separate rooms after vaccination. The piglets in the two inactivated vaccine groups were immunized once intramuscularly at 0 days post vaccination (dpv) with 2 mL of the appropriate prepared inactivated vaccine. The piglets in the mock control group were injected with PBS only. Serum samples were collected at 21 dpv for antibody detection. At 21 dpv, the piglets in each of the three groups were subdivided randomly into two parallel subgroups and were challenged orally with 3 mL of 1000 times the median pig diarrhea dose (PDD_{50}) of cell culture-adapted GIIa CH/HBXT/2018-P4 (original PDD_{50}: 8.63 $\log_{10}PDD_{50}$/3 mL) or GIIb CH/HNPJ/2017-P4 (original PDD_{50}: 7.68 $\log_{10}PDD_{50}$/3 mL) (Liu et al., 2018). After the challenge, the piglets were observed daily for 7 days for clinical signs of PEDV infection, and clinical scores of fecal consistency were determined as described in a previous study, as follows: 0 = normal; 1 = pasty; 2 = semiliquid; and 3 = liquid (Liu et al., 2015). Fecal samples were collected daily using rectal swabs and were tested immediately for the duration of the challenge to monitor viral shedding in feces.

2.4. Real-time PCR

Viral RNA was extracted from intestinal contents using an RNeasy Mini Kit (Qiagen, USA) according to the manufacturer's instructions. Real-time PCR targeting the PEDV N gene was performed as described previously (Zhang et al., 2017).

2.5. ELISA and virus neutralization (VN) test

PEDV-specific IgG antibody responses elicited by immunization with the inactivated GIIa CH/HBXT/2018-P20 and GIIb CH/HNPJ/2017-P20 vaccines were assessed with a commercial indirect ELISA (I-ELISA) kit (Swinecheck PED indirect, Biovet, GC Kerkrade, the Netherlands) according to the manufacturer's instructions.

Neutralizing antibody titers in serum samples collected from piglets in all groups were determined with a cell culture-adapted GIIa PEDV CH/HBXT/2018 strain-based VN test (HBXT VN) and a cell culture-adapted GIIb PEDV CH/HNPJ/2017 strain-based VN test (HNPJ VN) as described previously (Liu et al., 2018), with some modifications. Briefly, two-fold serial serum dilutions starting at 1:2 were coincubated at 37°C for 1 h with equal volumes of viral stock containing 200 $TCID_{50}$ of virus in 96-well plates (Corning, USA). Then, the mixture was inoculated into the Vero cell monolayers of a 96-well tissue culture plate, and the plate was washed thrice with PBS and incubated at 37 °C for 1 h. After incubation, the mixture was discarded, and the plate was washed thrice with PBS. Next, maintenance medium containing trypsin (20 μg/mL) was added to each well, and the plate was incubated for 5 days at 37°C. Neutralizing antibody titers were calculated as the reciprocal of the highest serum dilution that completely inhibited CPEs.

2.6. Cytokine detection (IFN-γ)

The levels of IFN-γ in piglet serum samples in all groups were collected at 21 dpv and were determined with a commercial porcine IFN-γ ELISA kit (Solarbio, China) according to the manufacturer's instructions.

2.7. Statistical analysis

Statistical analysis was performed using SPSS 16 software. Statistical significance among the different experimental groups was determined using one-way ANOVA with Tukey multiple comparison

Table 1
Summary of clinical scores and fecal viral shedding for the challenged piglets in each group.

DPI[a]	Inactivated CH/HBXT/2018 vaccine group (n=8)						Inactivated CH/HNPJ/2017 vaccine group (n=8)						Mock control group[d] (n=8)					
	GIIa Challenge[b] (n=4)			GIIb Challenge[c] (n=4)			GIIa Challenge (n=4)			GIIb Challenge (n=4)			GIIa Challenge (n=4)			GIIb Challenge (n=4)		
	NP[e]	CT[f]	CS[g]	NP	CT	CS	NP	CT	CS	NP	CT	CS	NP	CT	CS	NP	CT	CS
0	0/4	-[h]	0	0/4	-	0	0/4	-	0	0/4		0	0/4	-[h]	0	0/4	-	0
1	0/4	-	1	0/4	-	0	0/4	-	0	0/4		0	0/4	-	1	0/4	-	0
2	0/4	-	1	0/4	-	0	1/4	24.27	0	1/4	26.23	3	2/4	22.57-23.02	2-3	0/4	-	1
3	0/4	-	0	0/4	-	0	2/4	25.44	3	2/4	24.32	2	4/4	17.72-21.92	3	4/4	23.02-28.20	2-3
4	0/4	-	0	0/4	-	1	2/4	-	0	2/4	-	0						
5	0/4	-	0	0/4	-	0	3/4	24.58	3	2/4	-	0						
6	0/4	-	0	0/4	-	0	3/4	-	0	2/4	-	0						
7	0/4	-	0	0/4	-	0	3/4	-	1	2/4	-	0						

[a]Days post inoculation for piglets.
[b]Piglets were challenged orally with 3 mL of 1000 times the median pig diarrhea dose (PDD_{50}) of cell culture-adapted CH/HBXT/2018-P4 (original PDD_{50}: 8.63 $log_{10}PDD_{50}$/3 mL).
[c]Piglets were challenged orally with 3 mL of 1000 PDD_{50} of cell culture-adapted CH/HNPJ/2017-P4 (original PDD_{50}: 7.68 $log_{10}PDD_{50}$/3 mL).
[d]Piglets in the mock control group were inoculated orally with 3 mL of PBS.
[e]Number of PEDV-positive piglets.
[f]Cycle threshold value; a value greater than 30 was considered negative or below the detection limit of real-time PCR.
[g]Clinical score for fecal consistency, as follows: 0 = normal; 1 = pasty; 2 = semiliquid; and 3 = liquid.
[h]Samples with no Ct value (no PEDV RNA detected) are indicated with a (-).
The gray blocks indicate that the PEDV-positive piglets were euthanized after infection was confirmed by clinical symptoms and fecal viral shedding.

test. A difference was considered significant when the P value was less than 0.05.

3. Results

3.1. Clinical observations and fecal viral shedding

Before and after the experiment, all of the piglets were closely monitored. The results for the detection of PEDV-specific IgG antibodies and viral shedding in the feces of all piglets showed that all piglets in the three groups were seronegative for PEDV and exhibited no viral shedding in feces before vaccination. In addition, all piglets were apparently healthy and had no clinical symptoms before the challenge.

After the challenge, fecal viral shedding in each group was generally accompanied by clinical signs of disease (Table 1). In detail, during the experiment, no piglets in the inactivated GIIa CH/HBXT/2018 vaccine group (n = 8) showed any clinical signs of PED or any viral shedding in fecal samples after separate 1000 PDD_{50} cell culture-adapted GIIa CH/HBXT/2018-P4 and GIIb CH/HNPJ/2017-P4 challenges. Among the piglets (n = 4) in the inactivated GIIb CH/HNPJ/2017 vaccine group inoculated with the GIIa strain, three piglets showed watery diarrhea (clinical scores: 3) and viral shedding in feces (cycle threshold (CT) values: 24.27–25.44) between 2 and 5 days post inoculation (DPI); these piglets were euthanized after PEDV infection was confirmed (Table 1). In addition, among the piglets (n = 4) in the inactivated GIIb CH/HNPJ/2017 vaccine group inoculated with the GIIb strain, two PEDV-positive piglets were confirmed between 2 and 3 DPI (clinical scores: 2–3, CT values: 24.32–26.23) (Table 1). In the mock control group, all of the piglets (n = 4) inoculated with the GIIa strain tested PEDV-positive (clinical scores: 2–3, CT values: 17.72–23.02) between 2 and 3 DPI, while all of the piglets (n = 4) inoculated with the GIIb strain tested PEDV-positive (clinical scores: 2–3, CT values: 23.02–28.20) at 4 DPI.

3.2. Anti-PEDV IgG and neutralizing antibody levels in serum of vaccinated pigs

The serum antibody responses induced by the two experimental vaccines were assessed by I-ELISA and VN tests. As shown in Fig. 1a, the antiserum samples collected from the two inactivated vaccine groups at 21 dpv were positive for PEDV-specific IgG antibodies as determined by the commercial I-ELISA kit, while all samples from the mock control group were negative. Compared with those of the mock control group, the PEDV-specific IgG antibody levels of the two inactivated vaccine groups were significantly elevated ($p < 0.05$). However, there were no significant differences between the two inactivated vaccine groups ($p > 0.05$).

As shown in Fig. 1b and c, VN antibodies were detected at 21 dpv in the serum of the piglets vaccinated with either the GIIa CH/HBXT/2018 strain-based inactivated vaccine or the GIIb CH/HNPJ/2017 strain-based inactivated vaccine, regardless of whether testing was conducted with the HBXT VN assay or the HNPJ VN assay. The positive HBXT antiserum samples induced by the GIIa CH/HBXT/2018 strain-based inactivated vaccine had similar VN antibody titers in both the HBXT VN

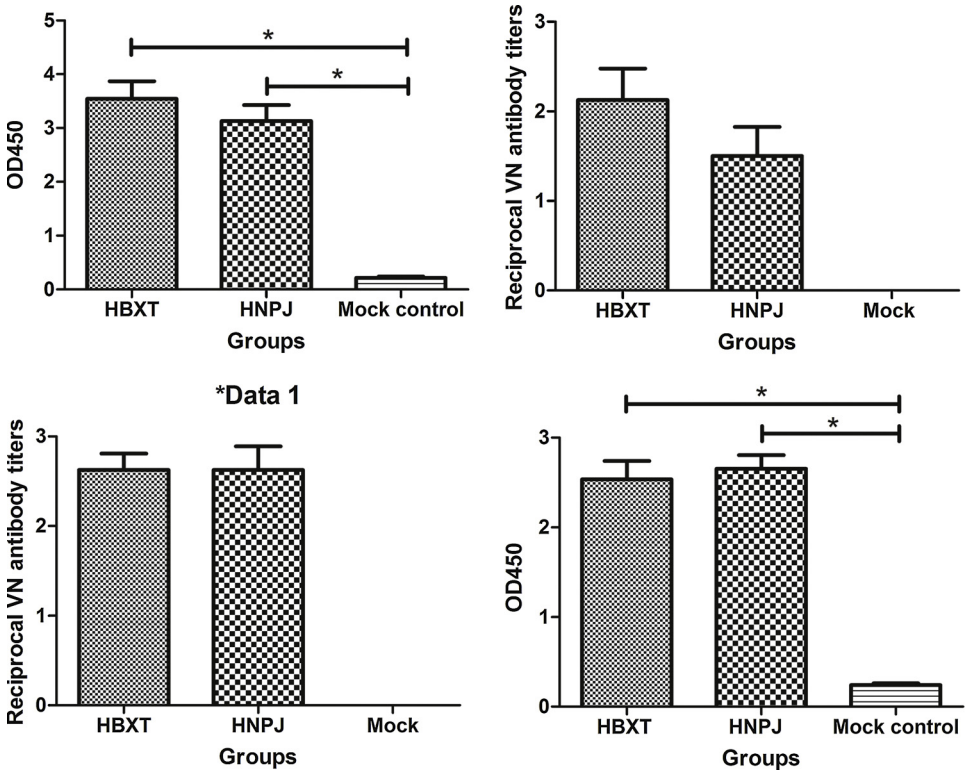

Fig. 1. Humoral and cellular immune response levels of piglets after vaccination with the GIIa (CH/HBXT/2018) and GII2b (CH/HNPJ/2017) PEDV strain-based inactivated vaccine candidates. Pigs were vaccinated at 0 days post vaccination (dpv) and then challenged with virulent homologous (GIIa strain CH/HBXT/2018 for the GIIa-vaccinated piglets) and heterologous (GII2b strain CH/HNPJ/2017 for the GIIa-vaccinated piglets) viruses. a: The IgG levels of piglets at 21 dpv. The asterisks indicate significant differences ($P < 0.05$). b and c: Neutralizing antibody titers against the GIIa (CH/HBXT/2018) and GII2b (CH/HNPJ/2017) PEDV strains. d: Levels of the cytokine IFN-γ in piglets at 21 dpv.

and HNPJ VN assays, and there was no significant difference between the two assays ($p > 0.05$) (Fig. 1b and c). However, the positive HNPJ antiserum samples had different VN antibody titers in both the HBXT VN and HNPJ VN assays, and overall, there was significant difference between the two assays ($p < 0.05$) (Fig. 1b and c). The VN antibody titers of the positive HNPJ antiserum samples tested with the homologous HNPJ VN assay were higher than the VN antibody titers of the positive HNPJ antiserum samples tested with the heterogenous HBXT VN assay (Fig. 1b and c). The antiserum samples collected from the mock control group (negative antiserum) were negative for antibodies in both HBXT VN and HNPJ VN assays at 21 dpv.

3.3. IFN-γ levels in vaccinated pigs

As shown in Fig. 1d, IFN-γ was detected in the serum of piglets vaccinated with either the GIIa CH/HBXT/2018 strain-based inactivated vaccine or the GIIa CH/HNPJ/2017 strain-based inactivated vaccine, while no IFN-γ was detected in the serum of the mock control group. The levels of IFN-γ in the two inactivated vaccine groups were significantly higher than that in the mock control group ($p < 0.05$). There was no significant difference between the two inactivated vaccine groups ($p > 0.05$) (Fig. 1d).

3.4. Protective efficacy in piglets after challenge

The protective efficacy of the two inactivated vaccines against challenge with virulent homologous and heterologous virus (1000 PDD_{50}/3 mL GIIa PEDV strain CH/HBXT/2018-P4) was determined by the absence of clinical signs and by decreased viral shedding during the 7-day observation period. The results showed that the GIIa CH/HBXT/2018 strain-based inactivated vaccine could protect all piglets (8/8) against virulent homologous and heterologous virus challenge (Table 1), while the GIIb CH/HNPJ/2017 strain-based inactivated vaccine protected only 2/4 and 1/4 piglets against virulent homologous and heterologous virus challenge (Table 1), respectively. No piglets in the mock control group were protected after virulent homologous or heterologous virus challenge (Table 1).

4. Discussion

The failure of live attenuated/inactivated classic PEDV strain-based vaccines and the severity of PED caused by highly virulent GII-genotype PEDV (field epidemic) strains make development of an efficacious vaccine imperative. Many epidemiological studies have shown that highly virulent GII PEDV strains have become pandemic in the swine population worldwide (Song and Park, 2012; Kim et al., 2015). In addition, according to previous studies, antigenic variations between classic and emerging PEDV strains may contribute to the failure of traditional attenuated vaccines in Asia (Lin et al., 2016). Therefore, our laboratory has previously isolated and serially propagated two highly virulent GII PEDV strains, CH/HBXT/2018 (GIIa) (data not shown) and CH/HNPJ/2017(GIIb) (Liu et al., 2018), in Vero cells for next-generation vaccine development. Furthermore, cross-protection experiments between GI and GII and between GIa and GIb have been performed (Lin et al., 2016) and have shown differences in cross-protective effects between the different genotype strains. However, to date, there is still no information on cross-protective effects between GIIa and GIIb. Therefore, in this study, the immunogenicity and cross-protective efficacy of two cell culture-derived GIIa and GIIb PEDV strain-based inactivated vaccines were evaluated in suckling piglets; furthermore, cross-neutralization between the two inactivated vaccines was evaluated.

Thus far, most PEDV challenge studies have chosen $TCID_{50}$ as the infectious titer for a PEDV challenge pool (Lohse et al., 2017; Lee et al., 2018). However, our previous studies showed that the infectious titer of the PEDV strain in pigs is higher than that in Vero cells (Liu et al., 2018). Therefore, to evaluate vaccine efficacy *in vivo*, it is necessary to challenge piglets with a standardized and validated dose of PEDV. Hence, in this study, we used the median pig diarrhea dose (PDD_{50}) as the standard infectious titer of inoculum to assess vaccine efficacy.

In many efficacy experiments for inactivated PEDV vaccines in pigs, a multiple-dose vaccination program involving 2 or 3 intramuscular

administrations of vaccines at 2- or 3-week intervals is commonly used to obtain high levels of neutralizing antibodies in serum and colostrum (Song and Park, 2012; Opriessnig et al., 2017; Lee et al., 2018; Sato et al., 2018). In addition, pregnant sows are optimal animal models for evaluating the efficacy of inactivated PEDV vaccines in target populations, such as neonatal piglets, because transfer of maternal antibodies to piglets *via* colostrum may play an important role in the development of protective immunity against PEDV in suckling piglets (Goede et al., 2015; Lee et al., 2018). In the present study, however, to overcome the constraints of housing and feeding and to comply with the relevant evaluation standards for Chinese veterinary bioproducts, we used newborn piglets (3–5 days old) to evaluate the protective efficacy of our experimental inactivated vaccine candidates, and all piglets received only one vaccination at 0 dpv. Hence, the levels of neutralizing antibodies determined in this study are lower than those reported in many previous related studies. In our future study, we will further evaluate the immunogenicity and protective efficacy of these two inactivated vaccine candidates using pigs in different growth phases.

5. Conclusion

The data in the present study indicate that the GIIa (CH/HBXT/2018) and GIIb (CH/HNPJ/2017) PEDV strain-based inactivated vaccine candidates both could induce high levels of PEDV-specific IgG antibodies and reduce levels of neutralizing antibodies at 21 dpv in suckling piglets. In addition, the two inactivated vaccine candidates both induced higher levels of IFN-γ than the mock control group. The GIIa-based inactivated vaccine protected all piglets (8/8) against virulent homologous and heterologous virus challenge, while the GIIb strain-based inactivated vaccine protected only 2/4 and 1/4 piglets against virulent homologous and heterologous virus challenge, respectively. Furthermore, the antibodies against the GIIa and GIIb strains cross-reacted and cross-neutralized both strains *in vitro*.

Declarations of interest

None.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (Grant No. 31602095), the National Key Research and Development Program (2016YFD0501505), the China Agriculture Research System (CARS-35) and the Central Public Interest Scientific Institution Basal Research Fund (Y2016CG23).

References

Chasey, D., Cartwright, S.F., 1978. Virus-like particles associated with porcine epidemic diarrhoea. Res. Vet. Sci. 25, 255–256.
Chen, Q., Gauger, P.C., Stafne, M.R., Thomas, J.T., Madson, D.M., Huang, H.Y., Zheng, Y., Li, G.W., Zhang, J.Q., 2016. Pathogenesis comparison between the United States porcine epidemic diarrhoea virus prototype and S-INDEL-variant strains in conventional neonatal piglets. J. Gen. Virol. 97, 1107–1121.
Crawford, K., Lager, K.M., Kulshreshtha, V., Miller, L.C., Faaberg, K.S., 2016. Status of vaccines for porcine epidemic diarrhea virus in the United States and Canada. Virus Res. 226, 108–116.
Goede, D., Murtaugh, M.P., Nerem, J., Yeske, P., Rossow, K., Morrison, R., 2015. Previous infection of sows with a "mild" strain of porcine epidemic diarrhea virus confers protection against infection with a "severe" strain. Vet. Microbiol. 176, 161–164.
Jarvis, M.C., Lam, H.C., Zhang, Y., Wang, L.Y., Hesse, R.A., Hause, B.M., Vlasova, A., Wang, Q.H., Zhang, J.Q., Nelson, M.I., Murtaugh, M.P., Marthaler, D., 2016. Genomic and evolutionary inferences between American and global strains of porcine epidemic diarrhea virus. Prev. Vet. Med. 123, 175–184.
Kim, S.H., Lee, J.M., Jung, J., Kim, I.J., Hyun, B.H., Kim, H.I., Park, C.K., Oem, J.K., Kim, Y.H., Lee, M.H., Lee, K.K., 2015. Genetic characterization of porcine epidemic diarrhea virus in Korea from 1998 to 2013. Arch. Virol. 160, 1055–1064.
Lee, C., 2015. Porcine epidemic diarrhea virus: an emerging and re-emerging epizootic swine virus. Virol. J. 12, 193.
Lee, S.H., Yang, D.K., Kim, H.H., Cho, I.S., 2018. Efficacy of inactivated variant porcine epidemic diarrhea virus vaccines in growing pigs. Clin. Exp. Vaccine Res. 7, 61–69.
Lin, C.M., Saif, L.J., Marthaler, D., Wang, Q., 2016. Evolution, antigenicity and pathogenicity of global porcine epidemic diarrhea virus strains. Virus Res. 226, 20–39.
Liu, X., Lin, C.M., Annamalai, T., Gao, X., Lu, Z., Esseili, M.A., Jung, K., El-Tholoth, M., Saif, L.J., Wang, Q., 2015. Determination of the infectious titer and virulence of an original US porcine epidemic diarrhea virus PC22A strain. Vet. Res. 46, 109.
Liu, X., Zhang, Q., Zhang, L., Zhou, P., Yang, J., Fang, Y., Dong, Z., Zhao, D., Li, W., Feng, J., Cui, B., Zhang, Y., Wang, Y., 2018. A newly isolated Chinese virulent genotype GIIb porcine epidemic diarrhea virus strain: biological characteristics, pathogenicity and immune protective effects as an inactivated vaccine candidate. Virus Res. 259, 18–27.
Lohse, L., Krog, J.S., Strandbygaard, B., Rasmussen, T.B., Kjaer, J., Belsham, G.J., Botner, A., 2017. Experimental infection of young pigs with an early European strain of porcine epidemic diarrhoea virus and a recent US strain. Transbound. Emerg. Dis. 64, 1380–1386.
Oka, T., Saif, L.J., Marthaler, D., Esseili, M.A., Meulia, T., Lin, C.M., Vlasova, A.N., Jung, K., Zhang, Y., Wang, Q., 2014. Cell culture isolation and sequence analysis of genetically diverse US porcine epidemic diarrhea virus strains including a novel strain with a large deletion in the spike gene. Vet. Microbiol. 173, 258–269.
Opriessnig, T., Gerber, P.F., Shen, H.G., de Castro, A.M.M.G., Zhang, J.Q., Chen, Q., Halbur, P., 2017. Evaluation of the efficacy of a commercial inactivated genogroup 2b-based porcine epidemic diarrhea virus (PEDV) vaccine and experimental live genogroup 1b exposure against 2b challenge. Vet. Res. 48, 69.
Park, S., Kim, S., Song, D., Park, B., 2014. Novel porcine epidemic diarrhea virus variant with large genomic deletion, South Korea. Emerg. Infect. Dis. 20, 2089–2092.
Sato, T., Oroku, K., Ohshima, Y., Furuya, Y., Sasakawa, C., 2018. Efficacy of genogroup 1 based porcine epidemic diarrhea live vaccine against genogroup 2 field strain in Japan. Virol. J. 15, 28.
Song, D., Park, B., 2012. Porcine epidemic diarrhoea virus: a comprehensive review of molecular epidemiology, diagnosis, and vaccines. Virus Genes 44, 167–175.
Song, D.S., Oh, J.S., Kang, B.K., Yang, J.S., Moon, H.J., Yoo, H.S., Jang, Y.S., Park, B.K., 2007. Oral efficacy of vero cell attenuated porcine epidemic diarrhea virus DR13 strain. Res. Vet. Sci. 82, 134–140.
Sun, R.Q., Cai, R.J., Chen, Y.Q., Liang, P.S., Chen, D.K., Song, C.X., 2012. Outbreak of porcine epidemic diarrhea in suckling piglets. China. Emerg. Infect. Dis. 18, 161–163.
Wang, L., Byrum, B., Zhang, Y., 2014. New variant of porcine epidemic diarrhea virus, United States, 2014. Emerg. Infect. Dis. 20, 917–919.
Wang, D., Fang, L., Xiao, S., 2016. Porcine epidemic diarrhea in China. Virus Res. 226, 7–13.
Zhang, Q.L., Liu, X.S., Fang, Y.Z., Zhou, P., Wang, Y.L., Zhang, Y.G., 2017. Detection and phylogenetic analyses of spike genes in porcine epidemic diarrhea virus strains circulating in China in 2016-2017. Virol. J. 14, 194.