Identification of a novel B-cell epitope in the spike protein of porcine epidemic diarrhea virus

Ning Kong 1,2†, Qiong Meng 1,3†, Yajuan Jiao 1†, Yongguang Wu 1, Yewen Zuo 1, Hua Wang 1, Dage Sun 1, Sujie Dong 1, Huanjie Zhai 1, Wu Tong 1,2, Hao Zheng 1,2, Hai Yu 1,2, Guangzhi Tong 1,2, Yongjie Xu 3* and Tongling Shan 1,2*

Abstract

Background: Porcine epidemic diarrhea virus (PEDV) infection causes an acute enteric tract infectious disease characterized by vomiting, anorexia, dehydration, weight loss and high mortality in neonatal piglets. During PEDV infection, the spike protein (S) is a major virion structural protein interacting with receptors and inducing neutralizing antibodies. However, the neutralizing B-cell epitopes within PEDV S protein have not been well studied.

Methods: To accurately identify the important immunodominant region of S1, the purified truncated S1 proteins (SA, SB, SC, SD and SE) were used to immunize BALB/c mice to prepare polyclonal antibodies. The antisera titers were determined by indirect ELISA, western blot and IFA after four immunizations to find the important immunodominant region of S1, and then purified the immunodominant region of S1 protein and immunized mice to generate the special antibodies, and then used recombinant peptides to determine the B-cell epitopes of monoclonal antibodies.

Results: Five antisera of recombinant proteins of the spike protein region of PEDV were generated and we found that only the polyclonal antibody against part of the S1 region (signed as SE protein, residues 666–789) could recognize the native PEDV. Purified SE protein was used to immunize BALB/c mice and generate mAb 2E10. Pepscan of the SE protein demonstrated that SE16 (722SSTFNSTREL731) is the minimal linear epitope required for reactivity with the mAb 2E10. Further investigation indicated that the epitope SE16 was localized on the surface of PEDV S protein in the 3D structure.

Conclusions: A mAb 2E10 that is specifically bound to PEDV was generated and identified a specific linear B-cell epitope (SE16, 722SSTFNSTREL731) of the mAb. The epitope region of PEDV S1 localized in the different regions in comparison with the earlier identified epitopes. These findings enhance the understanding of the PEDV spike protein structure for vaccine design and provide a potential use for developing diagnostic methods to detect PEDV.

Keywords: PEDV, Spike protein, Monoclonal antibody, Epitope

© The Author(s). 2020 Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated in a credit line to the data.
Background
Porcine epidemic diarrhea (PED) is an acute enteric tract infectious disease characterized by vomiting, anorexia, dehydration, weight loss and high mortality in neonatal piglets [1, 2]. The disease was reported in European and Asian pig industries over the last 30 years, with the virus firstly appearing in England and Belgium in the early 1970s [3, 4]. Porcine epidemic diarrhea virus (PEDV), although the etiologic agent of PED has become a severe problem in many Asian countries, including China, Korea, Japan and Thailand [5–8]. Since 2010, the virulent PEDV has become prevalent in swine herds and incurred huge economic losses to the swine industry [9–11]. Due to the lack of effective vaccines, PED is still circulating in the worldwide.

PEDV belonging to the genus Alphacoronavirus, family Coronaviridae, has an approximately 28 kb genome of single-stranded, positive-sense RNA [1]. The PEDV genome encodes two large polyproteins, an accessory protein and four structural proteins. The structural proteins contain glycosylated spike (S), envelope (E), glycosylated membrane (M) and RNA-binding nucleocapsid (N) proteins [12]. The spike gene can be divided into S1 and S2 domains, as in other coronaviruses and it has multiple functions that can interact with cellular receptors and regulating viral entry and containing neutralizing epitopes to induce neutralizing antibodies [13, 14].

In the present study, we expressed and purified the recombinant truncated PEDV S1 constructs (SA-SE) to immunize BALB/c mice and found that SE, one of the S1 construct (residues 666–789), was the immunodominant region of S1 protein. Furthermore, we utilized the SE protein to immunize BALB/c mice and obtained one SE specific mAb, 2E10. A novel linear B-cell epitope, (T722SSTFNSTREL734), was subsequently identified using the SE specific mAb 2E10. These results provide valuable information for virus diagnosis and vaccine design.

Methods
Cell lines, viruses and plasmids
African green monkey kidney cells (Vero E6) and SP2/0 myeloma cells were cultured in a humidified 5% CO₂ atmosphere at 37 ℃. All the culture media were Dulbecco’s modified Eagle’s medium (DMEM, Hyclone) supplemented with 10% fetal bovine serum (FBS) and antibiotics (0.1 mg/ml of streptomycin and 100 IU/ml of penicillin). PEDV strain JS-2013 was obtained from the Shanghai Veterinary Research Institute (CAAS, China). Plasmid DNA (pCold-TF) containing the S1 gene (1–2367 bp) of PEDV strain JS-2013 was constructed by our own laboratory.

Expression of the truncated PEDV S1 proteins
The five overlapping fragments, comprising partial length of PEDV S1 gene, were constructed and designated as SA, SB, SC, SD and SE. A BamH I site and sixteen extra bases that were homologous to the terminal sequence of the vector were added to the 5’ end. The sequences of the primers used for amplification of the gene in this study are shown in Table 1. All the recombinant plasmids were constructed by ClonExpress II One Step Cloning Kit (Vazyme Biotech, C112–02), according to the manufacturer’s instructions. The truncated segments SA-SE were cloned into pCold-TF vector and the confirmed recombinant plasmids were transformed into E. coli BL21 and induced by isopropyl-β-D-thiogalactoside (IPTG) at 16 ℃ for 24 h. The truncated PEDV S1 proteins were analyzed by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and western blot. All the recombinant proteins were purified by using Nickel Magnetic Beads (Biotool, Shanghai, China) to prepare polyclonal antibodies.

Identification of immunoactivity of truncated proteins
Groups of five 8-week-old female BALB/c mice were intraperitoneally immunized with 50 μg purified truncated PEDV S1 proteins. Antigens were emulsified in the same volume of complete Freund’s adjuvant (Sigma, USA) for the initial immunization, then emulsified in incomplete Freund’s adjuvant on subsequent immunizations at 2-week intervals for 6 weeks. Phosphate-buffered saline (PBS) was used for the controlled trial with the same procedures. Three days after the final boosting, the mice were narcotized and their blood samples were collected from the caudal vein. The collected antisera were diluted 1000-fold and used for indirect ELISA, western blot and IFA to detect the immunoactivity of truncated proteins.

Development of monoclonal antibody targeting PEDV
Female 8-week-old BALB/c mice were immunized with 50 μg purified protein emulsified in the same volume of complete Freund’s adjuvant via intraperitoneal injection. This procedure was followed by three additional injections at 2-week intervals with the same dose of antigen emulsified in incomplete Freund’s adjuvant. Three days after the final injection, spleen cells from immunized mice were fused with SP2/0 myeloma cells using polyethylene glycol 1450 (PEG4150, Sigma, USA), as previously described [15]. Then the hybridoma cells were seeded into 96-well plates and selected in hypoxanthine-aminopterin-thymidine (HAT) medium and hypoxanthine-thymidine (HT) medium. The cell culture supernatants of surviving clones were determined by indirect ELISA for antibody reactivity and specificity. Positive hybridomas were cloned four times by limiting dilution. Ascites fluids were produced in pristane induced BALB/c mice.
Indirect enzyme-linked immunosorbent assay (ELISA)

Indirect ELISA was used to identify the immune reactivity of the truncated proteins and the screen of positive hybridoma cells. The ELISA plates were plated with purified PEDV S1 protein or synthesized peptides (400 ng/well) in carbonate bicarbonate buffer (15 mM Na$_2$CO$_3$, 3.5 mM NaHCO$_3$ [pH 9.6]) and coated at 4 °C overnight. The plates were blocked with 5% non-fat dry milk in TBST (TBS (NC) membrane (GE Healthcare, USA). The membranes were subsequently incubated with horseradish peroxidase (HRP) -conjugated goat anti-mouse IgG (Proteintech Group, China) with 1:20,000 dilution in PBS (slowly) for 1 h at RT. The membranes were subsequently incubated with HRP-conjugated goat anti-mouse IgG (Thermo, USA) containing protease inhibitor cocktail (Bimake, USA) and phosphatase inhibitor cocktail (Bimake, USA) on ice for 5 min. The cell lysates were then separated by 10% SDS-PAGE and transferred to the nitrocellulose (NC) membrane (GE Healthcare, USA). The membranes were blocked with 5% non-fat dry milk in TBST (TBS with 0.1% Polysorbate-20) for 1 h at room temperature (RT). The membranes were subsequently incubated with HRP-conjugated goat anti-mouse IgG (Thermo Fisher Scientific, USA) according to the manufacturer’s instructions.

Indirect immunofluorescence assay (IFA)

Vero cells were plated in a six-well plate and infected with PEDV when the cells reached approximately 90% confluence. At 24 h postinfection, the cells were fixed with 4% paraformaldehyde (Sigma-Aldrich) for 15 min and permeabilized with 0.1% Triton X-100 (Sigma-Aldrich) for 10 min at room temperature. After being washed three times in phosphate-buffered saline (PBS), the cells were blocked with 10% bovine serum albumin (BSA) in PBS for 1 h at 37 °C and then incubated with the primary antibody for 1 h. After three washes with PBS, cells were incubated with Alexa Fluor 488 donkey

Table 1  Sequence of the primers used in this study

| Names | Primer sequences (5' → 3') | Position |
|-------|---------------------------|----------|
| SA    | F: TACCCCTCAGGGATCTAGTGAACATGTATTTAACAATCATCACCTCCTGCTGTGTAAGCA | 1–609    |
|       | R: GCTTGAATTTCGGATCC |         |
| SB    | F: TACCCCTCAGGGATCTAGTGAACATGTATTTAACAATCATCACCTCCTGCTGTGTAAGCA | 499–1071 |
|       | R: GCTTGAATTTCGGATCC |         |
| SC    | F: TACCCCTCAGGGATCTAGTGAACATGTATTTAACAATCATCACCTCCTGCTGTGTAAGCA | 964–1617 |
|       | R: GCTTGAATTTCGGATCC |         |
| SD    | F: TACCCCTCAGGGATCTAGTGAACATGTATTTAACAATCATCACCTCCTGCTGTGTAAGCA | 1513–2142 |
|       | R: GCTTGAATTTCGGATCC |         |
| SE    | F: TACCCCTCAGGGATCTAGTGAACATGTATTTAACAATCATCACCTCCTGCTGTGTAAGCA | 1999–2367 |
|       | R: GCTTGAATTTCGGATCC |         |
| SE1   | F: TACCCCTCAGGGATCTAGTGAACATGTATTTAACAATCATCACCTCCTGCTGTGTAAGCA | 1999–2333 |
|       | R: GCTTGAATTTCGGATCC |         |
| SE2   | F: TACCCCTCAGGGATCTAGTGAACATGTATTTAACAATCATCACCTCCTGCTGTGTAAGCA | 2068–2208 |
|       | R: GCTTGAATTTCGGATCC |         |
| SE3   | F: TACCCCTCAGGGATCTAGTGAACATGTATTTAACAATCATCACCTCCTGCTGTGTAAGCA | 2119–2283 |
|       | R: GCTTGAATTTCGGATCC |         |
| SE4   | F: TACCCCTCAGGGATCTAGTGAACATGTATTTAACAATCATCACCTCCTGCTGTGTAAGCA | 2224–2367 |
|       | R: GCTTGAATTTCGGATCC |         |
| SE5   | F: TACCCCTCAGGGATCTAGTGAACATGTATTTAACAATCATCACCTCCTGCTGTGTAAGCA | 2119–2178 |
|       | R: GCTTGAATTTCGGATCC |         |
| SE6   | F: TACCCCTCAGGGATCTAGTGAACATGTATTTAACAATCATCACCTCCTGCTGTGTAAGCA | 2149–2208 |
|       | R: GCTTGAATTTCGGATCC |         |

The introduced restriction enzyme sites BamH I are underlined. At 5' terminal of each sense strand, there is a sequence of TACCCTCGAG (bold) which is as same as the ends of carrier Pcold-TF. At 3' terminal of each reverse sense strand, there is a sequence of GCTTGAATTC (bold) complement with the ends of carrier Pcold-TF.

*Location of the synthesized peptides is based on the sequence of 5 protein of PEDV strain HeB/TS/2016/325 (GenBank accession no. KX907710.1)
anti-mouse IgG (H + L) antibody in the dark for 1 h at 37 °C. Following several washes, the fluorescence was visualized by using an Olympus® IX73 inverted microscope.

**Bioinformatics analysis**
The spatial position of the identified epitope was analyzed by mapping the location on the 3D structure model of PEDV S [17] by using PyMOL software [18, 19], and the secondary structure of amino acid sequences of the identified epitope was also analyzed by PROTEAN software (DNASTAR’s Lasergene, Inc., Madison, WI, USA) [20].

**Statistical analysis**
All results are representative of three independent experiments. Statistical analysis was performed using Prism 5.0 software (GraphPad). Significance was determined by two-tailed Student’s t test. Statistical significance: *p < 0.05, **p < 0.001.

**Results**
**Immunodominant region of S1 protein**
To accurately identify the immunodominant region of S1, the purified truncated S1 proteins (SA, SB, SC, SD and SE) were used to immunize BALB/c mice to prepare

---

**Fig. 1** Detection of recombinant proteins’ antigenicity for polyclonal antisera (PcAbs) by indirect ELISA, western blot and IFA. **a** Reactivity of antisera against the recombinant S1 protein by indirect ELISA. **b** Western blot analysis of the recombinant S1 protein with PcAbs. The recombinant S1 protein (approximately 140 kDa) was transferred to the nitrocellulose (NC) membrane, followed by the different PcAbs (negative control, SA polyclonal antisera, SB polyclonal antisera, SC polyclonal antisera, SD polyclonal antisera and SE polyclonal antisera) as primary antibody. **c** Immunofluorescence analysis of SE polyclonal antisera against PEDV. Vero cells were plated in six-well plates and inoculated with PEDV (0.01 MOI). Twenty-four hours later, cells were fixed and incubated with SE polyclonal antisera or normal mouse serum (negative control), and then incubated with Alexa Fluor 488 donkey anti-mouse IgG (H + L) antibody. Scale bars: 200 μm
polyclonal antibodies. The antisera titers were determined by indirect ELISA, western blot and IFA after four immunizations. The indirect ELISA and western blot analysis showed that the SE polyclonal antiserum had the highest antibody titers against PEDV S1 protein (Fig. 1a and b), suggesting that SE protein was the important immunodominant region of S1. The immunofluorescence signal for the PEDV S1 protein was also detected by the SE polyclonal antisera (Fig. 1c), further confirming that SE polyclonal antisera could recognize the native PEDV. Collectively, these results indicated that the region of SE (666–789 aa) was the immunodominant region of S1 protein.

Production and characterization of SE protein-specific mAb
Purified SE protein was used to immunize BALB/c mice to prepare mAbs, and then determined the antisera titers using indirect ELISA after four immunizations. The mouse with the highest antibody titers against SE protein was used for cell fusion. After being subcloned by limiting dilution and screening for four times, one positive mAb against SE protein was identified and named 2E10. The mAb 2E10 cell clone was used to prepare ascites containing mAbs. The ascites was collected and purified using Nab Protein G Spin Columns (Thermo Fisher Scientific, Rockford, IL). Western blot analysis (Fig. 2a) and immunofluorescence assay (IFA) (Fig. 2b) were used to identify the specificity of the mAbs against PEDV. The results suggested that the mAb 2E10 could be specifically reacted with native PEDV protein.

Analysis of the immunodominant region of SE with mAb
To identify the antigenic epitope recognized by mAb 2E10, four truncated and overlapping 6 × His-tagged peptides (SE1-SE4) spanning the SE fragments were designed (Fig. 3a) and expressed using the bacterial system. The sequences of the primers were also shown in Table 1. All fusion proteins were predominantly expressed in soluble form in bacterial cells. Subsequently, western blot was used to determine immune reactivity between the Mab 2E10 and these SE fragments. The results showed that MAb 2E10 reacted with fragments spanning aa 690 to 736 (SE2) and 707 to 761 (SE3) but not aa 667 to 711 (SE1) or 742 to 798 (SE4), suggesting that the epitope recognized by 2E10 was located in aa 707 to 736 of PEDV S1 protein (Fig. 3b). Furthermore, we generated two deletion constructs of SE (SE5, SE6) that span the overlaps of SE2 and SE3 (Fig. 3a) to identify the antigenic epitope. As demonstrated by western blot, SE6 was recognized by mAb 2E10 (Fig. 3c), which suggested that SE6 (722SSTFNSTRELPG736) epitope may be a harbored antigenic epitope.

Minimization of the epitope SE6 with mAb 2E10
In order to further minimize the epitope of SE6, two shortened peptides (SE7-SE8) were synthesized via solid-phase peptide synthesis (Table 2). Using indirect ELISA, we found that SE8 showed a strong reaction with mAb 2E10 as SE6 did (Fig. 4a). The result suggested that SE8 was the essential region for recognition by mAb 2E10. According to the results, four shortened peptides (SE9-SE12) by deleting three amino acids at either the amino or the carboxy terminus in sequence from the SE8 were synthesized (Table 2). In ELISA, only SE11 could be recognized by mAb 2E10, which indicates that the 2E10-specific epitope was 722SSTFNSTRELPG733 (Fig. 4a). With the same method, the other four shortened peptides (SE13-SE16) were synthesized according to the peptide sequence of SE8. The results showed that the peptide SE16 (722SSTFNSTREL731) was strongly recognized by the mAb 2E10 (Fig. 4a). Taken together, these results demonstrate that SE16 (722SSTFNSTREL731) is the minimal linear epitope required for reaction with the mAb 2E10.

Conservation and spatial distribution of the novel epitope
To localize the identified epitope SE16, a 3D structural model of PEDV S was obtained from the Protein Data Bank (PDB, ID: 6U7K), and the spatial distribution was
analyzed by PyMOL software. The structural visualization revealed that the identified epitope recognized by 2E10 was exposed on the surface of PEDV S structure (Fig. 4b) and partial peptide formed a beta-sheet structure (Fig. 4c). Moreover, the identified epitope SE16 had high antigenic index and hydrophilicity (Fig. 4d). It was suggested that the epitope can easily explore and induce host immune response in those infected animals.

**Discussion**

PEDV S protein, one of the most important glycoproteins, contains multiple neutralizing epitopes to induce neutralizing antibodies. The S protein is divided into S1 (residues 1–789) and S2 (residues 790–1383) domains which are defined by the conserved nonamer and the GxGx motifs in coronavirus group II members [21]. Based on the information of other coronaviruses, we found that the S1 domain is thought to contain multiple virus neutralization epitopes and receptor binding-domains [22]. The S2 domain forms the trans-membrane structure of the S protein, but can not induce neutralizing antibodies [23]. These properties make it possible that the S1 could be a suitable candidate for screening and identifying antigenic epitopes. Epitopes are important antigenic elements of virus structural proteins, which could induce antibody production and cell-mediated immunity against viruses. Therefore, epitopes are essential to develop epitope-based vaccines and diagnostics.

Preparation of mAb is required to identify the epitopes of the S1 protein. We had tried to use intact S1 protein to immunize mice, but it was unsuccessful to get the mice serum with immune activity. Because of unidentified but
complex factors, we chose to divide the S1 protein into five fragments (SA, SB, SC, SD, SE). The five truncated proteins were expressed and the immunogenicity of the proteins was identified. These results indicated that the SE protein (666–789 aa) had good reactivity as the immunodominant region of S1 protein. Then the SE protein was selected as an immunogen to elicit the formation of monoclonal antibody. After cell fusion and four times of selection, mAb 2E10 was chosen because of its specific reactivity with the SE protein as well as the native S protein PEDV.

Several domains containing neutralizing epitopes within the S protein were identified, such as residues 499–638 [24], residues 636–798 [25], residues 592–607 [26] and residues 575–639 [27]. These mimicking epitopes had antigenic similarities with the PEDV neutralizing epitopes. In this study, we expressed a series of truncated proteins (SE1-SE6) to map the epitopes of SE protein. The SE6 protein could be recognized by mAb 2E10. Generally, linear epitopes consist of six to nine or more continuous amino acid residues. So, the pepscan method was used to

| Names | Amino acids sequences and location on S1 protein | Location of the synthesized peptides is based on the sequence of S protein of PEDV strain HeB/TS/2016/325b (GenBank accession no. KX907110.1) |
|-------|--------------------------------------------------|------------------------------------------------------------------------------------------------|
| SE7   | 717-SSSLSSSFNSTREL721                             |                                                                                                  |
| SE8   | 722-SSFNSTRELPGFY736                             |                                                                                                  |
| SE9   | 725-SSFNSTRELPGFY736                             |                                                                                                  |
| SE10  | 728-TRELPGFY736                                  |                                                                                                  |
| SE11  | 725-SSFNSTRELPG733                               |                                                                                                  |
| SE12  | 722-SSFNSTREL730                                 |                                                                                                  |
| SE13  | 723-SSFNSTRELPG733                               |                                                                                                  |
| SE14  | 724-SSFNSTRELPG733                               |                                                                                                  |
| SE15  | 722-SSFNSTREL732                                 |                                                                                                  |
| SE16  | 722-SSFNSTREL731                                 |                                                                                                  |
| SE6   | 717-SSSLSSSFNSTRELPGFY736                        |                                                                                                  |

Table 2 Sequence of the synthesized peptides corresponding to the epitope SE6

Fig. 4 Pepscan of the epitopes SE6 and localization of the epitopes recognized by the mAb 2E10. a ELISA analysis of the truncated peptides SE6-SE16 with mAb 2E10 and PBS. The surface b and cartoon c from SE16 was labeled in the sequence chain view picture of PEDV S, obtained from the Protein Data Bank (PDB, ID: 6U7K). d The structural features of SE16 were predicted by PROTEAN software. The epitope SE16 was shown in the boxes.
truncate the epitope SE6 by deleting three amino acids at either the amino or the carboxy terminus in sequence respectively. A total of ten sub-segments (SE7-SE16) was synthesized and the results of ELISA suggested that SE16 could react with mAb 2E10. Above all these results, the epitope SE16 is the immunodominant region of PEDV S protein. The epitope SE16 (722SSTFNSTREL731) was highly conserved among different strains of virulent PEDV. Further, the 3D structural visualization showed that the epitope SE16 presented on the surface of PEDV S and had a feature of high antigenic index and hydrophilicity. The character of this location makes this epitope easily explore and induce host immune response in the infected animals.

Conclusions

We prepared a mAb 2E10 that is specifically bound to PEDV and identified a specific linear B-cell epitope (SE16, 722SSTFNSTREL731) of the mAb. The epitope region of PEDV S1 localized in the different regions in comparison with the earlier identified epitopes. Therefore, the identified region is a novel B-cell antigenic epitope region of PEDV S protein and the identified epitope has potential use for developing diagnostic reagent and effective vaccines for PEDV.

Abbreviations

ELISA: Indirect enzyme-linked immunosorbent assay; HAT: Hypoxanthine-aminopterin-thymidine; HT: Medium and hypoxanthine-thymidine; IFA: Indirect immunofluorescence assay; IPTG: Isopropyl-β-D-thiogalactoside; mAb: Monoclonal antibody; PBS: Phosphate-buffered saline; PED: Porcine epidemic diarrhea; PEDV: Porcine epidemic diarrhea virus; RT: Room temperature; SDS-PAGE: Sodium dodecylsulfate-polyacrylamide gel electrophoresis; TCD50: 50% tissue culture infectious doses

Acknowledgements

We thank Mr. Yongjun Chen for his assistance in animal feeding.

Authors’ contributions

TS, GT, NK and QM conceived and designed the experiments. QM, NK, YJ, YW, YZ and HW performed the experiments. DS, HZ, WT and HZ performed bioinformatic analysis. HY and YX provided nutritional advice. TS, GT, NK and QM wrote and revised the manuscript. All authors reviewed the manuscript. The author(s) read and approved the final manuscript.

Funding

This work was supported by the National Key Research and Development Programs of China (No. 2016YFD0500103); the National Natural Science Foundation (No. 31872478); the Natural Science Foundation of Shanghai (No. 16ZR1469100) and the China Postdoctoral Science Foundation (No. 2017M611074).

Availability of data and materials

Data generated and analyzed in this study are presented in this manuscript. Data can be obtained by contacting the corresponding author.

Ethics approval and consent to participate

The study was approved by the Ethics and Animal Welfare Committee of Shanghai Veterinary Research Institute, China and the animals were handled by the rules stipulated by the Ethics and Animal Welfare Committee of Shanghai Veterinary Research Institute, China.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

1Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Shanghai, People’s Republic of China. 2Jiangsu Co-Innovation Center for the Prevention and Control of Important Animal Infectious Disease and Zoonosis, Yangzhou University, Yangzhou, People’s Republic of China. 3College of Life Science, Xinyang Normal University, Xinyang, China.

Received: 7 December 2019 Accepted: 26 February 2020

Published online: 03 April 2020

References

1. Pensaat M, de Bouck P. A new coronavirus-like particle associated with diarrhea in swine. Arch Virol. 1978;58:243–7.
2. Ducatelle R, Coussemere W, Charlier G, Debouch P, Hooren J. Three-dimensional sequential study of the intestinal surface in experimental porcine CV 777 coronavirus enteritis. Zentralbl Vetmeder Heinforbl. 1981;28:483–93.
3. Chasey D, Cartwright SF. Virus-like particles associated with porcine epidemic diarrhea. Res Vet Sci. 1978;25:255–6.
4. Wood EN. An apparently new syndrome of porcine epidemic diarrhoea. Vet Rec. 1977;100:243–4.
5. Puranaveja S, Poolperm P, Lertwatcharasarakul P, Kesdaengsakonwut S, Boonsoongern A, Uraiong K, Kittipon P, Chojpai P, Kedkolvo R, Teanunkorn K, Thanawongnuwech R. Chinese-like strain of porcine epidemic diarrhoea virus, Thailand. Emerg Infect Dis. 2009;15:1112–5.
6. Chen JF, Sun DB, Wang CB, Shi HY, Cui XC, Liu SW, Qiu HJ, Feng L. Molecular characterization and phylogenetic analysis of membrane protein genes of porcine epidemic diarrhea virus isolates in China. Virus Genes. 2008;36:355–64.
7. Takahashi K, Okada K, Oshihara K. An outbreak of swine diarrhea of a newtype associated with coronavirus-like particles in Japan. Nihon Jukigaku Zasshi. 1983;45:829–32.
8. Chae C, Kim O, Choi C, Min K, Cho WS, Kim J, Tai JH. Prevalence of porcine epidemic diarrhoea virus and transmissible gastroenteritis virus infection in Korean pigs. Vet Rec. 2000;147:606–8.
9. Li W, Li H, Liu Y, Pan Y, Deng F, Song Y, Tang X, He Q. New variants of porcine epidemic diarrhea virus, China, 2011. Emerg Infect Dis. 2012;18:1350–3.
10. Stevenson GW, Hoang H, Schwartz KJ, Burrough ER, Sun D, Madson D, Cooper VL, Pillatzki A, Gauger P, Schmitt BJ, et al. Emergence of porcine epidemic diarrhea virus in the United States: clinical signs, lesions, and viral genomic sequences. J Vet Diagn Invest. 2013;25:649–54.
11. Islam MT, Kubota T, Ujike M, Yahara Y, Tajchichi H. Phylogenetic and antigenic characterization of newly isolated porcine epidemic diarrhoea viruses in Japan. Virus Res. 2016;222:1113–9.
12. Song D, Park B. Porcine epidemic diarrhoea virus: a comprehensive review of molecular epidemiology, diagnosis, and vaccines. Virus Genes. 2012;44:167–75.
13. Kang TJ, Han SC, Yang MS, Jang YS. Expression of synthetic neutralizing epitope of porcine epidemic diarrhea virus fused with synthetic B subunit of Escherichia coli heat-labile enterotoxin in tobacco plants. Protein Expr Purif. 2006;46:16–22.
14. Gallagher TM, Buchmeier MJ. Coronavirus spike proteins in viral entry and pathogenesis. Wrology. 2001;27:371–4.
15. Kong N, Meng Q, Wu Y, Wang Z, Zuo Y, Tong W, Zheng H, Li G, Yang S, Yu H, et al. Monoclonal antibody to bone marrow stromal cell antigen 2 protein of swine. Monoclon Antib Immunodiagn Immunother. 2016;15:172–6.
16. Kong N, Wu Y, Meng Q, Wang Z, Zuo Y, Pan X, Tong W, Zheng H, Li G, Yang S, et al. Suppression of virulent porcine epidemic diarrhoea virus proliferation by the PI3K/Akt/GSK-3β/beta pathway. PLoS One. 2016;11:e0161508.
17. Wrapp D, McLellan JS. The 3.1 A cryo-EM structure of the porcine epidemic diarrhoea virus spike protein in the prefusion conformation. J Virol. 2019;93:e00923–19.
18. Jiang N, Jin H, Li Y, Ge X, Han J, Guo X, Zhou L, Yang H. Identification of a novel linear B-cell epitope in nonstructural protein 11 of porcine reproductive and respiratory syndrome virus that are conserved in both genotypes. PLoS One. 2017;12:e0188946.

19. Bi C, Shao Z, Li J, Wang C. Identification of novel epitopes targeting non-structural protein 2 of PRRSV using monoclonal antibodies. Appl Microbiol Biotechnol. 2019;103:2689–99.

20. Xu JJ, Wu QJ, Cheng XF, Tong W, Zheng H, Zhu HJ, Liu YT, Jiang YF, Gao F, Yu H, et al. Identification of two novel epitopes targeting glycoprotein E of pseudorabies virus using monoclonal antibodies. Biochem Biophys Res Commun. 2019;519:330–6.

21. Follis KE, York J, Nunberg JH. Furin cleavage of the SARS coronavirus spike glycoprotein enhances cell-cell fusion but does not affect virion entry. Virology. 2006;350:358–69.

22. Delmas B, Laude H. Assembly of coronavirus spike protein into trimers and its role in epitope expression. J Virol. 1990;64:5367–75.

23. de Haan CA, Te Lintelo E, Li Z, Raaben M, Wurdinger T, Bosch BJ, Rottier PJ. Cooperative involvement of the S1 and S2 subunits of the muine coronavirus spike protein in receptor binding and extended host range. J Virol. 2006;80:10909–18.

24. Chang SH, Bae JI, Kang TJ, Kim J, Chung GH, Lim CW, Laude H, Yang MS, Jang YS. Identification of the epitope region capable of inducing neutralizing antibodies against the porcine epidemic diarrhea virus. Mol Cells. 2002;14:295–9.

25. Sun DB, Feng L, Shi HY, Chen JF, Liu SW, Chen HY, Wang YF. Spike protein region (aa 636789) of porcine epidemic diarrhea virus is essential for induction of neutralizing antibodies. Acta Virol. 2007;51:149–56.

26. Sun YG, Li R, Xie S, Qiao S, Li Q, Chen XX, Deng R, Zhang G. Identification of a novel linear B-cell epitope within the collagenase equivalent domain of porcine epidemic diarrhea virus spike glycoprotein. Virus Res. 2019;266:34–42.

27. Chang CY, Cheng IC, Chang YC, Tsai PS, Lai SY, Huang YL, Jeng CR, Fang VF, Chang HW. Identification of neutralizing monoclonal antibodies targeting novel conformational epitopes of the porcine epidemic diarrhoea virus spike protein. Sci Rep. 2019;9:2529.