



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



Development of a rapid immunochromatographic strip test for the detection of porcine epidemic diarrhea virus specific SIgA in colostrum



Jianbo Liu¹, Ran Gao¹, Hongyan Shi, Guangyi Cong, Jianfei Chen, Xin Zhang, Da Shi, Liyan Cao, Xiaobo Wang, Jialin Zhang, Zhaoyang Ji, Zhaoyang Jing, Li Feng*

State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute of the Chinese Academy of Agricultural Sciences, Harbin 150069, China

ARTICLE INFO

Keywords:

Porcine epidemic diarrhea virus
SIgA
Rapid immunochromatographic test strip
Diagnostics

ABSTRACT

Porcine epidemic diarrhea virus (PEDV) causes very high mortality in newborn piglets. The mucosal immune system in the gut must eliminate potential pathogens while maintaining a mutually beneficial relationship with the commensal microbiota. Antibodies derived from the secretory immunoglobulin A (SIgA) class, act as the first line of antigen-specific immunity in the gut by recognizing both pathogens and commensals. Therefore, the measurement of SIgA levels is an important index in evaluating PEDV infections and immune status. A simple and rapid method for the detection of PEDV-specific SIgA using an immunochromatographic test strip has been developed; incorporating a colloidal gold-labeled anti-SIgA secretory component (SC) mAb probe for the detection of anti-PEDV-specific SIgA in swine. On the strip, a gold-labeled anti-SIgA SC mAb was applied to a conjugate pad; purified PEDV particles and goat anti-mouse antibodies were blotted onto a nitrocellulose membrane to form the test and control lines, respectively. Results showed that the immunochromatographic test strip had high sensitivity and specificity. When compared with enzyme-linked immunosorbent assay, kappa value suggesting that the strip could be used to detect PEDV specific SIgA in colostrum samples. Furthermore, the strip assay is rapid and easy to perform with no requirement for professional-level skills or equipment. We found that the immunochromatographic test strip was a rapid, sensitive, and reliable method for the identification of PEDV specific SIgA, indicating its suitability for epidemiological surveillance as well as vaccine immunity when studying PEDV.

1. Introduction

Porcine epidemic diarrhea virus (PEDV) belongs to the family *Coronaviridae*, first recorded in pigs in England in 1971 (Pensaert and de Bouck, 1978), and subsequently spread to other European and Asian countries (Song and Park, 2012) and North America (Huang et al., 2013). Porcine epidemic diarrhea is a global infectious disease and is characterized by high morbidity and mortality in pre-weaned piglets, and causes serious economic losses to the swine industry in China (Gao et al., 2013; Li et al., 2014).

PEDV is an enveloped coronavirus with a 28 kb positive-stranded RNA genome, containing at least seven open reading frames (Kocherhans et al., 2001). These mainly encode four major structural proteins: the spike (S), nucleocapsid, membrane, and envelope proteins. The S protein is a glycoprotein and can form homotrimers to mediate membrane fusion and gain entry into host cells (Bosch et al., 2003). It is

known that the S protein of coronavirus plays a crucial role in the induction of neutralizing antibodies, and it has been used to prepare effective vaccines (Brian and Baric, 2005; Tuboly and Nagy, 2001). The amino-terminal portion of the S protein of several coronaviruses has been shown to contain key antigenic sites that are responsible for eliciting humoral and cellular immune responses (Gebauer et al., 1991).

The mucosal immune system in the gut faces the formidable task of eliminating potential pathogens while maintaining a mutually beneficial relationship with the commensal microbiota. Antibodies of the secretory immunoglobulin A (SIgA) class act as the first line of antigen-specific immunity in the gut, and can recognize both pathogens and commensals (Song and Park, 2012). In contrast to serum IgA, which is derived from plasma cells in the bone marrow, SIgA is generated locally by plasma cells in the lamina propria, which lies beneath the intestinal epithelium (Kaetzel, 2005). The protective action of SIgA in the infant gut is a result of many processes, including intracellular neutralization

* Corresponding author at: Harbin Veterinary Research Institute, The Chinese Academy of Agricultural Sciences, 678 Haping Road, Xiangfang District, Harbin, 150069, China.

E-mail address: fengli@caas.cn (L. Feng).

¹ The first two authors contributed equally to this work.

and viral particle excretion, immune exclusion, whereby SIgA agglutinates bacteria and viruses, as well as prevents the binding of pathogens to mucosal surfaces, and interference with bacterial motility (van Egmond et al., 2001v). At high endogenous concentrations of SIgA, infants are less likely to have experienced illness in the preceding and subsequent months (Breakey et al., 2015).

PEDV can affect the intestinal tract, and based on the above results, we can infer that the SIgA concentration in the colostrum and rectal swab is a better marker than IgA of protection and survival rate after virulent PEDV challenge. Tools to monitor PEDV mucosal immunity and colostrum immunity could be useful for the development of preventative programs on affected farms. Therefore, the aim of this study was to develop a simple and rapid immunochromatographic test strip incorporating a colloidal gold-labeled anti-SIgA SC mAb probe for the detection of anti-PEDV-specific SIgA in swine, and to compare its performance with an indirect SIgA ELISA based on the whole PEDV virus (Cong et al., 2019).

2. Materials and methods

2.1. Viral propagation and purification

Vero E6 cells (American Type Culture Collection no. CRL-1586) were used to propagate PEDV (GenBank accession no. KT323980). Virus was propagated according to the method described by Liu et al. (2017) with minor modifications. Briefly, growth medium was removed (containing 5 % FBS) when the cells formed a confluent monolayer and were then washed with phosphate-buffered saline (PBS, pH 7.2). Then, PEDV containing 1×10^5 plaque forming units (PFU) were diluted in 10 ml of DMEM (DMEM, Gibco BRL Life Technologies, USA) (containing 100 μ g trypsin), and added to each culture bottle and incubated at 37 °C. Virus was harvested and purified according to the methods described by Liu et al. (2017). The purified viruses were negatively stained with 3 % phosphotungstic acid (pH 7.0) for 30 s according to previously described procedures (Chen et al., 2014; Jung et al., 2014). And then identified by H-7650 electron microscopy (Hitachi, Japan).

2.2. Colostrum samples

SPF swine were used to produce colostrum. A total of four specific pathogen free swine (supplied by Experimental Animal Base, Harbin Veterinary Research Institute of the Chinese Academy of Agricultural Sciences) were used in this study. Three swine were immunized with PEDV (LNCT2 strain), transmissible gastroenteritis virus (TGEV) strain H16 (GenBank accession no. FJ755618), and porcine rotavirus (PoRV) A (GenBank accession no. JF781161), respectively. All the viruses were inactivated by 0.5 % methanol solution (PBS, pH 7.2) at 37 °C for 24 h, and then emulsified with Freund's complete adjuvant (Sigma-Aldrich, St. Louis, MO, USA). And they were performed by neck intramuscular injection. The fourth animal was immunized with DMEM as control and each swine was immunized twice. The first immunization was 40 days before delivery whereas the second immunization was 20 days before delivery. After delivery, colostrum from the four swine was collected and store at -20 °C. The protocols involving animal immunity in this study were approved by our institute's Animal Care and Use Committee and performed in accordance with standard ethical guidelines.

2.3. Preparation and purification of the anti-SC mAb

The hybridoma cell line 2F9, stably secreting mAb against SIgA SC protein (Gao et al., 2018) was cultured in the peritoneal cavities with Pristane (Sigma-Aldrich, St. Louis, MO, USA) primed BALB/c mice to obtain ascites fluid. The ascites fluid was purified by HiTrap protein G HP (GE healthcare) according to the manufacturer's instruction and the purified antibody was identified by SDS-PAGE. The gel was stained by coomassie blue.

2.4. Conjugation of mAb with colloidal gold

Colloidal gold with an average particle diameter of approximately 20 nm was purchased from Shanghai Kimbio Tech. Co. Ltd. (China). The colloidal gold-labeled mAb was prepared according to the methods described by Li et al. (2017) with some modifications. Briefly, 0.9 mL (1 mg/mL) of purified mAb was incubated with 100 mL of colloidal gold solution (pH 8.2) for 20 min at 20 °C. After addition of 1 mL of a 3% casein solution, the mixture was incubated at 20 °C for 10 min. Then, the mixture was centrifuged at $40,000 \times g$ for 10 min at 4 °C. The supernatant was discarded and the pellet of the colloidal gold-labeled mAb was suspended in 4 mL of 0.02 M sodium borate buffer (containing 0.1 % NaN₃, 2 % bovine serum albumin (BSA), 1 % sucrose) and stored at 4 °C.

2.5. Preparation of the test strips

The colloidal gold-based lateral flow test strips were generated according to the procedures described by Liang et al. (2015), with some modifications. Briefly, 25 \times 300-mm pieces of glass fiber was immersed in phosphate-buffered saline (0.2 M, pH 8.0, containing 10 mM EDTA, 1 % Tween-20, and 10 % BSA) for 2 h at 20 °C. Next, the pieces were dried for 2 h at 37 °C and stored for use as the sample pad. Glass fiber was also immersed in phosphate-buffered saline (0.1 M, pH 7.4, containing 2.5 % mycose, 1 % BSA, 1 % Tween-20) for 2 h at 20 °C and then dried for 2 h at 37 °C and stored for use as the conjugate pad. The conjugate pad was covered with an appropriate volume of colloidal gold-labeled anti-SIgA SC mAbs (2F9) using a XYZ3060 dispense platform (BioDot, Inc., Irvine, CA, USA), and then dried at 37 °C for 2 h and stored dry. Purified PEDV particles were dispensed onto the nitrocellulose (NC) membrane to serve as the test line, and goat anti-mouse IgG polyclonal antibody (pAb) was dispensed onto the NC membrane, (being separated by a distance of 7 mm) to serve as the control line. The PEDV particles and goat anti-mouse IgG pAb were diluted with coating buffer to a final concentration of 0.02 and 1.0 mg/mL, respectively. Then, the NC membrane was dried at 37 °C for 2 h and stored in the dry at room temperature until use.

2.6. Assembly principle and use of the test strips

The test strips were assembled and used as described by Liang et al. (2015). The sample pad, conjugate pad, NC membrane, absorbent pad and backing plate were assembled in sequence. The sample pad and the conjugate pad were overlapped by 2 mm with one end of the conjugate pad; the other end of the conjugate pad was overlapped (2 mm) with one end of the NC membrane underneath the conjugate pad; the absorbent pad was stuck to the other side of the NC membrane. After this, the whole plate was cut into 3 mm-wide strips, which were assembled in the strip cassettes with desiccant.

To test for colostrum, we diluted the samples 1: 20 in sample buffer and mixed thoroughly then, 100 μ L of the solution was dispensed onto the sample pad well of the strip apparatus to determine the presence of PEDV specific SIgA, as described above. With this system, the liquid migrates towards the absorption pad by capillary action. The SIgA in the colostrum samples will form a "gold-labeled antibody-SIgA" complex with the gold-labeled antibody on the conjugate pad. If the colostrum sample has PEDV specific SIgA present, the PEDV specific "gold-labeled antibody-SIgA" complex, while migrating to the NC membrane, will bind to PEDV, thereby displaying a red line in the T line area. Additional complex migrates further to react with the goat anti-mouse IgG pAb, showing another red line in the C line area. Negative samples do not produce a red line in the T area, whereas the C line will always show a red line. The results can be seen within eight minutes at room temperature.

2.7. Validation of specificity and sensitivity of the immunochromatographic strip test

PEDV SIgA positive colostrum, TGEV SIgA positive colostrum, PoRV SIgA positive colostrum, and PEDV SIgA negative colostrum were used to test the specificity of the strip. The sensitivity of the strip was evaluated by comparing the detection of the same samples with PEDV SIgA ELISA Kit. The detection limit of the strip was evaluated by using PEDV SIgA positive colostrum and colostrum titers determined by using an ELISA Kit (Cong et al., 2019) as the reference standard with minor modifications. In brief, sample buffer was used to dilute colostrum from 1:10 to 1:100, and 100 μ L of the solution was added into microtiter plates (Costar, Corning, NY, USA) incubated at 37 °C for 1 h. After a washing step, a horseradish peroxidase mouse anti-pig SIgA SC antibody 1: 3000 dilution was added and incubated at 37 °C for 45 min. After three times wash with PBST, the peroxidase reaction was visualized using 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (BBI, Shanghai, China) as the substrate for 15 min at 37 °C, and the reaction was stopped by adding 50 μ L of 2.5 M sulfuric acid to each well. Optical densities (ODs) were measured at 405 nm using an ELISA plate reader (BioTek Instruments, Winooski, VT, USA).

For the sensitivity of the ELISA kit, one PEDV specific SIgA positive colostrum was still positive when diluted at 1: 6400. For the specificity, the ELISA kit has no cross-reaction with TGEV as well as PoRV specific SIgA positive colostrum (Cong et al., 2019).

2.8. Colostrum assay performance using field samples

Sixty three colostrum field samples from Hebei, Shandong and Heilongjiang province were assayed by the strip and the PEDV specific SIgA ELISA (Cong et al., 2019). By comparing results obtained by the two methods, Kappa statistic value was used to evaluate the effectiveness of the test strip.

3. Results

3.1. Identification of the purified PEDV by electron microscopy

After removing sucrose, the bands between the 20 % and 40 % and 40 % and 60 % sucrose solutions were identified by electron microscopy. In the 40 % and 60 % sucrose solution band, PEDV particles were observed and their diameter was approximately 130 nm (Fig. 1).

3.2. Identification of the purified antibody by SDS-PAGE

Ascites fluid containing mAb 2F9 was purified by HiTrap protein G HP and the purified antibody was identified by SDS-PAGE. As the results show (Fig. 2), the purified antibody had two bands, a light chain and heavy chain. The purified antibody was stored until further use.

3.3. Specificity and sensitivity of the immunochromatographic strip

The assay specificity was determined using anti-PEDV specific SIgA positive colostrum, anti-TGEV specific SIgA positive colostrum, anti-PoRV specific SIgA positive colostrum, and anti-PEDV specific SIgA negative colostrum. As shown by the strips listed in Fig. 3, the anti-PEDV specific SIgA positive colostrum was positive at the test line; the other colostrum samples were negative, suggesting that the immunochromatographic strip had good specificity. Anti-PEDV specific SIgA positive colostrum was diluted to determine the sensitivity of the strip. According to the results shown in Fig. 4, the strip had a sensitivity of up to 1: 50, while the detection limits of the ELISA kit reached 1:160 dilution (Table 1).

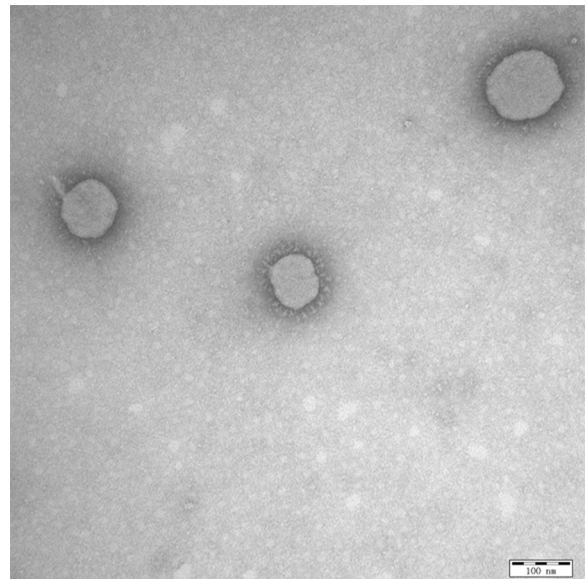


Fig. 1. Identification of purified PEDV by electron microscopy. The band between the 40 % and 60 % sucrose solutions was collected with a syringe, and after removing the sucrose the purified viruses were identified by electron microscopy. Scale bar = 100 nm.

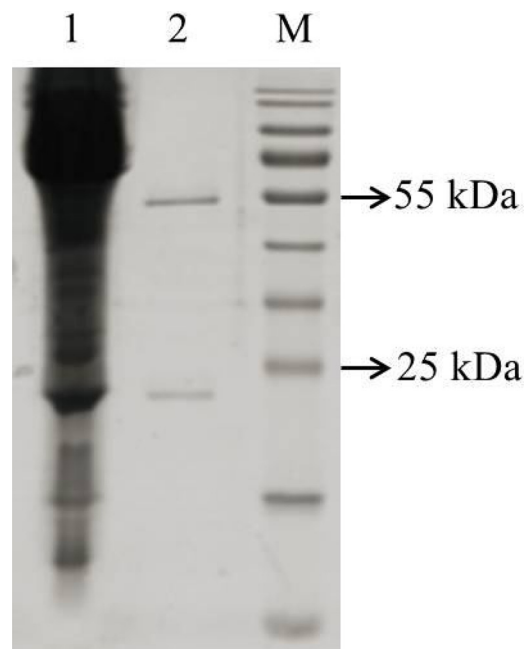


Fig. 2. Identification purified antibody by SDS-PAGE. Lane 1: ascites fluid of mAb 2F9 (2 μ L was added); Lane 2: purified antibody (1 mg/mL, and 2 μ L was added); M: Marker. The gel used was 12 % acrylamide and stained by coomassie blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

3.4. Comparison between the strip and ELISA for detection of colostrum in field samples

All of the 63 colostrum samples were detected by the strip and ELISA mentioned above. As shown in Table 2, 53 colostrum samples were anti-PEDV specific SIgA positive as determined by the strip assay, whereas 55 colostrum samples were anti-PEDV specific SIgA positive as detected by the ELISA. The kappa statistic value for the comparison of the strip versus the ELISA kit, was 0.612. For the interpretation of agreement of the kappa statistic, a value of 0.2 to 0.4 is considered fair,

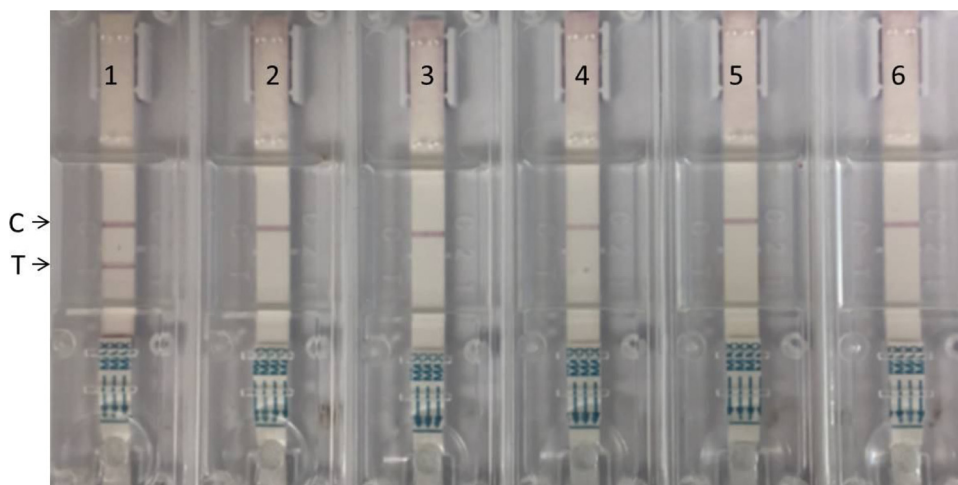


Fig. 3. Specificity of the immunochromatographic strip. Lanes 1 to 6: strip detection of the anti-PEDV specific SIgA positive colostrum, anti-TGEV specific SIgA positive colostrum, anti-PoRV specific SIgA positive colostrum, anti-PEDV specific SIgA negative colostrum, water, milk. The samples were diluted at 1: 20 in sample buffer and mixed thoroughly. Then, 100 μ L of the solution was dispensed onto the sample pad. C stands for control line, T stands for test line.

0.4 to 0.6 is moderate, 0.6 to 0.8 is good, and 0.8 to 0.9 is very good (Altman, 1990). The kappa statistic value was 0.612, suggesting that the strip could be used to detect PEDV specific SIgA in colostrum samples.

4. Discussion

Antiviral antibody detection is key for vaccine immunity surveillance and in determining pig exposure to PEDV. Currently, indirect ELISAs used to assay IgG, IgA, SIgA, have been established to monitor antibodies against PEDV (Fan et al., 2015; Li et al., 2015; Cong et al., 2019). Also, reverse transcription-polymerase chain reaction (RT-PCR) (Ishikawa et al., 1997), duplex RT-PCR (Kim et al., 2001), and other methods have been used to monitor PEDV. Although the presence of antibodies in serum is not directly related to protection of sows or piglets, serological examinations facilitate the assessment of the humoral responses to PEDV, elicited either through vaccination or natural infection. However, the above-mentioned methods are time-consuming and require professional/technical personnel and are mainly limited to laboratory use. The immunochromatographic test strip used to detect anti-PEDV specific SIgA described here is easy to operate, and sensitive.

It is known that PEDV is mainly transmitted by the fecal-oral route and disease is initiated following interaction with the mucosal surface lining the digestive tract. The primary defense used by this tissue is the mucosal immune system. Antibodies from the SIgA class act as the first line of antigen-specific immunity in the gut. They prevent pathogens from binding to mucosal surfaces, and agglutinate bacteria and viruses (van Egmond et al., 2001v). Therefore, SIgA levels are an important index by which to evaluate PEDV infection and immune states. In this study, SIgA, as opposed to IgG, detection was developed to assess PEDV infections and vaccination.

Here a PEDV SIgA-specific immunochromatographic test strip was developed. The purified PEDV-LNCT2 particles, which belongs to the G2 genotype, were used as capture antigen. The shared amino acid identity of the PEDV LNCT2 S protein and the PEDV CV777 (G1 genotype) S protein (GenBank accession number KT323979) is 93.2 %, whereas it is only 46.1 % for the PEDV LNCT2 S protein and the TGEV S protein (Purdue strain, GenBank accession number DQ811789). Reactivity using the polyclonal antibodies (PABs) revealed significant cross-reactivity between the two PEDV subtypes, although there was a two-fold difference in the antigenic responses based on PAB titers in the ELISA and IFA (Wang et al., 2016). Therefore, the ELISA antigen based on the PEDV-LNCT2 particles could detect both G1 and G2 genotype strains. Additionally, the PEDV particles used in the present study had no cross-reactivity with anti-TGEV positive specific colostrum or anti-PoRV positive specific colostrum (Fig. 3). Furthermore, the strip had a sensitivity of up to 1:50, whereas the ELISA kit limits for the same sample was 1:160 (Table 1). Based on these results, the anti-PEDV specific SIgA immunochromatographic test strip could be used to effectively monitor SIgA levels.

Previously, researchers have used ELISAs to study the effect of PEDV on mucosal immunity; an indirect ELISA based on the S1 protein of PEDV was used to detect IgA levels in colostrum and fecal samples to evaluate PEDV colostrum immunity (Gerber et al., 2014 and 2015). IgA plays an important role in providing protection at mucosal surfaces, and it is effective in neutralizing bacterial toxins. Furthermore, polymeric IgA has been shown to be more effective at neutralizing exotoxins from *Clostridium difficile* when compared to either monomeric IgA or IgG with the same variable regions (Stubbe et al., 2000). SIgA consists of the SC, two IgA molecules, and a linking chain. SC protects SIgA from proteolytic degradation (Kaetzel, 2005), making the SIgA more stable. It has been estimated that approximately 3 g of SIgA is transported daily

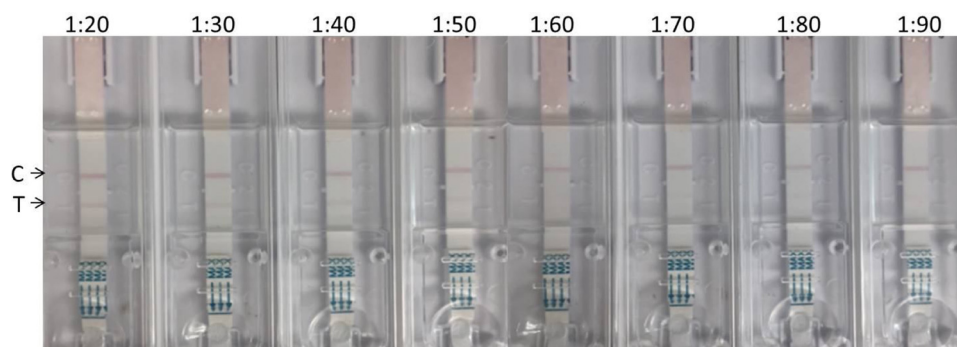


Fig. 4. Sensitivity of the immunochromatographic strip. Lanes 1 to 8: detection of different dilutions of anti-PEDV specific SIgA positive colostrum (1:20, 1:30, 1:40, 1:50, 1:60, 1:70, 1:80, and 1:90). The strip detection limit was 1:50 for anti-PEDV specific SIgA positive colostrum. C stands for control line, T stands for test line.

Table 1
Determination the anti-PEDV specific SIgA antibody titers using ELISA.

	Samples	Dilution						
		1:20	1:40	1:80	1:160	1:320	1:640	1:1280
Mean OD ₄₀₅ Value	anti-PEDV specific SIgA positive colostrum	1.318	1.153	1.093	0.619	0.368	0.267	0.198
	anti-TGEV specific SIgA positive colostrum	0.287	null	null	null	null	null	null
	anti-PoRV specific SIgA positive colostrum	0.265	null	null	null	null	null	null
	anti-PEDV specific SIgA negative colostrum	0.239	null	null	null	null	null	null

Null stands for not done. The cut off value of OD₄₀₅ value was 0.305. The colostrum samples with an OD₄₀₅ value ≥ 0.305 were considered to be positive while < 0.305 were considered to be negative.

Table 2
Comparison of the strip with ELISA for detection of colostrum field samples.

The strips	ELISA		Total	Kappa value
	Positive	Negative		
Positive	51	2	53	0.612
Negative	4	6	10	
Total	55	8	63	

into the intestines of the average adult (Mestecky et al., 1986; Conley and Delacroix, 1987), where SIgA forms the first line of antigen-specific immune protection against ingested, inhaled, or sexually transmitted pathogens and antigens at mucosal surfaces (Kaetzel, 2005), making it more important than IgA. In this study, we investigated the SC portion to detect SIgA in colostrum samples, as this can eliminate the effect of monomeric IgA.

After infection with coronaviruses, such as PEDV or TGEV, or after vaccination, SIgA is generated locally by plasma cells located in the lamina propria, which underlies the epithelium (Kaetzel, 2005). Therefore, the anti-PEDV specific SIgA immunochromatographic test strip established in this study could be used to detect infections and immune states. By determining the SIgA level, we could assess whether pig herds (Unvaccinated) have been infected by PEDV, as well as pigs (vaccinated) could be protected against PEDV, highlighting the versatility of our method.

Therefore, monitoring SIgA titers in colostrum and milk samples could be performed to ensure that piglets receive adequate passive immunity. The anti-PEDV specific SIgA immunochromatographic test strip for the detection of SIgA antibodies developed in our study provides a simple, sensitive and specific tool for monitoring passive immunity and PEDV infection.

Author statement

All authors have carefully revised the manuscript point-by-point according to the reviewers' comments. None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of this paper.

Declaration of Competing Interest

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of this paper.

Acknowledgments

This work was supported by grants from the National key R & D plan for the 13th five year plan (2017YFD0501603), National Natural Science Foundation of China (No. 31502066), the Natural Science Foundation of Heilongjiang (No. QC2016043).

References

Altman, D.G., 1990. Practical Statistics for Medical Research. Chapman and Hall, London, United Kingdom.

Bosch, B.J., van der Zee, R., de Haan, C.A., Rottier, P.J., 2003. The coronavirus spike protein is a class I virus fusion protein: structural and functional characterization of the fusion core complex. *J. Virol.* 77, 8801–8811.

Breakley, A.A., Hinde, K., Vallengia, C.R., Sinofsky, A., Ellison, P.T., 2015. Illness in breastfeeding infants relates to concentration of lactoferrin and secretory Immunoglobulin A in mother's milk. *Evol. Med. Public Health* 1, 21–31.

Brian, D.A., Baric, R.S., 2005. Coronavirus genome structure and replication. *Curr. Top. Microbiol. Immunol.* 287, 1–30.

Chen, Q., Li, G., Stasko, J., Thomas, J.T., Stensland, W.R., Pillatzki, A.E., Gauger, P.C., Schwartz, K.J., Madson, D., Yoon, K.J., Stevenson, G.W., Burrough, E.R., Harmon, K.M., Main, R.G., Zhang, J., 2014. Isolation and characterization of porcine epidemic diarrhea viruses associated with the 2013 disease outbreak among swine in the United States. *J. Clin. Microbiol.* 52 (1), 234–243.

Cong, G., Liu, J., Shi, H., Zhang, X., Chen, J., Feng, L., 2019. Establishment and application of indirect ELISA for detection of porcine epidemic diarrhea virus specific SIgA. *Chin. J. Prev. Vet. Med.* 41, 812–817.

Conley, M.E., Delacroix, D.L., 1987. Intravascular and mucosal immunoglobulin A: two separate but related systems of immune defense? *Ann. Intern. Med.* 106, 892–899.

Fan, J., Zuo, Y., Shen, X., Gu, W.Y., Di, J.M., 2015. Development of an enzyme-linked immunosorbent assay for the monitoring and surveillance of antibodies to porcine epidemic diarrhea virus based on a recombinant membrane protein. *J. Virol. Methods* 225, 90–94.

Gao, Y., Kou, Q., Ge, X., Zhou, L., Guo, X., Yang, H., 2013. Phylogenetic analysis of porcine epidemic diarrhea virus field strains prevailing recently in China. *Arch. Virol.* 158, 711–715.

Gao, R., Liu, J., Chen, J., Shi, H., Zhang, X., Shi, D., Cao, L., Dong, S., Feng, L., 2018. Preparation and identification of monoclonal antibodies against porcine SIgA SC fragment. *Chin. J. Prev. Vet. Med.* 40, 455–458.

Gebauer, F., Posthumus, W.P., Correa, I., Suñé, C., Smerdou, C., Sánchez, C.M., Lenstra, J.A., Meloen, R.H., Enjuanes, L., 1991. Residues involved in the antigenic sites of transmissible gastroenteritis coronavirus S glycoprotein. *Virology* 183, 225–238.

Gerber, P.F., Opriessnig, T., 2015. Detection of immunoglobulin (Ig) A antibodies against porcine epidemic diarrhea virus (PEDV) in fecal and serum samples. *MethodsX* 2, 368–373.

Gerber, P.F., Gong, Q., Huang, Y., Wang, C., Holtkamp, D., Opriessnig, T., 2014. Detection of antibodies against porcine epidemic diarrhea virus in serum and colostrum by indirect ELISA. *Vet. J.* 202, 33–36.

Huang, Y.W., Dickerman, A.W., Pineyro, P., Li, L., Fang, L., Kiehne, R., Meng, X.J., 2013. Origin, evolution, and genotyping of emergent porcine epidemic diarrhea virus strains in the United States. *MBio* 4, e00737–13.

Ishikawa, K., Sekiguchi, H., Ogino, T., Suzuki, S., 1997. Direct and rapid detection of porcine epidemic diarrhea virus by RT-PCR. *J. Virol. Methods* 69, 191–195.

Jung, K., Wang, Q., Scheuer, K.A., Lu, Z., Zhang, Y., Saif, L.J., 2014. Pathology of US porcine epidemic diarrhea virus strain PC21A in gnotobiotic pigs. *Emerg. Infect. Dis.* 20, 662–665.

Kaetzel, C.S., 2005. The polymeric immunoglobulin receptor: bridging innate and adaptive immune responses at mucosal surfaces. *Immunol. Rev.* 206, 83–99.

Kim, S.Y., Song, D.S., Park, B.K., 2001. Differential detection of transmissible gastroenteritis virus and porcine epidemic diarrhea virus by duplex RT-PCR. *J. Vet. Diagn. Invest.* 13, 516–520.

Kocherhans, R., Bridgen, A., Ackermann, M., Tobler, K., 2001. Completion of the porcine epidemic diarrhoea coronavirus (PEDV) genome sequence. *Virus Genes* 23, 137–144.

Li, R., Qiao, S., Yang, Y., Su, Y., Zhao, P., Zhou, E., Zhang, G., 2014. Phylogenetic analysis of porcine epidemic diarrhea virus (PEDV) field strains in central China based on the ORF3 gene and the main neutralization epitopes. *Arch. Virol.* 159, 1057–1065.

Li, Y., Zheng, F., Fan, B., Muhammad, H.M., Zou, Y., Jiang, P., 2015. Development of an indirect ELISA based on a truncated S protein of the porcine epidemic diarrhea virus. *Can. J. Microbiol.* 61, 811–817.

Li, H., Yang, J., Bao, D., Hou, J., Zhi, Y., Yang, Y., Ji, P., Zhou, E., Qiao, S., Zhang, G., 2017. Development of an immunochromatographic strip for detection of antibodies against porcine reproductive and respiratory syndrome virus. *J. Vet. Sci.* 18, 307–316.

Liang, R.L., Xu, X.P., Liu, T.C., Zhou, J.W., Wang, X.G., Ren, Z.Q., Hao, F., Wu, Y.S., 2015. Rapid and sensitive lateral flow immunoassay method for determining alpha fetoprotein in serum using europium (III) chelate microparticles-based lateral flow test

- strips. *Anal. Chim. Acta* 891, 277–283.
- Liu, J., Shi, H., Chen, J., Zhang, X., Ji, Z., Yuan, J., Shi, D., Cao, L., Zhu, X., Dong, H., Wang, X., Zhang, J., Feng, L., 2017. Neutralization of genotype 2 porcine epidemic diarrhea virus strains by a novel monoclonal antibody. *Virology* 507, 257–262.
- Mestecky, J., Russell, M.W., Jackson, S., Brown, T.A., 1986. The human IgA system: a reassessment. *Clin. Immunol. Immunopathol.* 40, 105–114.
- Pensaert, M.B., de Bouck, P., 1978. A new coronavirus-like particles associated with diarrhea in swine. *Arch. Virol.* 58, 243–247.
- Song, D., Park, B., 2012. Porcine epidemic diarrhoea virus: a comprehensive review of molecular epidemiology, diagnosis, and vaccines. *Virus Genes* 44, 167–175.
- Stubbe, H., Berdoz, J., Kraehenbuhl, J.P., Corthesy, B., 2000. Polymeric IgA is superior to monomeric IgA and IgG carrying the same variable domain in preventing *Clostridium difficile* toxin A damaging of T84 monolayers. *J. Immunol.* 164, 1952–1960.
- Tuboly, T., Nagy, E., 2001. Construction and characterization of recombinant porcine adenovirus serotype 5 expressing the transmissible gastroenteritis virus spike gene. *J. Gen. Virol.* 82, 183–190.
- van Egmond, M., Damen, C.A., van Sriel, A.B., Vidarsson, G., van Garderen, E., van de Winkel, J.G., 2001. IgA and the IgA fc receptor. *Trends Immunol.* 22, 205–211.
- Wang, X., Chen, J., Shi, D., Shi, H., Zhang, X., Yuan, J., Jiang, S., Feng, L., 2016. Immunogenicity and antigenic relationships among spike proteins of porcine epidemic diarrhea virus subtypes G1 and G2. *Arch. Virol.* 161, 537–547.