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Indirect ELISA for Detecting Specific IgA antibody to Porcine Epidemic Diarrhea Virus

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Abstract: The object of this study is to establish an indirect ELISA method for detecting IgA antibody against porcine epidemic diarrhea virus (PEDV) and provide a technological means to PEDV infection detection and immune effect evaluation. Antigen coating concentration, blocking and dilution buffer, optimal dilutions of test serum and enzyme-labeled antibody were determined and the indirect ELISA method for detecting PEDV-IgA antibody was developed. Furthermore, the specificity, intra- and inter-batch repeatability tests of the ELISA were examined, the coincidence rate were measured between the ELISA and the existing ELISA kit, and the correlation between the specific IgA and the neutralizing antibodies in serum was analyzed. The results showed that the optimal antigen coating concentration of the ELISA was 1μg/mL, the blocking and dilution buffers were PBS containing 5% calf serum and 0.05% Tween-20, and the working concentrations of tested serum and enzyme-labeled antibody were 1:40 and 1:1500, respectively. The method could be used to detect the PEDV specific antibodies without cross reactions with those antibodies against porcine reproductive and respiratory syndrome virus, classical swine fever virus, pseudorabies virus, and porcine circovirus type 2 using the ELISA.The coefficients of variation (CV) of intra- and inter-batch repetitive tests of the ELISA were less than 10%. The coincidence rate was 94.8% when the same serum samples were detected using the ELISA and existing ELISA kit for detecting PEDV antibody. The level of specific IgA antibody was positively correlated with that of the neutralizing antibody in serum ($r=0.69$, $p<0.001$).

Keywords: Porcine Epidemic Diarrhea Virus; S1 Protein; Indirect ELISA; IgA; Neutralizing Antibody; Correlation

Introduction

Porcine epidemic diarrhea virus (PEDV) can infect pigs of all ages. Epidemic diarrhea (PED)[1,2]. Sick pigs have clinical symptoms such as vomiting, diarrhea, loss of appetite and dehydration. 2010 and Year PEDV and mutant strains are widely prevalent in China, resulting in a large number of newborn piglets' morbidity and mortality as high as 100%[3,4]. At present, PED is the main cause of diarrhea and death in piglets, which seriously threatens the economic benefits of pig farms. PEDV belongs to Coronaviridae (Coronavirus), member of Coronavirus (Coronavirus), genome and RNA. The total length is about 28 kb, including at least 7 open reading frames (ORFs), encoding 4 structural proteins (fibronectin S, capsular protein E, membrane protein M and nucleocapsid protein N) and 3 non-structural proteins (replicases 1a, 1b and ORF3)[5,6]. The S and S proteins are glycoproteins located on the surface of virus particles. They are composed of 1383 aa and can be divided into S1 (1 ~ 789AA) and S2 (790 ~ 1383AA) two domains[7,8]. S protein plays a key role in mediating virus infection, invading cells and inducing the body to produce neutralizing antibodies, as well as strain variation, antigen and virulence changes,[9-12]. Therefore, S protein is the main target protein for vaccine and anti-body diagnostic technology research,[13,14]. PEDV mainly invades small intestine,

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local mucosal immunity and specificity of intestinal tract IgA antibody plays an important role in anti-infection[15-17]. It is known that serum specific antibody level and PEDV IgA antibody level are positively correlated with local mucosal immune level. Immunization of pregnant sows and passive immunization of newborn piglets through breast milk are effective measures to protect piglets from disease. Therefore, the level of specific antibody in maternal antibody, especially colostrum, is directly related to passive immune effect[15,16,18-20]. Although there are reports of detection of antibodies in milk, PEDV IgA, indirect ELISA, and research reports on methods[21] ,the detection of colostrum fails to know the antibody level of immunized sows in advance and the immune status of sows in time, and supplements or other preventive measures are taken for sows with low immune level. In this study, recombinant PEDV S1 protein is used as the detection antigen. By determining the coating concentration of antigen, blocking solution, antibody diluent and working concentration of serum and enzyme-labeled antibody to be detected, the detection methods of PEDV IgA antibody indirect ELISA are established to provide technical support for the detection and effect evaluation of PEDV infection and host anti-infection immune level.

1. Materials and methods

1.1 Main materials

E.coli BL21 competent cells and pET-28a-PEDV-S1 recombinant expression plasmids were prepared and stored in this laboratory. HRP- sheep anti-pig IgA, purchased from Abcam Company; IPTG, TMB substrate chromogenic solution and BCA protein concentration determination kit, purchased from Beijing Solebo Biotechnology Co., Ltd.; Ni-Agarose His tag protein purification kit, purchased from Beijing kang for trial Agent Biotechnology Limited; PEDV and positive and negative antisera Antiserums to viruses such as (PRRSV), classical swine fever virus (CSFV), and pseudorabies virus (PRV) are American Edwardson. Products of the Material Science and Technology Company; Antiserum of porcine circovirus 2 type (PCV2) is a product of Shenzhen lvshiyuan biotechnology co., ltd.; PEDV IgA, Antibody, ELISA and Test Kit are BIONOTE and Company Products.

1.2 Preparation of protein

According to the method of reference and 22, the pET-28a-PEDV-S1 recombinant plasmid is transformed into e coli, bl21 and fine competence cell, pick out positive individual colonies and inoculate them into Kan ,+,/LB culture medium, 37℃ 200 r min ,-1 shake culture to logarithmic growth phase (OD600nm=0.6~0.8), IPTG was added at the final concentration of 05>5 mmol L-1, 230 r min-1 at 28℃ for 5 hours. collect The thallus was purified according to the instructions of Ni-Agarose His tag protein purification kit, and the protein concentration was determined by BCA protein concentration determination kit. after subpackaging, the protein was stored at -80℃ for later use.

1.3 Determination of antigen coating concentration and sealing solution

Using square titration, different concentrations of antigen and different blocking solutions are formed into a square matrix, and the best antigen coating concentration and blocking solution are screened. The main operation steps are as follows: respectively adding S1, protein and 100 μL of the concentrations of 1, 2, 3, 4, 5μ g ml-1 to the corresponding wells of the enzyme standard plate, and repeating the 1 and wells for each concentration; Placing enzyme-labeled plates at 4℃ and coating overnight; Abandoning the blocking solution, adding the positive and negative serum diluted 1:40 times, 100 μL, 37℃ reaction and 45 min with the above different blocking solutions; The enzyme-labeled plate was washed according to the above method, and 100 μL 1:1500 times diluted HRP- sheep anti-pig IgA ,37℃ reaction 45 min were added to each well. Wash the enzyme-labeled plate, add 100 μL TMB to each well, develop color at room temperature in the dark and 15 min; Add 100 μ L 2 m h, 2, SO and 4 to each well to terminate the reaction. The OD, 450 nm and light absorption values" of each well were measured with an microplate reader. When the ratio of positive serum OD 450 nm value to negative serum OD 450 nm rt0P/N) is maximum, the antigen coating concentration and blocking solution are the best antigen coating concentration and blocking solution.

1.4 Determination of working concentration of serum to be detected and enzyme-labeled antibody

Take the enzyme-labeled plate coated with antigen, and follow the operation steps described in square titration and

1.3 respectively, and compare with 1:40, 1:80, and the serum to be tested diluted 1:160, 1:320 and 1:640 times reacts with enzyme-labeled antibody diluted 1:1000, 1:1500 and 1:2000 times. Select the best working concentration of serum to be tested and enzyme-labeled antibody.

2. Results

2.1 PEDV antibody indirect ELSIA method

The best antigen coating concentration for the established ELISA method is 1 μ g ml-1, the blocking solution and antibody diluent are PBST containing 5% calf serum, the serum to be tested and enzyme-labeled antibody are 1:40 and 1:1500 times dilution. The main operating procedures of ELISA are as follows: coating the enzyme-labeled plate with 1 μ g ml, 1 ,S1, protein ,100 μL and coating overnight at 4℃; After washing, add 100 μL and blocking solution, 37℃ blocking, 1 h to each well; Abandon the blocking solution, and add 100 μL1:40 diluted serum to be tested, 37℃ reaction, 45 min to each well; After washing, add 100 μL 1:1500 times diluted HRP- sheep anti-pig IgA ,37℃ reaction 45 min into each well; After washing, add 100 μL TMB color development at room temperature and away from light 15 min to each well. Add 100 μ L 2 m h, 2, SO and 4 to each well to terminate the reaction. The OD, 450 nm and light absorption values of each well were measured with an microplate reader.

2.2 Specificity

The OD450nm value was small when the antisera PRRSV, CSFV, PRV and PCV2 were detected by the established ELISA method. At 0.304, it was negative, indicating that the established ELISA method had no cross reaction with other antiserums (Figure 1).

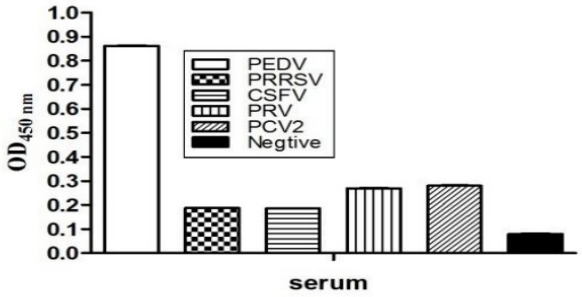

Figure 1. The specific detection of ELISA.

2.3 Repeatability

The enzyme-labeled plates are coated with the same batch or 3 and batch proteins, and the same serum is detected respectively. Its CV is between 2.90%~6.91% and 1.92%~7.44%, which are less than 8%, indicating that the established method has good repeatability.

2.4 Compliance rate

96 clinical serum samples were detected by the established method and foreign kits respectively. A total of 46 positive serum samples were detected by the established method and 45 positive serum samples were detected by the kits. The positive coincidence rate of the two was 95.6%, the negative coincidence rate was 94.1%, and the total coincidence rate was 94.8%.

2.5 Specific IgA correlation between levels and serum neutralizing antibody

By analyzing the correlation between the level of PEDV specific IgA antibody and neutralizing antibody in 94 serum samples, it can be seen that the level of specific IgA antibody in serum is positively correlated with neutralizing antibody ($r=0.69$, $p<0.001$) (Figure 2).

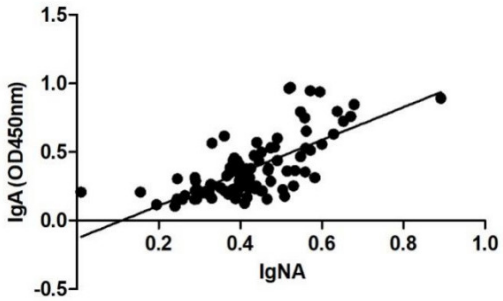

Figure 2. Correlation between the specific IgA levels and the neutralizing antibodies.

3. Discussion

In the winter of 2010 and 2010, pig epidemic diarrhea caused by PEDV and mutant strains broke out in pig farms all over China, causing a large number of newborn piglets to die and causing huge economic losses to China's pig production[3,4] Immunoprophylaxis is the key means to effectively prevent and control PED ,but there is currently a lack of ideal immune monitoring means. It is known that newborn piglets are most susceptible to PEDV and PEDV. Viruses mainly infect intestinal tract through digestive tract. Local immunity and specificity of intestinal mucosa IgA and antibodies play a major role in host anti-infection process[15,16]. The immune characteristics of this infection determine that the passive immunization of maternal antibody via breast milk is to prevent the early occurrence of newborn piglets PED. Effective measures to reduce morbidity and mortality. Therefore, the immune response level of sows, especially the specific antibody level in colostrum, is closely related to the level of primary and secondary antibodies.

Passive immune protection obtained by piglets is closely related, and it is of great practical significance to carry out specific antibody immune monitoring on sows.

Although PEDV S protein and N protein can all be used as coating antigens for the establishment of ELISA antibody detection methods, the S protein ratio N protein has higher sensitivity and specificity, the anti S protein antibody lasts longer in the infected body, and the S protein is located on the surface of PEDV particles. There are virus, receptor binding domain and antigen epitopes including neutralization epitopes, so the antibody detection technology based on S, protein, ELISA has more advantages. It can not only detect the infection of PEDV, but also monitor the immune response level of immunized pigs and evaluate the protective effect of passive immunization for newborn piglets. Experiments have proved that S1 and domain have abundant antigen epitopes, can induce the body to produce neutralizing antibodies, and has repeatability, and is expected to be further developed into a kit.

The serum of pregnant sows is positively correlated with the level of specific antibody in colostrum or milk. Specific IgG antibody absorbed from colostrum of medium-sized piglets is proportional to its serum specific IgG level, which can protect piglets from virus systemic infection, and specific IgA antibody in milk can provide passive protection for piglets' intestinal tract locally[16,29]. Ding Zhenjiang et al.,[21] have established indirect ELISA and ELISA methods for detecting specific antibodies and IgA in milk. However, if we know in advance whether newborn piglets can obtain passive immune protection, we must know the level of serum specific antibodies of pregnant sows before delivery, while specific IgA plays a key role in protecting piglets from intestinal infection. In view of this, this study has established a serum specific IgA antibody ELISA detection method, and found that the specific IgA antibody level detected by this method is significantly positively correlated with serum neutralization activity. Therefore, the established ELISA method can not only be used for PEDV and infection detection and epidemiological investigation, but also meet the practical needs of monitoring the immune status of pregnant sows and predicting piglets to obtain maternal passive immune protection, providing important technical support for the effective prevention and treatment of PEDV.

4. Conclusion

In this paper, by optimizing the concentration of antigen coating, blocking solution, diluent and the optimal working concentration of serum to be tested and enzyme-labeled antibody, An indirect ELISA method for detecting PEDV IgA antibody with strong specificity and good repeatability was established. The serum specific IgA antibody level was positively correlated with neutralizing antibody ($r=0.69$, $p<0.001$). This method can not only be used for

PEDV and infection detection and epidemiological investigation, but also can monitor the immune status of pregnant sows and predict the effect of piglets on maternal passive immune protection.

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