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RESEARCH ARTICLE

Antibody against Porcine Parvovirus VP2 Protein Differentiated This Virus from Other Viruses

Weijuan Zhu[§], Yudong Ren[§1], Guangxing Li, Dingding Su[2], Qing Yang[3] and Xiaofeng Ren*

College of Veterinary Medicine; [1]College of Engineering, Northeast Agricultural University, 59 Mucai Street, Xiangfang District, Harbin 150030, China; [2]Hunan Provincial Key Laboratory for Germplasm Innovation and Utilization of Group; [3]College of Veterinary Medicine, Hunan Agricultural University, 1 Nongda Road, Furong Disctrict, Changsha 410128, China
* Corresponding author: E-mails: rxfemail@yahoo.com.cn

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ABSTRACT

Viral protein 2 (VP2) of porcine parvovirus (PPV) is the major viral structural protein and responsible for eliciting neutralizing antibodies in immunized animals. In this study, the gene encoding VP2 of PPV was amplified by PCR. The VP2 gene was then cloned into the prokaryotic expression vector, pET-32a followed by expression in *Escherichia coli* Rosetta. The VP2 protein expression was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Rabbit polyclonal antiserum was generated using the recombinant VP2 protein. The optimal titer of the anti-VP2 antibody was determined by ELISA. The anti-VP2 antibody was able to distinguish PPV from other viruses in ELISA.

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INTRODUCTION

Porcine parvovirus (PPV) is a major cause of the syndrome of reproductive failure in sows. PPV can cross the placental barrier, infecting embryos and leading to fetal death, stillbirths and so on, although infection by PPV does not result in significant clinical symptoms (Rueda *et al*., 2000). In addition, PPV may also cause dermatitis and diarrhea (Kresse *et al*., 1985; Allan *et al*., 1999; Krakowka *et al*., 2000). Widespread of PPV is rather detrimental to pig industry, leading to substantial economic losses (Parke and Burgess, 1993; Gardner *et al*., 1996).

Structurally, PPV is a non-enveloped, single-stranded, negative-sense small DNA virus. Three viral proteins named VP1, VP2, and VP3 can form viral capsids. VP2, the major structural protein of PPV, is responsible for eliciting neutralizing antibodies in immunized animals (Martinez *et al*., 1992; Kamstrup *et al*., 1998). It has been documented that baculovirus expressed PPV VP2 may assembled into virus-like particles (VLPs) with similar morphology to the native capsid and identical hemagglutination activity compared with active PPV (Martinez *et al*., 1992).

In this report, high-level expression of VP2 protein of PPV was achieved in a bacterial expression system. Rabbit polyclonal anti-serum was generated by immunizing the VP2 protein into a rabbit. We optimized the anti-VP2 antibody-based ELISA and established a differentiating ELISA for PPV.

MATERIALS AND METHODS

Amplification of PPV VP2 gene by PCR: The plasmid VP2-T bearing full-length VP2 gene was used as a template to amplify the PPV VP2 fragment for cloning by PCR using the following specific primers, Forward: 5'-GGATCCATGAGTGAAAATGTGGAA-3', Reverse: 5'-CCCCGTCGACTTAGTATAATTT TCTTGG -3'. The amplification was performed at 94^{o}C for 5 min; 30 cycles of 94 ^{o}C for 40 s, 54^{o}C for 30 s, and 72 ^{o}C for 2 min; 72^{o}C for 15 min for a final extension. PCR product was identified by DNA sequencing.

Construction of recombinant plasmid and protein expression: The PCR fregment of VP2 was cleaved with BamH1 and Sal Ⅰ restriction endonuclease (TaKaRa, Dalian, China) and inserted into the corresponding sites of pET-32a, giving rise to pET-VP2. The recombinant plasmid was transformed into *Escherichia coli* (*E. coli*) Rosetta cells and positive transformants were double checked by PCR using specific primers for PPV VP2 gene and restriction

[§]These authors equally contributed to this study.

analysis. Empty vector transformed *E. coli* as the control. The transformed *E. coli* was cultured in Luria-Bertani (LB) medium supplemented with ampicillin (60 µg/mL) at 37°C. When OD_{600} reached 0.6~0.8, isopropyl beta-D-thiogalactoside (IPTG, final concentration 0.6 mM) was added to the medium to induce protein expression for 0, 1, 2, 3, 4 and 5 h. Cell lysates were subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE), gel purification and renaturation performed as our previously reported protocols (Liu *et al*., 2009).

Generation of polyclonal antibody to PPV VP2: To generate the polyclonal antiserum to PPV VP2, A New Zealand rabbit was used. Rabbit was injected subcutaneously with 2 mL of PPV VP2 protein (1 mg/mL) emulsified with equal amount of Freund's complete adjuvant. A booster was given at day 10 with the same amount of antigen mixed with equal volume of incomplete adjuvant followed by a second booster at day 17. Control animal was inoculated with 2 ml PBS and served as negative control. Blood was collected at day 20 and serum was store at -20°C until use.

ELISA analysis: ELISA was performed to determine the titration of the antiserum and the reaction of the antiserum with VP2 protein. ELISA plates were coated with 5 µg/well purified VP2 at 4°C overnight. After three washes with PBS-0.1% Tween20 (PBST), wells were incubated with blocking buffer containing 5% non-fat milk in PBST at 4°C overnight. Serially diluted polyclonal antibody was added into the wells and incubated at 37°C for 1 h followed by three PBST washes. Bound antibodies were detected using HRP-conjugated goat anti-rabbit IgG (1:5,000, Zhongshan, China) at 37°C for 1 h. Wells were washed three times with PBST, followed by color development using *o*-Phenylenediamine dihydrochloride (OPD, 100 µL/well, Sigma) and stopping reaction with 2 M H_2SO_4. Absorbance was measured at 490 nm. To test the reactivity of the anti-VP2 antibody to PPV, the purified PPV isolate PPV2010 (0.8 µg/mL) were coated onto ELISA plates, and the antiserum and control serum were diluted to the VP2 serially from $1:2^6$ to $1:2^{17}$. Other procedures were performed as above.

Immunoreactivity of polyclonal antibody to PPV VP2 with other viruses: Immunoreactions of the antiserum with other viruses were evaluated using ELISA. The selected viruses included PPV isolate PPV2010, Porcine pseudorabies virus (PrV) strain Kaplan, Porcine circovirus type 2 (PCV2) strain PCV2-LJR, Porcine reproductive and respiratory syndrome virus (PRRSV) strain JilinTN1 PRRSV, Porcine epidemic diarrhea virus (PEDV) strain HLJBY, Porcine transmissible gastroenteritis virus (TGEV) strain HR/DN1, and Porcine rotavirus (PRV) strain DN30209. Cell lysates containing 10 µg of each pathogen were coated onto the ELISA plate at 4°C overnight, blocked with nonfat milk for 3 h, followed by incubation with anti-VP2 rabbit antibody (1:1,000). Other steps were performed as above. DMEM or lysis buffer was used as negative control. Ratios generated from the OD_{490} value of tested samples (P)/the OD_{490} value of negative control, coating buffer (N) above 2 were judged as positive. The experiment was performed in triplicate.

RESULTS

Amplification of PPV VP2 gene and construction of recombinant plasmid pET-VP2: The purified PCR product was confirmed by DNA sequencing which showed that product was coincided with the expected VP2 fragment with a size of 1740 bp (data not shown). The recombinant plasmid pET-VP2 was identified by BamH I and Sal I enzyme digestion. Two fragments were generated with sizes of apprxo. 1800 bp and 5900 bp (Fig. 1). The authenticity of the insert was confirmed by DNA sequencing.

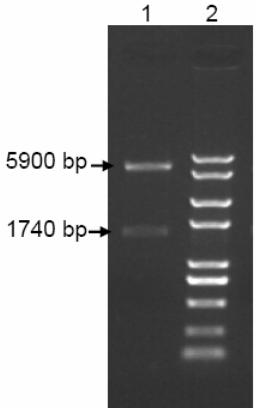

Fig. 1: Identification of recombinant plasmid pET-VP2. PPV VP2-containing pET-VP2 was digested with BamH I and Sal I, generating a fragment VP2 (1740 bp) and a vector fragment (5900 bp). The agarose gel picture is provided; lane 1, digestion of pET-VP2 by BamH I and Sal I; lane 2, DNA marker.

Expression of polyhistidine-tagged PPV VP2: In the present study, *E. coli* system was used to express the His-tagged VP2 protein. Result from the SDS-PAGE showed that the fusion protein was ~ 82 kDa as expected which could be induced by IPTG with a time-dependent manner (Fig. 2). Protein accumulated as inclusion bodies in ultrasonicated bacteria. No target protein band was observed in the *E. coli* transformed with empty vector.

Generation of anti-VP2 antibody: A rabbit anti-VP2 antibody was generated by conventional animal immunization and its titer was determined using an indirect ELISA. As shown in Figure 3, the antibody showed a high valence, the optimal antibody dilution was$1:2^{13}$ with a fixed concentration of PPV VP2 protein.

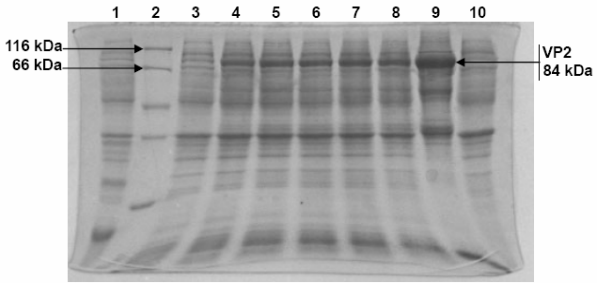

Fig. 2: Expression of VP2 protein in *E.coli.* Expression of VP2 protein was detected by a Coomassie blue staining SDS-PAGE. Lane 1, crude bacterial lysate from cells containing non-recombinant vector; lane 2, protein marker; lane 3, non-induced crude pET-VP2-containing bacterial lysate; lanes 4-8, unlysed VP2-containing bacteria at 1-5 h post-induction (hpi); lane 9, lysed VP2-containing bacterial inclusion bodies at 5 hpi; lane 10, lysed VP2-containing bacterial supernatant at 5 hpi; The size of protein marker and the VP2 protein is indicated.

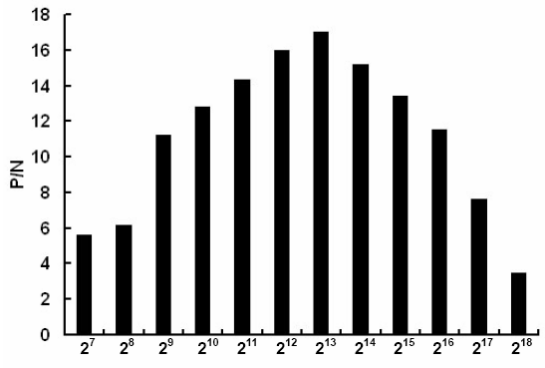

Fig. 3: Titration of anti-VP2 antibody in ELISA . Purified VP2 protein (5 μg/well) was diluted and coated in ELISA wells, and serially diluted anti-VP2 antibody was used as primary antibody. Dilutions of the anti-VP2 antibody are indicated. The OD_{490} value of tested samples (P)/the OD_{490} value of negative control, coating buffer (N)>2 is judged as positive. The P/N values were the mean values from three independent assays.

ELISA for detection of PRV based on anti-VP2 antibody: The anti-VP2 antibody was used as primary antibody in an ELISA to differentiate PPV from other viruses. The results indicated that the antibody showed a significant reactivity with PPV and no positive P/N value was obtained with other viruses and controls (Fig. 4).

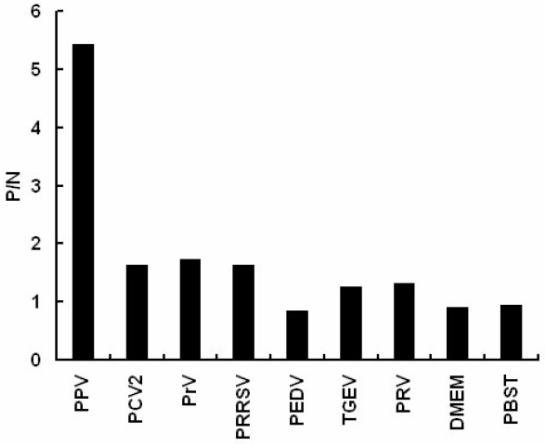

Fig. 4: Detection of PPV by VP2 antibody-based ELISA. As stated in Materials and Methods, PPV, PCV2, PrV, PRRSV, PEDV, TGEV, PRV, DMEM and PBST were used as coating antigen in ELISA. These ELISA plates were incubated with the anti-VP2 antibody followed by incubation of HRP-conjugated secondary antibody. OD_{490} value of the tested samples (P)/the OD_{490} value of negative control, coating buffer (N)>2 is judged as positive. The P/N values were the mean values from three independent assays.

DISCUSSION

As infection of PPV has important impact on pig husbandry, surveillance of this disease may be a supplementary approach in addition to vaccination. Because VP2 is the main structural protein of PPV particles, constituting most of viral capsids (Pan *et al*., 2008), the aim of the current study was to express VP2 protein of PPV, generate anti-VP2 antibody and establish an antibody-based ELISA for detection of PPV.

Compared with other available foreign protein expression systems, *E. coli* system has several advantages such as low costs, high production, and manipulation convenience, etc (Yin *et al*., 2007). Previously, we have expressed several heterologous proteins in this system (Ren *et al*., 2010 a and b; Ren *et al*., 2011 a and b; Zhu and Ren, 2012). Previously, Qi and Cui had tried to express PPV VP2 in *E. coli*, and they proved that only if the VP2 gene was cloned into pET-32a vector, the protein of interest could be expressed in *E.coli* (Qi and Cui, 2009). Therefore, we used the same vector to express the VP2 protein; high-level bacterial expression of PPV VP2 further confirmed that expression of the VP2 gene may require codon-optimized *E. coli* cells. High-level of rabbit anti-VP2 antibody was also achieved using the purified VP2 as immunogen.

It was reported that PPV was able to increase effects of porcine circovirus type 2 (PCV2) infection in the clinical course of postweaning multisystemic wasting syndrome (Allan *et al*., 1999; Krakowka *et al*., 2000). In addition, co-infection of PPV with other porcine viruses is also possible. Therefore, discrimination between this virus and other viruses is important for clinical diagnosis. We and others have used ELISA for detection of pathogens due to its simplicity, convenience and sensitivity (Ren *et al*., 2011b; Li *et al*., 2010; Sande *et al*., 2011), therefore, we used the anti-VP2 antibody to establish a discrimination ELISA. Our results indicated that the anti-VP2 antibody distinguished PPV from other control viruses in the antigen-capture ELISA. Further work has to focus on testing the sensitivity of the discrimination ELISA using more PPV clinical isolates.

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