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

Weijuan Zhu, Yudong Ren, Guangxing Li, Dingding Su, Qing Yang and Xiaofeng Ren

College of Veterinary Medicine; 1College of Engineering, Northeast Agricultural University, 59 Mucai Street, Xiangfang District, Harbin 150030, China; 2Hunan Provincial Key Laboratory for Germplasm Innovation and Utilization of Group; 3College of Veterinary Medicine, Hunan Agricultural University, 1 Nongda Road, Furong District, 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.

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'-CCCCGGTACGTTAGTATAATTT TCTTGG -3'. The amplification was performed at 94°C for 5 min; 30 cycles of 94°C for 40 s, 54°C for 30 s, and 72°C for 2 min; 72°C for 15 min for a final extension. PCR product was identified by DNA sequencing.

Construction of recombinant plasmid and protein expression: The PCR fragment 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 OD₆₀₀ 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 H₂SO₄. 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 1:2⁶ to 1:2¹⁷. 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 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 OD₆₅₀ value of tested samples (P)/the OD₆₅₀ 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 BamHI I and Sal I enzyme digestion. Two fragments were generated with sizes of approx. 1800 bp and 5900 bp (Fig. 1). The authenticity of the insert was confirmed by DNA sequencing.

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 1:2¹³ with a fixed concentration of PPV VP2 protein.
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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REFERENCES


