

DOI 10.24425/pjvs.2023.148280

Original article

Expression and self-assembly of virus-like particles from porcine parvovirus and its application in antibody detection

Y. Li^{1*}, Q. Wang^{2*}, W. Yue¹, X. Li¹, Y. Chen¹, Y. Gao¹

¹ Beijing Biomedicine Technology Center of JoFunHwa Biotechnology (Nanjing Co. Ltd.);
No.25 Xiangrui Street Daxing District, Beijing 102600 China

² State Key Laboratory for Animal Disease Control and Prevention,
Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin 150001, China

Abstract

Porcine parvovirus (PPV) is a major causative agent in reproductive pig disease. The swine industry faces a significant economic and epizootic threat; thus, finding a reliable, quick, and practical way to detect it is essential. In this investigation, recombinant PPV VP2 protein was expressed in the *Escherichia coli* (*E. coli*) expression systems. As shown by electron microscopy (TEM), Western blot, and hemagglutination (HA) assays, the recombinant VP2 protein was successfully assembled into virus-like particles (VLPs) after being expressed and purified. These VLPs had a structure that was similar to that of real PPV viruses and also exhibited HA activity. These VLPs induced high levels of PPV-specific antibody titers in mice after immunization, indicating that the VLPs may be beneficial as potential candidate antigens. VLPs were used as the coating antigens for the VLP ELISA, and the PPV VLPs-based ELISA displayed a high sensitivity (99%), specificity (93.0%) and agreement rate (98.3%) compared to HI assay, and the agreement rate of this ELISA was 97.5% compared to a commercial ELISA kit. Within a plate, the coefficient of variation (CV) was 10%, and between ELISA plates, the CV was 15%. According to a cross-reactivity assay, the technique was PPV-specific in contrast to other viral illness sera. The PPV VLP indirect-ELISA test for PPV detection in pigs with an inactivated vaccine showed that the PPV-positive rate varied among different sample sources from 88.2 to 89.6%. Our results indicate that this ELISA technique was quick, accurate, and repeatable and may be used for extensive serological research on PPV antibodies in pigs.

Keywords: porcine parvovirus, virus-like particles, indirect ELISA

Introduction

Porcine parvovirus (PPV) is one of the major pathogens of reproductive failure in pregnant sows, which is characterized by stillbirth, mummification, embryonic death, and infertility (Oravainen et al. 2005). The virus has been endemic in the majority of the world's regions and can be found in all types of pig herds, including boars and fattening pigs, thus the swine industry has suffered enormous financial losses (Mengeling et al. 2000, Zeeuw et al. 2007). Therefore, it is necessary to create a fast and reliable PPV diagnosis.

To date, the haemagglutination inhibition (HI) assay and enzyme-linked immunosorbent assay (ELISA) are the two leading serological diagnosis procedures for PPV (Jenkins 1992, Oravainen et al. 2006). HI is a commonly used standard approach for quantifying PPV-specific antibodies, but it involves multiple time-consuming procedures and can be impacted by incubation temperature and erythrocyte source (Mengeling, Cutlip 1976). Due to its easier standardization and suitability for clinical field testing, ELISA is typically recommended over other techniques for the diagnosis of PPV (Hohdatsu et al. 1988, Westenbrink et al. 1989). Recombinant proteins as diagnostic antigens would avoid those limitations since these approaches typically need the synthesis of the entire virus as a diagnostic antigen, posing biosafety concerns. Recombinant VP2, for instance, was created in an insect-baculovirus system and was used as a diagnostic antigen in indirect-ELISA (Kong et al. 2014). However, the preparation of recombinant proteins by using a baculoviral system is complicated and costly compared with a prokaryote expression system.

The single-stranded DNA genome of PPV is 4-6.3 kb in size and has two open reading frames (ORFs) that code for the structural proteins VP1, VP2, and VP3 and a non-structural protein (NS1) (Allander et al. 2005). Among these, VP2 is immunogenic and has significant antigenic regions for receptor binding. Because of this, it has been utilized as a form of alternative antigen for PPV diagnosis and to produce subunit vaccinations (Xu, Li 2007, Kong et al. 2014). Particularly noteworthy is that the recombinant PPV VP2 protein can assemble into VLPs on its own (Ji et al. 2017). VLPs, such as PCV2 VLPs, are well known as a highly effective alternative antigen for serological assays and are frequently employed for the generation of viral antigens (Marceková et al. 2009). The above studies suggest that recombinant VLPs are an improved option over separately produced recombinant proteins. However, there has yet been no information about using PPV-VLP as an antigen for PPV-specific antibody detection.

In the present study, PPV VLPs were created by employing a prokaryote expression system, allowing for large yields and cost-effective manufacture. To replace the PPV antigens currently used in ELISA assays, we also constructed an indirect ELISA with PPV VLPs as a coated antigen and assessed its validity, sensitivity, and specificity.

Materials and Methods

Serum Samples

Fifty negative serum samples were collected from specific-pathogen-free (SPF) piglets which were obtained from the Harbin Veterinary Research Institute of the Chinese Academy of Agricultural Sciences (Harbin, China). Samples were stored at -70°C before testing. One hundred and twenty clinical serum samples stored at Beijing Biomedicine Technology Center of JoFunHwa Biotechnology (Nanjing Co. Ltd.) were tested by HI for PPV indirect ELISA development.

In the retrospective serologic study, a total of 173 field pig serum samples were collected from farms from Heilongjiang Province (68 samples), Hunan Province (58 samples), and Henan Province (47 samples) in China, on which the animals had been vaccinated with an inactivated PPV vaccine (QiLu Animal Health Production Co Ltd., Jinan, China). The serum samples were tested using the PPV indirect ELISA established in this study.

Preparation of mouse serum

Each of three female BALB/c mice (aged 6-8 weeks) was intraperitoneally injected with PPV VLPs (50 µg) and an equal volume of Freund's complete adjuvant to induce antibody production against the antigen. Two booster immunizations with the same dose of VLPs plus Freund's incomplete adjuvant were intraperitoneally administered at 2-week intervals. Two weeks after the final booster injection, the blood of the mouse was collected, and the sera were prepared and stored at -80°C before analysis.

This study followed the guidelines in the Guide for the Care and Use of Laboratory Animals of Beijing Kemufeng Biopharmaceutical Co., Ltd (approval number DW202003-011).

Construction and expression of recombinant VP2 protein in *E. coli*

The gene encoding PPV VP2 protein with a His-tag in the N-terminal was optimized according to the codon preference of *E. coli* and subcloned into the pET28a

vector. The recombinant plasmids were confirmed and transformed into BL21(DE3) cells (Takara, China). Moreover, the expression of PPV VP2 protein was induced in the control of 0.01 mM isopropyl- β -D-thiogalactopyranoside (IPTG) at 16°C for 20 h under conditions of 220 rpm shaking speed. The bacteria were then harvested by centrifugation at 5000 \times g for 10 min at 4°C. The cell pellet was resuspended in Tris-HCl buffer (pH 8.0, 50 mM Tris-HCl, 150 mM NaCl) and sonicated on ice until the supernatant became clear using a Cell Ultrasonic Crusher (Cole Parmer, USA). Lysates were divided into supernatant and pellet by centrifugation at 8000 \times g for 20 min at 4°C. Protein expression was analyzed using 10% SDS-PAGE and, subsequently, Coomassie Brilliant Blue staining.

Purification of PPV VLPs

The supernatant mentioned above was purified in an automated FPLC system (AKTA, GE-Healthcare Life Sciences, USA) using High-Affinity Ni-NTA Resin. The protein concentration was determined with a Bicinchoninic Acid Assay Kit (Beyotime, Shanghai, China). Finally, fractions containing pure target protein were analyzed using SDS-PAGE. The purified protein was stored at -80°C for next usage.

Western blot

The recombinant PPV VP2 protein was electrophoresed onto 12% SDS/PAGE and transferred to polyvinylidene difluoride membranes (EMD Millipore, Billerica, MA, USA). The membrane was blocked with 5% skim milk in PBST at 4°C overnight. After washing with PBST [phosphate buffer solution (PBS) containing 0.1% Tween-20] three times, it was then incubated with specific pig-positive serum (1/1000 dilution in PBS) at 4°C overnight. Then, after washing three times with PBST, it was incubated with HRP-conjugated goat anti-pig IgG (1/2000 dilution in PBS) for 1 h at room temperature. Protein expression was detected using an enhanced chemiluminescence system (Bio-Rad Clarity Western ECL; Bio-Rad Laboratories Inc.).

Electron microscopy

The purified PPV VLPs were coated onto formvar carbon film of 400-mesh formvar copper grids for 1 min at room temperature, gently air-dried, and negatively stained with 2% phosphotungstic acid, pH 7.0, and examined using a transmission electron microscope (H7650, HITACHI, Japan).

Haemagglutination (HA) assay and Hemagglutination inhibition (HI) test for the detection of PPV VLPs

The HA test was carried out using 0.6% (v/v) guinea pig erythrocytes as previously described (Feng et al. 2014). The antigen content was expressed as log₂ HA unit per 50 μ L. The detection limit was 1 log₂. Samples showing <1 log₂ were considered negative. HI test assay for PPV was performed as described previously (Zheng et al. 2020). 50% of RBCs showing agglutination inhibition was used as the endpoint, and HI titer was defined as the reciprocal of the highest dilution inhibiting HA. PPV NADL-2 strain was obtained from the China veterinary culture collection center (Beijing, China). The guinea pigs were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd (Beijing, China).

Optimization of indirect ELISA working conditions

An indirect ELISA was established using PPV VLPs for serological surveillance. The optimal dilutions of antigen and sera were determined through standard checkerboard titration procedures (Crowther 2000). Briefly, purified PPV VLPs (ranging from 0.5, 1.0, 1.5, 2.0, 2.5 to 5 μ g/ml) were used to coat 96-well ELISA plates. Correspondingly, sera dilution in PBS ranged from 1:100, 1:200, 1:400 to 1:800 (v/v). The optimal dilutions were determined based on the OD₄₅₀ values between PPV-positive and PPV-negative serum (P/N). The conditions that gave the highest OD₄₅₀ ratio between positive and negative serum (P/N value) and the OD₄₅₀ value of positive serum close to 1.0 was scored as optimal working conditions (Shang et al. 2008). In addition, the reaction temperature, time, and other conditions were also optimized.

Establishment of ELISA

VLPs (200 ng) prepared previously was coated onto 96-well plates with 100 μ L per well at 4°C overnight. After washing with PBST three times, the plates were blocked with 5% bovine serum albumin (BSA) at 37°C for 1.5 h. The plates were then washed with PBST three times, and 100 μ L of diluted serum samples were added and incubated at 37°C for 1 h. After washing with PBST three times, the plates were then incubated with 100 μ L diluted HRP-conjugated goat anti-pig IgG (1:30000; Solarbio, Beijing, China) at 37°C for 1 h. The HRP-catalyzed color reaction was performed by using a TMB solution (Tiangen, Beijing, China). The reaction was stopped with 2 M sulfuric acids, and the absorbance was measured at 450 nm using a Model 550 microplate reader (Bio-Rad, Hercules, CA, USA).

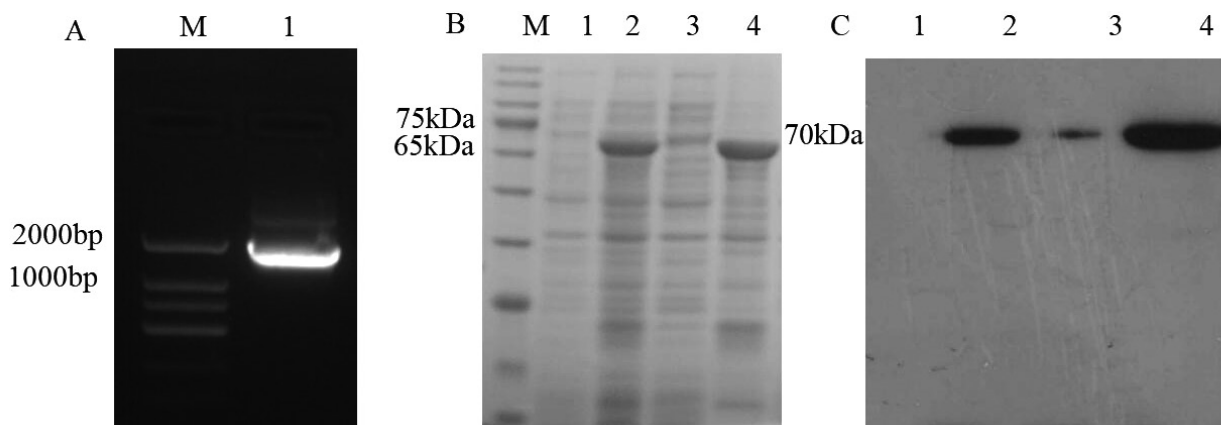


Fig 1. SDS-PAGE and Western blot analysis of VP2 protein expressed in *E. coli*. A. PCR verification of N-terminal his-tagged VP2 clones, B. SDS-PAGE of protein preparation and staining with Coomassie blue (Protein molecular marker (M), non-IPTG-induced lysated recombinant *E. coli* (lane 1); Lane 2: IPTG-induced recombinant *E. coli* (lane 2), (lane 3) pellet and supernatant (lane 4) of lysated recombinant *E. coli*. C. Expression of porcine parvovirus (PPV) virus-like particles (VLPs) was verified by Western blot with swine PPV-specific positive serum (Western blot analysis; lane 1-4, samples corresponding to lane 1-4).

Determination of cut-off value

Fifty negative serum samples were used to determine the cutoff value. These serum samples were collected from SPF piglets. The PPV antibody status of the sera was analyzed using an HI assay. All sera were subjected to PPV VLP-ELISA three times. The OD450 of the samples were converted to S/P value using the following formulation: $S/P = (\text{OD450 value of sample} - \text{OD450 value of negative value}) / (\text{OD450 value of positive value} - \text{OD450 value of negative value})$. The cut-off ratio was determined based on the mean S/P ratio plus three standard deviations (SD).

Sensitivity and Specificity of ELISA test

To determine the sensitivity of this ELISA method, 100 μL of each mixture was analyzed by indirect-ELISA using a serial of two-fold dilutions of the PPV-positive sera ranging from 1:200 to 1:25600. In addition, to evaluate the specificity of the indirect ELISA, the positive sera of PPV, porcine reproductive and respiratory syndrome virus (PRRSV), porcine epidemic diarrhea virus (PEDV), transmissible gastroenteritis coronavirus (TEGV), *pseudorabies* virus (PRV), and classical swine fever virus (CSFV) were used to assess its specificity within the same ELISA method with triplicate experiments, and the S/P value was used to characterize whether the samples were positive or negative.

Repeatability of the indirect ELISA

Five positive serum samples of PPV were used for the repeatability experiment. For intra-assay repeatability, three replicates of each serum sample were assigned to the same plate. For inter-assay, three

replicates of each sample were run in different plates. The mean P/N ratio, SD, and coefficient of variation (CV) were calculated. The dilution of serum was measured by indirect ELISA.

Statistical analysis

All statistical data were analyzed using GraphPad Prism v 5.03 (GraphPad Software Inc., San Diego, CA). Cohen's kappa coefficient value was calculated according to the guidelines of Landis and Koch. Data are expressed as the mean \pm SD. The coefficient of variation (CV) was used to evaluate the intra- and inter-assay variation. $p < 0.05$ was considered statistically significant.

Results

Preparation of PPV VLPs from *E. coli*

To express the VP2 protein of PPV in *E. coli*, the 1710 bp VP2 gene was partially optimized based on factors such as codon bias and GC content, and then constructed into a prokaryotic expression vector (Fig. 1A) and transformed into BL21 (DE3) cells for recombinant protein expression by induction with IPTG. With an estimated molecular mass of about 70 kDa, SDS-PAGE examination revealed that the protein was primarily expressed in the supernatant of *E. coli* (Fig. 1B). Furthermore, the VP2 reacted with PPV positive-serum, according to Western blot results (Fig. 1C). Collectively, PPV VP2 was successfully produced, had good antigenicity, and could be utilized to make ELISA antigens.

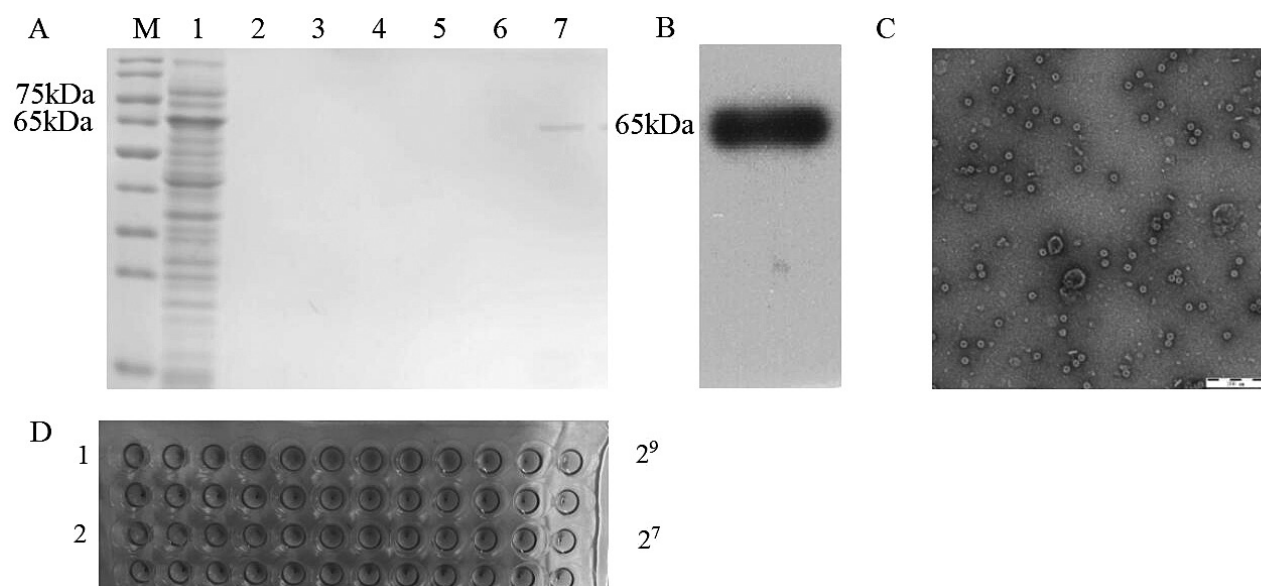


Fig. 2. Analysis and purification of Transmission electron microscope (TEM) analysis. A. Soluble, his-tagged VP2 was purified via chromatography using Ni-NTA agarose beads and the different fractions were analyzed via SDS-PAGE with Coomassie blue. B. Expression of PPV VLPs was verified by western blot with swine PPV-specific positive serum. C. TEM images of purified PPV-VP2. The bar indicates 200 nm. D. Hemagglutination assay of the PPV-VP2 protein. 1, purified PPV-VP2 protein; 2, positive control of PPV NADL-2.

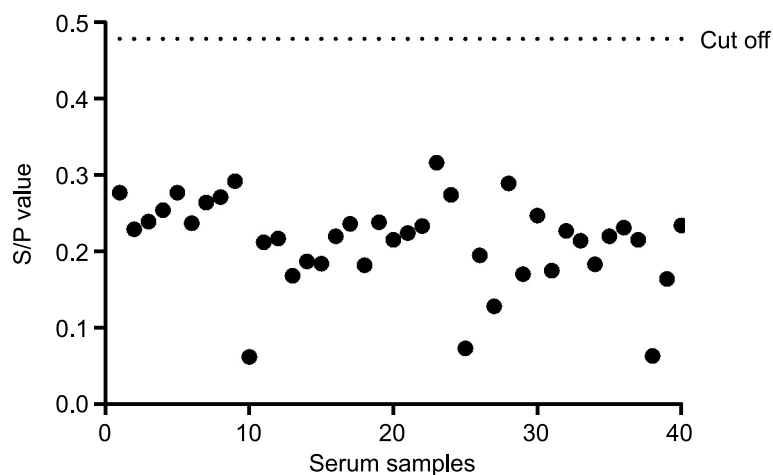


Fig. 3. Evaluation of the cut-off value of the indirect ELISA. The black dotted line presents the S/P cut-off value (0.478).

Formation of PPV VLPs

SDS-PAGE analysis of purified PPV VLPs from the supernatant of *E. coli* revealed a large protein band at approximately 70 kDa, which is consistent with the theoretical mass of PPV VP2 (Fig. 2A). Western blot was also used to corroborate the PPV VLPs' positivity (Fig. 2B). The pure VP2 protein generated as described above was studied under a Transmission electron microscope (TEM) to see if the expressed structural proteins self-assemble into VLPs. With external widths of around 25 nm, we discovered many regularly shaped particles that were comparable to PPV's natural virions (Fig. 2C). In addition, the purified VP2 protein showed high HA titers reaching 2^9 , similar to the natural PPV (Fig. 2D). To sum up, we successfully obtained PPV VLPs by using the prokaryotic expression system.

Antigenic specificity of PPV VLPs

We used the PPV VLPs as the antigen to coat microplates in order to investigate the antigenic specificity, and then looked at the reactivity to PPV-specific antibodies. Sera samples from three mice immunized with PPV VLPs were submitted to ELISA at twice-serial dilutions. Three mouse serum samples were found to be becoming more reactive, as shown in Fig. 3 (ELISA titers of 1:12800 for mice #1 through #3, respectively), proving that the VLPs had antigenicity comparable to that of native PPV and may be used as an antigen to identify PPV-specific antibodies.

Table 1. Checkerboard titration.

	Serum dilution			
	1:100	1:200	1:400	1:800
0.5	0.796/0.101	0.704/0.065	0.427/0.054	0.252/0.032
	P/N	7.88	10.83	7.904
1.0	0.916/0.111	0.743/0.070	0.463/0.056	0.312/0.035
	P/N	8.25	10.61	8.26
1.5	1.031/0.120	0.802/0.078	0.546/0.063	0.335/0.041
	P/N	8.59	10.28	8.67
2.0	1.101/0.101	0.853/0.081	0.581/0.057	0.377/0.042
	P/N	11.0	10.5	10.19
2.5	1.18/0.149	0.862/0.082	0.630/0.075	0.458/0.047
	P/N	7.92	10.5	8.4
5.0	1.232/0.184	0.910/0.100	0.728/0.081	0.541/0.051
	P/N	6.70	9.1	8.98

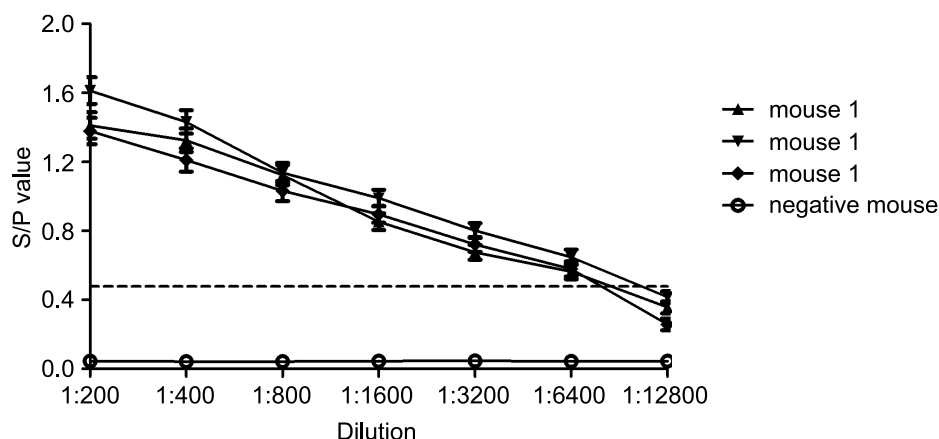


Fig. 4. Determination of antibody titers in immunized mice. BALB/c mice were intraperitoneally injected with PPV VLPs. The sera of mice were then prepared after final immunizations with the same dose of VLPs. The antibody titers of the sera were determined using the indirect-ELISA developed.

Development of an indirect ELISA for the detection of PPV antibodies

Using OD_{450nm} and P/N values, the ideal reaction conditions for the PPV VLP indirect-ELISA were identified. The coated antigen PPV VLPs' ideal dilution was found to be 0.20 $\mu\text{g}/\text{well}$ (2.0 $\mu\text{g}/\text{mL}$) using checkerboard titration, while the ideal serum sample dilution was 1:100 (Table 1). Other reaction conditions were also improved as well. In a nutshell, an overnight temperature of 4°C was ideal for coating. The 1% BSA in PBS was chosen as the optimum blocking solution. For serum, secondary antibodies, and TMB solution, the ideal reaction durations were 60 min, 60 min, and 15 min at 37°C, respectively. Finally, the best working dilution of the HRP-rabbit anti-pig IgG was 1:40,000.

Determination of the PPV VLP indirect-ELISA cut-off value

To establish the cutoff value of PPV VLP indirect-ELISA, 50 negative sera samples were tested. The mean of the OD_{450nm} values for these samples, as detected using the PPV VLP indirect-ELISA, had an average absorbance of 0.174, with a standard deviation of 0.101. The cutoff value was determined as mean + 3 standard deviations (SD) of negative sera according to Gaussian population distribution. Therefore, this ELISA threshold was $0.174 + 3 \times 0.101 = 0.478$, by which serum samples were considered as positive when the OD_{450nm} value was ≥ 0.478 . The serum was considered as negative less than 0.478 (Fig. 4).

Sensitivity and specificity of this ELISA

To determine the sensitivity of this ELISA method, dilutions of immunized mouse serum against VP2 pro-

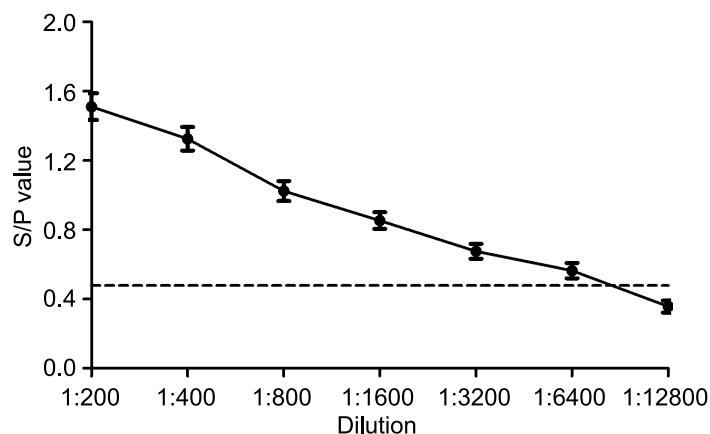


Fig. 5. Testing of the sensitivity of the indirect-ELISA. The PPV-positive sera were diluted in serial twofold dilutions from 1:200 to 1:25600, and the diluted serum was tested by indirect-ELISA.

Table 2. OD_{450} value of PPV VLP I-ELISA for other pathogens.

Pathogen	OD_{450nm}
PCV2	0.067±0.043
PRV	0.116±0.032
PRRSV	0.088±0.026
CSFV	0.071±0.027
TGEV	0.067±0.038
PEDV	0.072±0.038

Table 3. Comparison among PPV VLP I-ELISA, the commercial ELISA kit and HI test for the detection of the PPV antibodies in swine sera.

		No. of serum samples HI			No. of serum samples Commercial ELISA kit		
		Positive	Negative	Total	Positive	Negative	Total
ELISA	Positive	104	1	107	103	1	104
	Negative	1	14	13	2	14	16
Total		105	15	120	105	15	120
		99%	93.3%				
Agreement		98.3%		Agreement	97.5%		

tein from 1:200 to 1:12800 were used to detect the VP2 protein. The highest dilution for specific detecting serum anti-PPV antibodies was determined to be 1:6400 for the positive pig serum using the cut-off value of 0.478 (Fig. 5).

In addition, to evaluate the specificity of this indirect ELISA, the positive serum of PCV2, PRRSV, PEDV, TEGV, PRV, and CSFV was used in the established indirect ELISA method. PPV positive and negative serum served as positive and negative controls, respectively. Table 2 indicates that there was no cross-reactivity between the PPV and any of the anti-sera including PCV2, PRRSV, PEDV, TEGV, PRV, and CSFV, proving that the domain antigen was specific for antibody to PPV.

Concordance between VLP indirect-ELISA, HI and commercial indirect ELISA

The developed PPV VLP indirect-ELISA was applied to 120 serum samples with varied PPV antibody statuses. The coincidence rates tested by this ELISA compared with the commercial indirect ELISA kit (MingJing Biology, Shanghai, China) and HI, the traditional diagnostic assay, are shown in Table 3. The agreement between the double-antigen sandwich ELISA and HI was excellent, with a kappa value of 0.838, and that between the ELISA and the commercial indirect ELISA kit was also excellent, with a kappa value of 0.848, indicating very good compatibility of these tests.

Table 4. Repeatability test of PPV VLP I-ELISA.

Sample ID	Intra-batch repeatability			Inter-batch repeatability		
	X ^a	SD ^b	CV% ^c	X	SD	CV%
#1	0.759	0.045	5.9	0.856	0.035	4.1
#2	1.157	0.047	4.1	0.894	0.037	4.1
#3	1.107	0.031	2.8	0.890	0.044	4.9
#4	0.860	0.029	3.4	0.830	0.027	3.3
#5	1.165	0.043	3.7	1.050	0.033	3.1

^a Mean P/N value

^b Standard deviation

^c Coefficient of variation

Table 5. Prevalence of antibodies to PPV in pig sera collected from Henan, Hunan, and Heilongjiang.

Areas	Positive	Negative	Total	Positive rate
Hunan province	60	8	68	88.2%
Henan province	52	6	58	89.6%
Heilongjiang province	41	6	47	87.3%
Total	153	20	173	88.4%

The repeatability of this ELISA

In addition, the intra-assay CV% of five serum samples ranged from 2.8 to 5.9, whereas the inter-assay CV% of these samples was between 3.1 and 4.9. The coefficients of variation of intra- and inter-batch reproducibility tests of the method were less than 15% (Table 4). The purified PPV VLPs with varied batches were tested as a coating antigen of indirect-ELISA, the CV% were less than 10.0 and 15.0, respectively, demonstrating high reproducibility and low CV.

Serological survey of PPV-vaccinated pigs using the indirect-ELISA

A total of 173 swine serum samples collected from three provinces in China were tested using the PPV VLP indirect-ELISA (Table 5). The OD_{450nm} cutoff value at 0.478 for differentiating PPV-positive and -negative serum samples showed that 153 samples were positive (88.4%) and 20 samples (11.6%) were negative for PPV antibodies. Regionally, the positive rate was 88.2% (60/68) in Hunan province, 89.6% (52/58) in Henan province, and 87.2% (41/47) in Heilongjiang province. These data suggest good herd immunity after vaccination.

Discussion

Using recombinant PPV VP2 proteins made from prokaryotic expression system, the established indirect ELISA was with good repeatability, high sensitivity and high specificity for detecting PPV antibody in serum

samples. This indirect ELISA was successfully used to detect antibodies in clinical samples from several provinces in China, suggesting that this approach may be used for PPV vaccine evaluation or field infection surveillance.

PPV infection is one of the most important causes of reproductive failure in pigs impacting the piggery industry globally with huge economic losses (Meszaros et al. 2017). According to Streck et al. 2015, PPV infection in pregnant pigs can lead to mummification, stillbirth, abortion, and deformed fetuses. Additionally, PPV frequently co-infects pigs with PCV2 and other infections, amplifying PCV-2's role in the emergence of post-weaning multisystemic wasting syndrome (PMWS) (Allan et al. 1999, Choi,Chae 2000, Ellis et al. 2000, Kennedy et al. 2000). As a result, early diagnosis might be a useful strategy for PPV infection management. Although several serological methods such as hemagglutination inhibition (HI), serum neutralization (SN), and modified direct complement-fixation (MDCF) are still used in research and practice in some countries, today the enzyme-linked immunosorbent assay (ELISA) is the most frequently applied test to detect PPV-specific antibodies (Cartwright et al. 1971, Joo et al. 1976, Roic et al. 2005, Jozwik et al. 2009). Currently, recombinant nonstructural polyprotein 1 (NS1) synthesized in baculovirus or entire virus antigens in crude form are employed as a diagnostic tool for PPV antibody detection (Qing et al. 2006). To achieve high-quality diagnostic results, it is, however, necessary to continuously update the various approaches. In this study, the recombinant expression plasmid carrying VP2 was co-transformed into host

cells, activated, and self-assembled, resulting in the formation of PPV-VLPs. In guinea pigs and swine, a prior investigation showed that the bacterially originated PPV-VLPs had outstanding immunogenicity, pointing to a promising vaccine candidate (Hua et al. 2020). The reactogenicity of PPV-VLPs as a coating antigen for the creation of ELISA remains nevertheless, unknown.

The ability to produce antibodies is known to increase with antigenicity, and therefore the resulting antibody will likewise have an easier time performing the specific binding with the antigen. Indeed, according to our findings, PPV-VLP had antigenicity that was comparable to that of native PPV. Interesting results from testing the indirect ELISA reactivity of PPV VLPs with a panel of serum samples that had been well described revealed that the VLPs obtained would be suitable antigens for use in routine serological detection of PPV infections. The effectiveness of this novel method was demonstrated with each and every serum sample, and a strong connection was seen between HI and ELISA tests employing native antigens. The PPV-VLP has the potential to be used as an antigen to replace the native virus in sero diagnostics based on all of the outcomes of its characterization. Before replacing traditional antigens with VLPs, these results although encouraging must be confirmed with further studies.

In conclusion, the full-length recombinant VP2 protein of PPV has been produced using a bacterial expression technique. Here, a low-cost growth technique was employed in conjunction with the codon optimization strategy to produce large yields of recombinant capsid protein. Additionally, the *E. coli*-expressed recombinant VP2 protein self-assembled into VLPs and had antigenic characteristics similar to those of intact PPV virions. Additionally, an indirect ELISA was created using the VLP as an antigen for the specific and accurate detection of PPV antibodies. To our knowledge, this is the first investigation into the utility of VLPs produced from bacteria for PPV diagnosis.

Acknowledgements

We are deeply grateful for the support and assistance of all staff in Beijing Biomedicine Technology Center of JoFunHwa Biotechnology (Nanjing Co. Ltd.)

References

- Allan GM, Kennedy S, McNeilly F, Foster JC, Ellis JA, Krakowka SJ, Meehan BM, Adair BM (1999) Experimental reproduction of severe wasting disease by co-infection of pigs with porcine circovirus and porcine parvovirus. *J Comp Pathol* 121(1): 1-11.
- Allander T, Tammi MT, Eriksson M, Bjerkner A, Tiveljung-Lindell A, Andersson B (2005) Cloning of a human parvovirus by molecular screening of respiratory tract samples. *Proc Natl Acad Sci U S A* 102(36): 12891-12896.
- Cartwright SF, Lucas M, Huck RA (1971) A small haemagglutinating porcine DNA virus. II. Biological and serological studies. *J Comp Pathol* 81(1): 145-155.
- Choi C, Chae C (2000) Distribution of porcine parvovirus in porcine circovirus 2-infected pigs with postweaning multisystemic wasting syndrome as shown by in-situ hybridization. *J Comp Pathol* 123(4): 302-305.
- Crowther JR (2000) The ELISA guidebook. *Methods Mol Biol* 149: III-IV, 1-413.
- Ellis JA, Bratanich A, Clark EG, Allan G, Meehan B, Haines DM, Harding J, West KH, Krakowka S, Konoby C, Hassard L, Martin K, McNeilly F (2000) Coinfection by porcine circoviruses and porcine parvovirus in pigs with naturally acquired postweaning multisystemic wasting syndrome. *J Vet Diagn Invest* 12(1): 21-27.
- Feng H, Hu GQ, Wang HL, Liang M, Liang H, Guo H, Zhao P, Yang YJ, Zheng XX, Zhang ZF, Zhao YK, Gao YW, Yang ST, Xia XZ (2014) Canine parvovirus VP2 protein expressed in silkworm pupae self-assembles into virus-like particles with high immunogenicity. *PLoS One* 9(1): e79575.
- Hohdatsu T, Baba K, Ide S, Tsuchimoto M, Nagano H, Yamagami T, Yamagishi H, Fujisaki Y, Matumoto M (1988) Detection of antibodies against porcine parvovirus in swine sera by enzyme-linked immunosorbent assay. *Vet Microbiol* 17(1): 11-19.
- Hua T, Zhang D, Tang B, Chang C, Liu G, Zhang X (2020) The immunogenicity of the virus-like particles derived from the VP2 protein of porcine parvovirus. *Vet Microbiol* 248: 108795.
- Jenkins CE (1992) An enzyme-linked immunosorbent assay for detection of porcine parvovirus in fetal tissues. *J Virol Methods* 39(1-2): 179-184.
- Ji P, Liu Y, Chen Y, Wang A, Jiang D, Zhao B, Wang J, Chai S, Zhou E, Zhang G (2017) Porcine parvovirus capsid protein expressed in *Escherichia coli* self-assembles into virus-like particles with high immunogenicity in mice and guinea pigs. *Antiviral Res* 139: 146-152.
- Joo HS, Donaldson-Wood CR, Johnson RH (1976) A standardised haemagglutination inhibition test for porcine parvovirus antibody. *Aust Vet J* 52(9): 422-424.
- Jozwik A, Manteufel J, Selbitz HJ, Truyen U (2009) Vaccination against porcine parvovirus protects against disease, but does not prevent infection and virus shedding after challenge infection with a heterologous virus strain. *J Gen Virol* 90(Pt 10): 2437-2441.
- Kennedy S, Moffett D, McNeilly F, Meehan B, Ellis J, Krakowka S, Allan GM (2000) Reproduction of lesions of postweaning multisystemic wasting syndrome by infection of conventional pigs with porcine circovirus type 2 alone or in combination with porcine parvovirus. *J Comp Pathol* 122(1): 9-24.
- Kong M, Peng Y, Cui Y, Chang T, Wang X, Liu Z, Liu Y, Zhu Y, Luo Y, Tang Q, Feng L, Cui S (2014) Development and evaluation of the rVP-ELISA for detection of antibodies against porcine parvovirus. *J Virol Methods* 206: 115-118.

Allan GM, Kennedy S, McNeilly F, Foster JC, Ellis JA, Krakowka SJ, Meehan BM, Adair BM (1999) Experimental reproduction of severe wasting disease by co-infection

- Marcekova Z, Psikal I, Kosinova E, Benada O, Sebo P, Bumba L (2009) Heterologous expression of full-length capsid protein of porcine circovirus 2 in *Escherichia coli* and its potential use for detection of antibodies. *J Virol Methods* 162(1-2): 133-141.
- Mengeling WL, Cutlip RC (1976) Reproductive disease experimentally induced by exposing pregnant gilts to porcine parvovirus. *Am J Vet Res* 37(12): 1393-1400.
- Mengeling WL, Lager KM, Vorwald AC (2000) The effect of porcine parvovirus and porcine reproductive and respiratory syndrome virus on porcine reproductive performance. *Anim Reprod Sci* 60-61: 199-210.
- Meszaros I, Olasz F, Csagola A, Tijssen P, Zadori Z (2017) Biology of porcine parvovirus (Ungulate parvovirus 1). *Viruses* 9(12): 393.
- Oravainen J, Hakala M, Rautiainen E, Veijalainen P, Heinonen M, Tast A, Virolainen JV, Peltoniemi OA (2006) Parvovirus antibodies in vaccinated gilts in field conditions-results with HI and ELISA tests. *Reprod Domest Anim* 41(1): 91-93.
- Oravainen J, Heinonen M, Tast A, Virolainen J, Peltoniemi O (2005) High porcine parvovirus antibodies in sow herds: prevalence and associated factors. *Reprod Domest Anim* 40(1): 57-61.
- Qing L, Lv J, Li H, Tan Y, Hao H, Chen Z, Zhao J, Chen H (2006) The recombinant nonstructural polyprotein NS1 of porcine parvovirus (PPV) as diagnostic antigen in ELISA to differentiate infected from vaccinated pigs. *Vet Res Commun* 30(2): 175-190.
- Roic B, Cajavec S, Toncic J, Madic J, Lipej Z, Jemersic L, Lojkic M, Mihaljevic Z, Cac Z, Sostaric B (2005) Prevalence of antibodies to porcine parvovirus in wild boars (*Sus scrofa*) in Croatia. *J Wildl Dis* 41(4): 796-799.
- Shang SB, Li YF, Guo JQ, Wang ZT, Chen QX, Shen HG, Zhou JY (2008) Development and validation of a recombinant capsid protein-based ELISA for detection of antibody to porcine circovirus type 2. *Res Vet Sci* 84(1): 150-157.
- Streck AF, Canal CW, Truyen U (2015) Molecular epidemiology and evolution of porcine parvoviruses. *Infect Genet Evol* 36: 300-306.
- Westenbrink F, Veldhuis MA, Brinkhof JM (1989) An enzyme-linked immunosorbent assay for detection of antibodies to porcine parvovirus. *J Virol Methods* 23(2): 169-178.
- Xu Y, Li Y (2007) Induction of immune responses in mice after intragastric administration of *Lactobacillus casei* producing porcine parvovirus VP2 protein. *Appl Environ Microbiol* 73(21): 7041-7047.
- Zeeuw EJJ, Leinecker N, Herwig V, Selbitz HJ, Truyen U (2007) Study of the virulence and cross-neutralization capability of recent porcine parvovirus field isolates and vaccine viruses in experimentally infected pregnant gilts. *J Gen Virol* 88(Pt 2): 420-427.
- Zheng HH, Wang LQ, Fu PF, Zheng LL, Chen HY, Liu F (2020) Characterization of a recombinant pseudorabies virus expressing porcine parvovirus VP2 protein and porcine IL-6. *Virol J* 17(1): 19.