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Research Article

Development of PPA-ELISA for Diagnosing *Streptococcus suis* Infection Using Recombinant Sao-M Protein As Diagnostic Antigen

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Abstract

Streptococcus suis, an important zoonotic agent, is responsible for outbreaks of human infections. The accurate and rapid detection of *S. suis* may help control the potential outbreak and ameliorate patient outcomes. In the present study, Sao-M was used to establish a horseradish peroxidase enzyme-linked staphylococcal protein A immunosorbent assay (PPA-ELISA) for the diagnosis of *S. suis* infection. Results of chessboard titration test showed that the optimal concentration of coating antigen and dilution of serum were 8 μg/ml and 1:80, respectively. The cut-off was confirmed as OD450≥0.351 for positive response. The specificity of test indicated that rSao-M had no cross-reaction with antisera against the other 6 species of pathogens. The variation coefficient of intra-batch and inter-batch in the repeating tests was less than 9.5%. Comparative analysis by using conventional ELISA kit and established GDH-based ELISA showed that the present PPA-ELISA has higher specificity and sensitivity than GDH-based ELISA. A total seroprevalence of 6.6% in 500 pig serum samples indicated the method's applicability to detect *S. suis* infection. Cumulatively, the results suggested that the PPA-ELISA is a rapid, sensitive and specific diagnostic method and could be used as a new tool for large-scale epidemiological surveys and serological diagnosis of *S. suis* infection.

Keywords: *Streptococcus suis, PPA-ELISA, Sao-M, Diagnosis*

Diagnostik Antijen Olarak Rekombinant Sao-M Protein Kullanılarak *Streptococcus suis*'in Tanısı Amacıyla PPA-ELİSA Geliştirilmesi

Özet

Streptococcus suis, insanlarda enfeksiyona neden olan salgınlardan sorumlu önemli bir zoonotik ajandır. *S. suis*'in doğru ve hızlı bir şekilde tespit edilmesi enfeksiyona bağlı salgınları kontrol edebilir ve hastalığa bağlı etkileri azaltabilir. Bu çalışmada, Sao-M, *S suis* enfeksiyonunun teşhisi amacıyla peroksidaz enzim-bağlı stafilokokal protein A immün testi (PPA-ELİSA) için kullanılmıştır. Satranç tahtası titrasyon testinin sonuçları, kaplama antijeninin optimal konsantrasyonu ve serum sulandırılmasını sırasıyla 8 μg/mL ve 1:80 olarak göstermiştir. Pozitif cevap için eşik değeri OD450≥0.351 olarak belirlendi. Testin spesifitesi rSao-M'nin diğer 6 patojen türe karşı kullanılan antiserum ile çapraz reaksiyon vermediğini gösterdi. Tekrar testlerinde yığın içi ve yığınlar arası varyasyon katsayısı %9.5'ten az idi. Geleneksel ELİSA kiti ve kurulan GDH tabanlı ELİSA'nın karşılaştırmalı analizinde, mevcut PPA-ELİSA testinin GDH tabanlı ELİSA testinden daha yüksek spesifite ve sensitiviteye sahip olduğunu gösterdi. 500 domuz serumu örneğinde toplam seroprevalansın %6.6 olduğu ve bunun yöntemin *S. suis* enfeksiyonunun saptanmasında uygunluğunu gösterdi. Sonuçlar, PPA-ELİSA'nın hızlı, duyarlı ve spesifik bir tanı yöntemi olduğunu ve *S. suis* enfeksiyonunun serolojik tanısı için büyük kapsamlı epidemiyolojik araştırmalarda yeni bir araç olarak kullanılabilirliğini göstermektedir.

Anahtar sözcükler: *Streptococcus suis, PPA-ELISA, Sao-M, Tanı*

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INTRODUCTION

Streptococcus suis (S. suis), a Gram-positive bacteria, can cause pig meningitis and pneumonia, sepsis, sudden death and other diseases [1], also infect humans via impaired skin or mucosa, is an important zoonotic infectious disease pathogen [2]. Before 2005, *S. suis* was classified into 35 serotypes based on differences in capsular polysaccharide antigens [1,3,4]. In 2005, serotypes 32 and 34 were suggested to class into *Streptococcus orisratti* [4], and subsequently serotypes 20, 22, 26 and 33 were also removed from the *S. suis* taxon [5,6]. Hence, there are currently 29 remaining true *S. suis* serotypes [7]. Serotypes 1/2, 2, 3, 4, 5, 7, 8, 9, 21 and 31 have been determined to cause aggressive disease both in humans and/or pigs. Among these, serotype 2 is the most commonly associated with disease [8]. *S. suis* was initially reported as an etiological agent in 1954, and subsequently frequently-occurring bacterial infection [1,8]. Since originally reported in 1968, over 700 human cases of *S. suis* infection have been described in 2009 and further reached a staggering 1642 in 2013 [1,3,9]. *S. suis* is the most frequent cause of adult meningitis in Vietnam and the third most common cause of community-acquired bacterial meningitis in Hong Kong [10-12]. Of note, two large-scale outbreaks of lethal SS2 infection with a hallmark of streptococcal toxic shock-like syndrome (STSLS) occurred in China in 1998 and 2005, respectively, raising grave concerns in public health [13-15]. Unfortunately, there is no effective/specific human vaccine or therapeutics against *S. suis* infections is available so far [16]. Considering the high pathogenicity of *S. suis* infection in humans and pigs, it is crucial to develop a method for rapid and convenient diagnosis [16].

Standard bacteriological and biochemical analysis are routinely used to isolate and identify *S. suis* from clinical samples such as cerebrospinal fluid and blood samples, which are laborious and time consuming, and of low sensitivity [2,17,18]. At present, molecular biology technique based on PCR is the most commonly technique which is used for *S. suis* detection. Okwumabua et al.[17] developed a PCR method that targeted gdh encoding glutamate dehydrogenase, designated gdh PCR. Ishida et al.[19] also developed a PCR method targeting the recN gene, designated recN PCR. In addition, colloidal gold-based immunochromatographic strip test, and amperometric immunosensor techniques were also used for the detection of *S. suis* infection [2,20]. These methods could be used for the rapid and sensitive detection of *S. suis*; however, most of these methods are mainly used by professional technicians and require expensive instruments [2]. Therefore, establishing a simple, rapid, and cost-effective assay for the rapid detection of *S. suis* is an urgent problem to be solved in livestock production and scientific study.

Over the past few decades, the ELISA technique is the most universally used immunoassay in the laboratories to detect both bacteria and viruses. This method has the advantages of low cost, fast application, high sensitivity, ease of use and high reliability, and is possible to detect antibodies or antigens while screening large numbers of samples in a single experiment [7,21]. Surface antigen one (Sao) was identified by screening of a phage display library using convalescent swine sera [22]. Sao is a common surface protein, containing a C-terminal membrane anchoring Leu-Pro-X-Thr-Gly (LPXTG) motif and therefore is anchored to the cell wall peptidoglycan by the housekeeping sortase A, which mediates numerous virulence factors during *S. suis* infection [23]. Sao is highly conserved protein among most *S. suis* strains and has become an important candidate for the subunit *S. suis* vaccines [22]. Immunization with recombinant Sao protein was capable of provoking strong humoral antibody responses, diminish clinical signs and bacterial dissemination, improve survival rates and provide cross-serotype protection in pig and mouse vaccination protocols [24], indicating rSao is a suitable antigen for subunit *S. suis* vaccine development [25]. Sao protein is encoded by three allelic variants of gene of difference lengths, Sao-S (1.5 kb), Sao-M (1.7 kb) and Sao-L (2.0 kb), and Sao-M is the most prevalent variant comprising about 80% [26]. We suspect that Sao-M has great potential to become a diagnostic antigen for detection of *S. suis* infection.

Therefore, the objective of the present study was to develop an indirect ELISA test for the serodiagnosis of *S. suis* infection and to optimize the conditions for its use in basic clinical laboratories or in the field with basic levels of equipment. Furthermore, the performance of this test was evaluated and compared with the standard ELISA test.

MATERIAL and METHODS

Bacterial Strains and Serum (Samples)

In this experimental study, the strain *S. suis* SC22, an MRP^+ EF^+ SLY^+ strain, was provided by Shandong Binzhou Animal Science & Veterinary Medicine Academy (Binzhou, China). It was isolated from a diseased pig in Sichuan Province. Strain SC22 was cultured in Todd-Hewitt broth supplemented with 0.6% yeast at 37°C. The serum against *S. suis* was collected from pigs experimentally infected with the SC22 strain and saved by the aforementioned institute. Antisera against *S. enterica*, *E. coli*, *A. pleuropneumoniae*, *S. aureus*, *S. zooepidemicus*, and *S. equisimilis* were stored by the aforementioned institute. Clinical serum samples were collected from growing pigs from various geographical locations (Shandong, Henan, Anhui, Jiangsu, Zhejiang and Hubei).

Reagents

Horseradish peroxidase-labeled Staphylococcal protein A (HRP-PPA) was purchased from Boster (Wuhan, China). Porcine *S. suis* Antibody ELISA Test Kit, which uses the whole-bacteria lysis as antigen to for detect porcine *S.*

suis all sub-types, was purchased from SenBeiJia Bioloical Technology Co., Ltd (Nanjing, China). Twain-20, bovine serum albumin (BSA) were purchased from Solarbio LIFE SCIENCES Co. Ltd (Beijing, China). Skim milk was purchased from Becton, Dickinson and Company (Franklin Lakes, NJ, USA). Other reagents, imported or domestically produced, were of analytical grade. Preparation of coated solution, sealing solution, diluent and washing solution were refer to reference Xia [7].

Preparation of Recombinant Sao-M Protein

Specific primer was designed based on sequence of Sao-M coding gene from GenBank accession no. JF 810176. The primer of the Sao-M gene was: Sao-F: GCGGGATCC ATGAATACTAAGAAATGGAG and Sao-R: CAGAAGCTTGAACTA ATTTACGTTTACGTG [25]. The forward and reverse primers contained *Bam*H I and Hind III recognition sequences, respectively. The PCR product was purified by SanPrep PCR Cleanup kit (Sangon, Shanghai, China) and cloned into the prokaryotic expression vector pET-30a according to the manufacturer's instructions (Novagen, Darmstadt, Germany). The identity of the insert in pET30a was verified by DNA sequence analysis (Sangon, Shanghai, China). The recombinant plasmid was transformed into the *Escherichia coli* (E. coli) strain BL21 (DE3) and cultured in LB at 37°C until the absorbance was in the range of 0.6-1.0 at 600 nm. IPTG was added at a final concentration of 0.8 mmol/L and the culture was further incubated at 37°C for 5 h with shaking at 200 r/min. The recombinant Sao-M protein domain (rSao-M) was efficiently expressed in *E. coli* BL21 (DE3), and nickel ion-agarose affinity chromatography was subsequently used to obtain highly pure recombinant protein as described in a previous study [7]. The concentration of total bacterial protein and purified protein were measured using NanoDrop 2000/2000C spectrophotometer (Thermo Scientific; Boston, USA) to determine the concentration of target protein in total bacterial protein.

Procedure of Indirect PPA-ELISA

The purified rSao-M was appropriately diluted in carbonate buffer solution (CBS) and coated onto a polystyrene 96-well microtiter plates at 4°C, overnight. After the plates were washed four times with Phosphate buffered saline (PBS) -Tween-20 (PBST), 5% (w/v) skim milk-PBST was used to block non-specific binding sites at 37°C for 2 h. After washing three times with PBST, 100 µl of serum sample with a titer of 1:80 was then added to the plates and incubated at 37°C for 1 h. 96-well plates were washed three times and further incubated for 1 h with horseradish peroxidase-labeled PPA diluted in PBST-2.5% skim milk. After further washing, the reaction was visualized by the addition of a TMB substrate for 10 min at room temperature (RT). The reaction was terminated with Stop Solution and the results were revealed by Enzyme-linked immune apparatus. The preimmunization serum of New Zealand rabbits and the sera of immunized rabbits were served as negative and positive controls, respectively. Simultaneously, sterile carbonate buffer solution was served as a non-antigen-coated control and PBST-2.5% skim milk was used for the blank control (no serum or no conjugate added).

Optimization for PPA-ELISA

The primary antibody (serum came from a diseased pig in Sichuan Province as described above) and rSao-M were used at twofold dilutions from 1:20 to 1:320 and from 0.5 µg/ml to 64 µg/mL, respectively. The serum and coating antigen dilution corresponding to the largest value of P/N at the value of OD_{450} of positive serum was about 1 and the negative serum with a lower value of OD_{450} were considered as optimal serum dilution and optimal antigen concentration. PBS and CBS were selected as washing and coating buffers, respectively. Horseradish peroxidase-labeled Staphylococcal protein A (PPA) was used as second antibody at a 1:2000 dilution according to the instruction of the manufacturer (BOSTER, China). Serial dilutions of bovine serum albumin, skim milk, horse serum and fetal calf serum were used to screen the best blocking buffer condition. Dilutions were made in PBST. Effects of temperature and time on the performance of the PPA-ELISA were also optimized. The optimal conditions were used for all subsequent PPA-ELISA tests.

Determination of Positive and Negative Thresholds of PPA-ELISA

Indirect ELISA was carried out with 40 pig serum samples in triplicate (all samples were negative in the commercial ELISA kit analysis). The end results of OD_{450}nm were computed as mean (x) and standard deviation (S). According to statistical principles, the thresholds were defined depending on the criteria as follows: a sample OD_{450}nm value $\geq x + 3S$ was regarded as positive; a sample OD_{450}nm value $\leq x + 2S$ was regarded as negative; and the values between $x + 3S$ and $x + 2S$ were regarded as doubtful. The doubtful samples were double tested and regarded as positive if the value still suspicious. In this situation, clinical symptoms were considered for diagnosis if applicable, and further monitoring was performed.

Evaluation of PPA-ELISA

Cross-reaction was assessed by testing of sera from positive controls and antisera against *S. enterica*, *E. coli*, *A. pleuropneumoniae*, *S. aureus*, *S. zooepidemicus*, and *S. equisimilis* [17]. In blocking test, first, the rSao-M protein and *S. suis* positive serum were diluted with PBS at the optimal dilution. Second, the diluted serum was divided into two groups: A and B. Third, 50 µl of diluted rSao-M protein was mixed well with 50 µL A (P) and then the mixture was incubated at 37°C for 1 h. A mixture of 50 µL of B and 50 µL of CBS (N) served as the control. The percentage inhibit (PI, PI = (OD_{450}nm value of N - OD_{450}nm value of P)/OD_{450}nm value of N) was calculated.

At the identical experimental conditions, five negative serum samples and five positive serum samples were chosen randomly and each sample was examined 5 times. For inter-batch experiment, five batches of the purified rSao-M protein were performed at different plate on different occasions at different time. The coefficient of variation was computed (C.V %).

The storage conditions (-20°C, 4°C, 25°C, 37°C) and the duration (1-6 months) of studies were calculated to reflect the analytical stability of the assay.

The analytical sensitivity of the system was evaluated by measuring the reactivity between rSao-M antigen with twofold serial diluted (ranging from 1:20 to 1:1280) specific anti-*S. suis* serum and negative control serum with a 40-fold dilution.

To validate the indirect PPA-ELISA as a clinical diagnostic tool, 160 clinical samples from cases of suspected of *S. suis* infection were tested using our indirect PPA-ELISA and commercial ELISA. Confirmed *S. suis*-positive and negative sera served as controls, and the results obtained from PPA-ELISA and commercial ELISA were compared to computed their detection coincidence.

Clinical Application of the Indirect PPA-ELISA Test

500 sera from growing pigs from various geographical locations (Shandong, Henan, Anhui, Jiangsu, Zhejiang and Hubei) were tested to evaluate the positive rate of *S. suis* infection and further to understand the relationship between the *S. suis* infectivity and Sao-M.

Statistical Analysis

The data were presented as mean ± SD. All graphical illustrations were constructed either by GraphPad Prism5 software or in Microsoft Excel sheet. Student's t-test was used for all statistical comparisons. Significance (P) value summary: $P \leq 0.05$; $P \leq 0.01$.

RESULTS

Expression and Purification of Recombinant Protein rSao-M

E. coli BL21 (DE3) harboring the pET30a-Sao-M plasmid displayed efficient expression after induced by IPTG, an approximately 110 kDa protein band appeared as evidenced by SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) electrophoresis. The protein bands were analyzed using Alpha VIEW software, and the results indicated that the soluble Sao-M fusion protein accounted for 44.8% of the total bacterial protein in supernatant. The expressed Sao-M fusion protein was purified using nickel ion-agarose affinity chromatography according to the instructions *(Fig. 1*, line 5-9). As shown by SDS-PAGE, the purified protein was manifested as a single band, about 110 kDa *(Fig. 1*, line 5-9). The protein concentration was 1.16 mg/mL as measured using NanoDrop 2000/2000C spectrophotometer.

Standardization of PPA-ELISA

The Indirect PPA-ELISA was standarded with the conditions of an 80-fold dilution of swine serum and 8 μg/mL of antigen by the square titration experiments. The optimal conditions for the orthogonal experiments were as follows: reaction temperature was 37°C, blocking solution was 5% (v/v) BSA, blocking time was 2 h, primary/second antibody incubation time was 1 h, and chromogenic time was 10 min.

Establishment of Positive and Negative Thresholds

The range of OD_{450}nm values was between 0.138~0.338 with an average of 0.216 and a standard deviation of 0.045, as measured from 30 negative serum samples. The threshold between negative and positive samples was 0.306 which indicates that, a serum sample can be regarded as positive at OD_{450}nm ≥0.351, or negative at OD_{450}nm ≤0.308, or as suspicious with a value between 0.308-0.363. The suspicious sample was measured again and considered as positive with a questionable value.

Specificity and Sensitivity of Indirect PPA-ELISA

According to established PPA-ELISA conditions, all the antibodies against *S. enterica, E. coli, A. pleuropneumoniae, S. aureus, S. zooepidemicus,* and *S. equisimilis* showed negative OD_{450}nm values, indicating a negative cross-reactivity of this antigen with above sera (data not shown). Our blocking test also showed that the OD_{450}nm value of the positive samples sharply reduced after blocking with rSao-M protein *(Table 1)*, thereby indicating that the rSao-M may be specific.

Evaluation of Assay Repeatability

The variation coefficient is less than 8.5% within intra-batch experiments, whereas less than 9.5% with inter-batch experiments using different batches of purified recombinant antigens *(Table 2)*. These results suggested a high reproducibility of this study.

Evaluation of Assay Stability

The stability test was conducted by comparing results from coated microtiter plates tested at four different temperatures for six different times. The results showed that the sensitivity of diagnostic test antigen remained unchanged for 6 months after storing at -20°C and 4°C. However, after storage for 6 months at RT and 37°C the assay showed a slight decrease in the development of the discs, the latter decrease by even more; the results showed that the assay has a good "shelf life" *(Table 3)*.

The Coincidence Rate Between PPA-ELISA and Two Established ELISA

The commercial ELISA Kit for detection of all subtypes

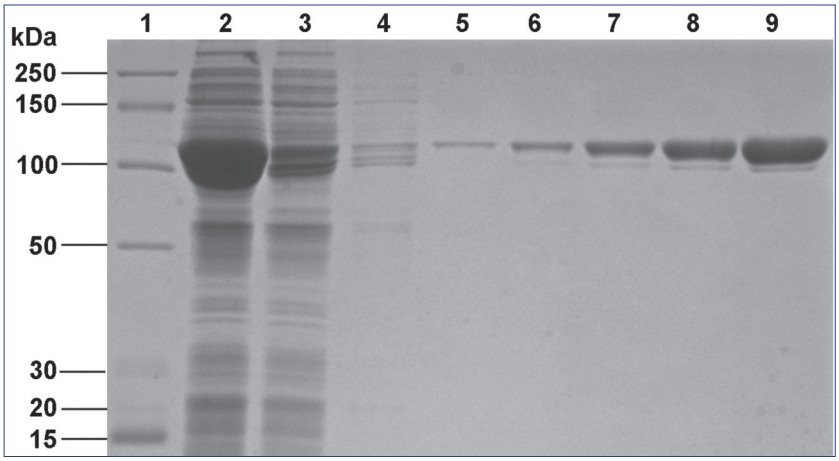

Fig 1. SDS-PAGE of BL21/pET-30a-Sao-M cell lysate. Line 1. Protein ruler; Line 2. All fraction of BL21/pET-30a-Sao-M cell lysate; Line 3. Insoluble fraction of BL21/pET-30a-Sao-M cell lysate; Line 4. Precipitation fraction of BL21/pET-30a-Sao-M cell lysate; Line 5-9. Purified His recombinant proteins with different concentration gradient imidazole

Table 1. *Special-blocking test of PPA-ELISA*

Positive Sera and Treatment		Dilution of Serum Sample			
		1:40	1:800	1:160	1:320
Sample 1	(N-P)/N	61.8%	65.1%	68.1%	71.5%
Sample 2	(N-P)/N	63.3%	67.2%	69.5%	76.1%
Sample 3	(N-P)/N	64.5%	67.6%	71.3%	77.1%

Table 2. *The different values among wells in plate and among plates for PPA-ELISA*

Sample NO.	Repeat Detections					Average X	Standard Deviation	CV Value (%)
	1	2	3	4	5			
Intra-batch experiments								
1	0.802	0.899	0.845	0.861	0.950	0.871	0.056	6.43
2	1.245	1.006	1.135	1.122	1.049	1.111	0.092	8.28
3	1.081	1.066	1.069	1.071	1.125	1.078	0.016	4.12
Inter-batch experiments								
4	0.756	0.689	0.613	0.777	0.748	0.717	0.066	9.20
5	1.033	1.061	1.038	1.145	1.046	1.085	0.063	5.80
6	1.250	1.155	1.239	1.151	1.140	1.187	0.053	4.47

Table 3. *Stability of being coated ELISA plate*

Preserving Time/month	Serum /Batch	Titer Changes Detected by ELISA Plate Stored in Different Preserving Conditions			
		-20°C	4°C	Room Temperature	37°C
1	1	2^8	2^8	2^8	2^8
	2	2^9	2^9	2^9	2^9
3	1	2^8	2^8	2^8	2^8
	2	2^9	2^9	2^9	2^8
6	1	2^8	2^8	2^8	2^8
	2	2^9	2^9	2^9	2^7
9	1	2^8	2^8	2^7	2^7
	2	2^9	2^9	2^8	2^6

Table 4. *Comparison among three kinds of testing methods for detecting sera samples*

Total Number of Samples	No. of Samples Positive by Commercial ELISA Kit	No. of Samples Positive by Indirect Dot-ELISA	No. of Samples Positive by Indirect PPA-ELISA
True- positive	119	115	117
False-positive	0	3	2
False-negative	0	4	2
True-negative	41	38	43
Specificity (%)	100	92.68	95.56
Sensitivity (%)	100	96.64	98.32

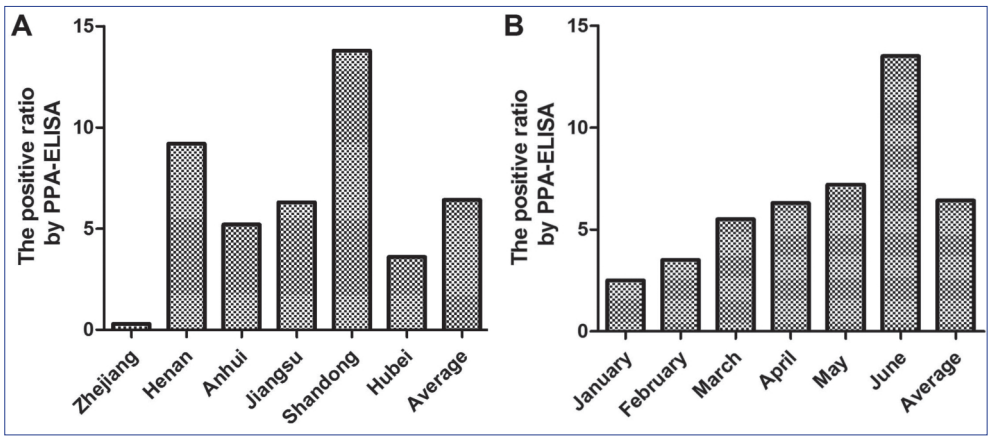

Fig 2. The positive detecting of *S. suis* by PPA-ELISA in 2015. A. The positive detecting of *S. suis* by PPA-ELISA in various geographical locations in 2015; B. The positive detecting of *S. suis* by PPA-ELISA in various months in 2015

of porcine *S. suis* was used as the standard method for confirmation to establish the authenticity of the PPA-ELISA. A total of 160 clinical samples from cases of suspected of *S. suis* infection were examined simultaneously using the indirect PPA-ELISA developed in this study, Dot-ELISA developed in our laboratory and commercialized ELISA diagnostic kit. Among the 160 immune sera samples in the study, positive reactions for *S. suis* were detected in 117 (73.13%) by PPA-ELISA. Two (1.25%) of the samples were negative in the PPA-ELISA but were positive when tested with the commercial kit. Apart from 41 negative samples, two (1.25%) showed a false-positive reaction, these samples were negative in the commercial ELISA Kit. The PPA-ELISA was positive with 73.13% of the samples while commercial Indirect ELISA Kit was positive in 74.38%. Thus, the PPA-ELISA developed in our laboratory showed a specificity of 95.56% and a sensitivity of 98.32% (Table 4), which higher than that of the Dot-ELISA developed in our laboratory [7].

Clinical Application of the Indirect PPA-ELISA Test

A total of 500 serum samples from growing pigs from various geographical locations (Shandong, Henan, Anhui, Jiangsu, Zhejiang and Hubei) were analyzed for the occurrence of *S. suis* infection by the PPA-ELISA method developed in the present study. The result showed that the total seroprevalence was 6.6% (33/500) *(Fig. 2A)*. The positive rate of sera in Shandong Province was 13.8%, only 0.3% in Zhejang Province and less than 10% in the other provinces *(Fig. 2A)*. As shown in *Fig. 2B*, from January to June in 2015, PPA-ELISA positive rate of SS infection displayed an upward trend, and the positive rate in January and February was low and significantly increased in June. These results indicated that the prevalence of *S. suis* is closely related to that of climate, the positive rate of *S. suis* will generally increase in high fever and high humidity environment.

DISCUSSION

Due to its fast spread and high mortality, *S. suis* infection has become a huge threaten for economic and healthy [27]. Therefore, serological surveillance is useful in determining the infection status and could play an important role in the control of *S. suis* in both pigs and humans [28]. ELISA has been confirmed to be a rapid and sensitive method for serological surveillance and detection of bacterial pathogens [28]. In this study, the Sao-M was applied as a diagnostic antigen to develop an accurate and rapid PPA-ELISA detection method, which can be conveniently applied in serological surveillance, with high sensitivity, specificity, and feasibility.

The diagnosis of *S. suis* infection is essentially based on the results of traditional microbiological and biochemical analysis. However, these methods are routine and complex and are unable to accurately distinguish *S. suis* from other related bacteria [17]. Moreover, numerous advanced molecular techniques, including PCR, colloidal gold-based immunochromatographic strip test, and amperometric

immunosensor techniques, could be used for the rapid and sensitive detection of *S. suis* yet require expensive equipment and highly skilled personnel [2,18,20]. ELISA becomes a widely used serological diagnostic technique, as also recommended by OIE International Trade, in animals quarantine for infectious diseases and epidemical investigation due to its strong specificity, sensitivity, objective criteria, and so on [29]. Based on the above, we turned our attention to ELISA which is an essential immunological technology for diagnosis of the diseases.

In addition to serotypes 2, serotypes 1/2, 3, 4, 5, 7, 8, 9, 21 and 31 possess a certain degree of virulence to both in humans and pigs and cause highly hazardous to porcine industry worldwide. Rapid detection of all subtypes of *S. suis* is of great significance for epidemiological survey of *S. suis* and widely monitoring the prevalence of *S. suis* in swine. The Sao protein is highly conserved among *S. suis* strains and Sao-specific antibodies have been shown to react with cell lysates of 28 of 33 *S. suis* serotypes and 25 of 26 serotype 2 isolates in immunoblots [22]. Sao protein is encoded by three allelic variants of gene of difference lengths, Sao-S (1.5 kb), Sao-M (1.7 kb) and Sao-L (2.0 kb) and Sao-M is the most common type of Sao [24]. Moreover, Sao-M in *S. suis* is well conserved and the protein exhibits strong immunogenicity [24]. Based on these observations, Sao-M was chosen as a diagnostic antigen for the development of a serodiagnostic test to detect *S. suis* in this study. Comparing with the GDH-based ELISA measuring system, which previously developed in our laboratory [7], the present PPA-ELISA has higher specificity and sensitivity.

The accuracy of the Indirect ELISA test was compared to the commercial ELISA kit to test the accuracy, our results showed the present Indirect ELISA showed 95.56% sensitivity and 98.32% specificity. Only 1.25% false negativities were observed. Cross-reactivity was not observed between *S. suis* antibody and other bacterial genera. Moreover, a total of 500 swine serum samples from various geographical locations were measured by Indirect PPA-ELISA to determine the seroprevalence of *S. suis*. The results showed that the total seroprevalence was 6.6% (33/500). Furthermore, our results indicated that the prevalence of *S. suis* is closely related to that of climate, the positive rate of *S. suis* generally increases in high fever and high humidity environment, which is in agreement with previous studies [30-32].

In 2007, Li et al. established Sao-based ELISA for detecting titers of Sao-specific total IgG and IgG subclasses in mouse and swine sera [24]. In 2012, Hsueh et al. [25] established ELISA using Sao-L and Sao-M for detecting titers of Sao-L-specific total IgG and Sao-M-specific total IgG. In the present study, Sao-M was used to establish PPA-ELISA for the diagnosis of *S. suis* infection. Compared with the former two groups the present PPA-ELISA displayed less operation time, since the optimized parameters. In addition, Staphylococcal protein A (SPA) could bind to Fc segments of serum IgG in human and various mammals (pigs, dogs, rabbits, monkeys, mice, mice and bovine), and the enzyme-labeled SPA (HRP-SPA) could replace the second antibody for ELISA, can be achieved at the same time on guinea pigs, rabbits, pigs and other animal serum detection. Moreover, the present PPA-ELISA was used in clinical test. Enolase, an immunodominant antigen involved in the virulence of Streptococcus species [33,34] was also used as diagnostic antigen for ELISA and used in clinical test [35]. However, they just tested small amount of samples in Jiangsu province. In our study, 500 sera from growing pigs from six geographical locations (Shandong, Henan, Anhui, Jiangsu, Zhejiang and Hubei) were tested and further to probe whether the prevalence of *S. suis* is associated with climate.

Some limitations of this study should be noted. Firstly, the present PPA-ELISA failed to distinguish wild-type from vaccine strains of the *S. suis*. Secondly, sample sizes was still small though 500 samples were tested in this study. In the future, we will increase the sample sizes from more geographical locations to enhance the practicability of the PPA-ELISA.

In conclusion, our study establishes an indirect ELISA method for detection of *S. suis* antibody, which provides a technical support for quarantine, diagnosis, antibody surveillance, which may prevent the further spread of this emerging pathogen.

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Conflicts of Interest

All authors declared that there are no conflicts of interest.

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