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Immunogenicity and cross-protective efficacy of double-mutant *Streptococcus suis* Δ*SspepO*/Δ*SspspC* serotypes 2 and 7

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ABSTRACT

Streptococcus suis serotype 2 (*S. suis* 2) is a major pathogen causing *streptococcosis* in swine, resulting in significant losses in swine breeding worldwide. We previously reported that the non-antibiotic-resistant double-mutant strain Δ*SspepO*/Δ*SspspC* can be used as a live vaccine, providing effective protection against *S. suis* 2 infections in mice. This study aimed to understand the characteristics of *streptococcosis* and develop vaccine candidates for immunization. Intramuscular injection of live *S. suis* Δ*SspepO*/Δ*SspspC* in pigs induced discernable antibody production and provided cross-protection against challenges by a type 2 strain (100% protection) and a type 7 strain (60% protection). Protection was evaluated via clinical, bacteriological, serological, and post-mortem examinations. Furthermore, vaccination induced the production of opsonizing antibodies against serotypes 2 and 7. Analysis of IgG subclasses (IgG1 and IgG2a) revealed that both Th1 and Th2 responses were induced by *S. suis* Δ*SspepO*/Δ*SspspC*, although the IgG2a (Th1) response predominated over the IgG1 (Th2) response. This study is the first, to our knowledge, to establish a live vaccine candidate to protect against two major *S. suis* serotypes. Further studies are required to assess these candidate vaccines and examine their feasibility in providing cross-protection against *S. suis*.

1. Introduction

Steptococcus suis is a major zoonotic pathogen responsible for significant economic losses in swine rearing worldwide and numerous infections in humans [1,2,3,4]. Infections in pigs may result in sudden death or meningitis, arthritis, septicemia, pneumonia, and endocarditis [3,4,5,6]. Based on differences in capsular polysaccharides, *S. suis* comprises 33 serotypes (types 1–31, 33, and 1/2) [5,7]. Among them, serotype 2 is considered the most virulent and most frequently associated with diseases worldwide [5]. By 2012, the total number of *S. suis* infections in humans approached 1600 cases [6]. In 1998 and in 2005, *S. suis* outbreaks occurred in Jiangsu and Sichuan respectively, resulting infections in 240 individuals 52 mortalities in Sichuan and infections in 215 individuals 38 mortalities in Jiangsu, causing widespread concern worldwide. *S. suis* infections in humans were clinically associated with streptococcal toxic shock syndrome. Moreover, *S. suis* was the foremost cause of adult meningitis in Vietnam, the second leading cause of adult meningitis in Thailand, and the third most frequent cause of community-acquired bacterial meningitis in Hong Kong [2].

We previously generated a deletion mutant Δ*SspepO* strain (SSU05_0153) and reported that its virulence in piglets decreased significantly along with its adhesiveness to Hep-2 cells in comparison with the wild strain SC19. Hence, *SspepO* was an important virulence factor in *S. suis* 2 [8]. Li et al. identified a novel recombinant *SspepO* via prokaryotic expression, which protected mice and pigs against infection of lethal dose of *S. suis* 2 and inhibited *S. suis*-induced bacteremia [9]. Liu et al. reported that *SspepO* was a fibronectin-binding protein, which contributing to the development of *S. suis* 2-induced meningitis [10].

Yuan et al. investigated the interaction between *SspspC* (SSU05_0196) and host cell surface glycosaminoglycans (GAGs) via indirect immunofluorescence and cell adhesion inhibition assays [11]. They reported that the positively charged residues on the exposed surface of the 18-kDa domain, mostly lysine

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residues, play a critical role in the *SspspC*–heparin interaction and bacterial–host cell adhesion [11,12]. Zhang et al. reported that *SspspC* enhances CcpA activity to regulate genes involved in carbohydrate utilization and capsular polysaccharides synthesis, thereby contributing to the virulence of *S. suis* [12].

Our previous studies demonstrated that the double mutant ΔSspepO/ΔSspspC, which was less virulent compared with the wild type strain, clearly provoked an antibody response. Furthermore, ΔSspepO/ΔSspspC exposure induced a protective response against a lethal dose of fully virulent *Streptococcus suis* serotype 2 in 90% of mice [13]. In this study, we confirm that ΔSspepO/ΔSspspC can be used as an effective live vaccine and provide a novel strategy against infection of *S.suis* 2 and 7 in pigs.

Although conventional inactivated vaccines protect against homologous bacterial infections, most reports are inconsistent regarding protection against heterologous bacterial infections, thereby necessitating studies on novel genetically engineered vaccines against *S. suis*. Hence, this study aimed to understand the characteristics of the popular Chinese strain SC19 via a series of experiments to understand this disease and to develop appropriate vaccines.

2. Material and methods

2.1. Bacterial strains and culture

S. suis 2 was isolated from the brain of a diseased piglet collected in Sichuan outbreak in China in 2005 (Accession NO. MNPY00000000 in GenBank) [14]. *S. suis* 7 is a typical China strain isolated from a diseased pig [15]. The ΔSspepO/ΔSspspC, *S. suis* 2 and 7 strains were grown overnight on tryptic soy agar (TSA) plates (Difco, Detroit, MI, USA) at 37 °C, and isolated colonies were inoculated into 10 mL tryptic soy broth (TSB; Difco, Detroit, MI, USA). The culture was incubated without shaking for 8 h at 37 °C. Working cultures were prepared by transferring 10 mL 1:1000 diluted 8 h cultures into 50 mL TSB and incubating without shaking for 16 h at 37 °C with 5% CO_2. The bacteria were washed once in PBS (pH 7.4; Invitrogen). The pellet was then resuspended in 10 mL PBS. Serial dilutions of the suspension were plated onto TSA plates to determine the number of CFU/mL^{-1}.

2.2. Determination of antibody titers

Blood samples were obtained from piglets by vena cava anterior venipuncture on day 14 after the second immunization. To get serum samples, the blood samples were left to stand at 4 °C overnight, and obtained the supernatant. Briefly, 100 μL of coating buffer, consisting of 50 mM sodium carbonate buffer (pH 9.6) and 121 °C high-pressure SS2 heat-stable antigens, was added to each well of a 96 well ELISA plate and incubated at 4 °C overnight. The plates were washed 3 times with PBST (PBS supplemented with 0.05% Tween 20) and blocked for 1 h at 37 °C with PBS containing 5% bovine serum albumin. After washing again with PBST, diluted serum samples were added to each well and the plates were incubated at 37 °C for 1 h. After washing, horseradish peroxidase (HRP)-conjugated goat anti-porcine IgG antibodies (diluted 1:5000 in PBST) were added to each well. The serum antibody IgG concentration was determined by measuring absorbance at 630 nm. Mouse anti-pig IgG1-HRP (AbD Serotec, United Kingdom) or IgG2a-HRP (AbD Serotec) antibodies were used in the ELISA to determine sera IgG subtypes. After washing, 3,3′,5,5′-tetramethyl benzidine (TMB) and H_2O_2 were added and plates were incubated at room temperature for 30 min. SDS (1%) was added to each well to terminate the reactions and the plates were read at an absorbance of 450 nm [16].

2.3. Whole-blood bacterial killing assay

Whole-blood bacterial killing assays were performed as described previously with minor modifications [17]. Blood samples were obtained from piglets on day 14 after the second immunization [13]. Briefly, 10 μL (10^6 cells) of cultures of SS2 cells in the late logarithmic phase was mixed with 190 μL of each inactivated serum sample. After incubation (30 min, 37 °C), heparinized non-immune pig whole blood (100 μL) was added. The mixture was incubated at 37 °C for 1 h. The bacteria that survived was diluted and plated on trypticase soy agar +10% newborn calf serum [13]. The results are expressed as percentage killing in accordance with the following formula: (CFU after 1 h of growth in control serum)-(CFU after 1 h of growth with immunized serum)/CFU after 1 h of growth with control serum × 100% [13].

2.4. Animal experiments

Forty 4–5-week-old healthy piglets testing negative upon ELISA for *S. suis* 2 and *S. suis* 7 antibodies were used; all animal experiments were conducted in accordance with the guidelines and approved protocols of the Laboratory Animal Monitoring Committee of Huazhong Agricultural University. The piglets were randomly divided into eight experimental groups each of 5 (Table 1). Groups 1 and 5 were vaccinated twice intramuscularly (i.m.) with 4×10^8 CFU of ΔSspepO/ΔSspspC in 2 mL PBS. Groups 2 and 6 (Positive control) were vaccinated twice intramuscularly (i.m.) with 3×10^9 CFU of commercial *S.suis* 2 and 7 inactive vaccines (Keqian, Wuhan, Hubei, China). Groups 3 and 7 (Negative control) were injected intramusculary with 2 mL PBS. Groups 4 and 8 (Blank control) as a blank check. A second identical immunization was performed 14 days after the primary vaccination. Based on the comparative evaluation of experimental infection with the serotype 2 and the serotype 7 reference strains, the piglets were challenged intranasally 2 weeks after the second immunization with either 1×10^6 CFU of serotype 2 or 1.5×10^{10} CFU of serotype 7 [18]. The health status of the animals was monitored every 12 h. If piglets developed any of the following symptoms, they were removed from the study and euthanized: high fever (40.5 °C), apathy, anorexia, clinical signs of acute polyarthritis, or clinical signs of severe meningitis. Pigs were infected again with the *S. suis* challenge strain, which was isolated from pigs immunized with ΔSsPepO/ΔSsPspC. The infected pigs were sacrificed and tonsil, lung, serosa, spleen, liver, CSF, and joint fluid samples were collected and weighed. After homogenization in 1 mL PBS, the samples were serially diluted and plated on TSA to determine the number of viable bacteria. Multiplex PCR for cps1, cps2, cps7, mrp, sly, arcA, gdh, epf, and cps9 was used to confirm the isolation of the challenge strains [19]. All surviving piglets were euthanized at 12 d post-infection [18]. Lungs harvested from surviving piglets were fixed in 10% neutral-buffered formalin and embedded in paraffin. Tissue sections (4-μm-thick) were stained with hematoxylin and eosin in accordance with a standard protocol and examined via light microscopy [13].

2.5. Statistical analysis

Statistical analysis was conducted using GraphPad Prism software (San Diego, USA), and data are expressed as the mean ± standard deviation values. Between-group differences were analyzed using analyzed using Student's *t*-test, while multiple-group differences were analyzed using two-way ANOVA. Log rank tests were performed for survival analysis. The level of significance was set at $P < 0.05$, and $P < 0.01$ was considered extremely significant.

Table 1
Evaluation of protection induced by Δ*SsPepO*/Δ*SsPspC* in *Streptococcus suis* serotype 2 and 7 challenge experiments of growers[a].

S.suis challenge serotype[b] and immunization antigen	No.of piglets	*S.suis* challenge		Morbidity[c]	Mortality[c]	No. of piglets with clinical symptom(s)			No. of piglets with condition/total no. of piglets[d]			
									Max. body temp:		Max. WBC:	
		Route of administration[e]	CFU			CNS[f]	Lameness	Unspecific	<40 °C	≧40.5 °C	<22 × 10^9/liter	≧22 × 10^9/liter
Serotype 2												
Δ*SsPepO*/Δ*SsPspC*	5	i.v.	10^6	0/5	0/5	0/5	0/5	0/5	3/5	2/5	3/5	2/5
positive control	5	i.v.	10^6	0/5	0/5	0/5	0/5	0/5	4/5	1/5	4/5	1/5
negative control	5	i.v.	10^6	5/5	5/5	2/5	2/5	1/5	0/5	5/5	0/5	5/5
blank control	5	i.v.	10^6	5/5	5/5	1/5	3/5	2/5	0/5	5/5	0/5	5/5
Serotype 7												
Δ*SsPepO*/Δ*SsPspC*	5	i.v.	1.5×10^{10}	2/5	2/5	1/5	1/5	1/5	1/5	4/5	3/5	2/5
positive control	5	i.v.	1.5×10^{10}	0/5	0/5	0/5	0/5	0/5	3/5	2/5	4/5	1/5
negative control	5	i.v.	1.5×10^{10}	5/5	5/5	3/5	2/5	2/5	0/5	5/5	1/5	4/5
blank control	5	i.v.	1.5×10^{10}	5/5	5/5	1/5	4/5	1/5	0/5	5/5	0/5	5/5

[a] Landrace piglets from a herd free of sly^+ epf^+ mrp^+cps 2 and cps 7 *S. suis* strains.
[b] The highly virulent serotype 2 strain (mrp^+ epf^+ sly^+ cps 2) and the moderately virulent serotype 7 strain (mrp* sly^+ cps7) were used in homologous and heterologous challenge experiments, respectively.
[c] No. of piglets affected/total no. of piglets.
[d] Max., maximum. White blood cell (WBC) counts were performed on days 2, 4, 6, and 10 postinfection. All piglets had WBCs below 20 × 10^9/liter preinfection.
[e] i.v., intravenous.
[f] CNS, central nervous system.

3. Results

3.1. Satety test

Piglets were infected with 2×10^8 CFU and 4×10^8 CFU of Δ*SspepO*/Δ*SspspC*. A week after the challenge, the body temperature of the piglets normalized rapidly after a slight increase by 1 °C, feeding of piglets was normal (Fig. 1).

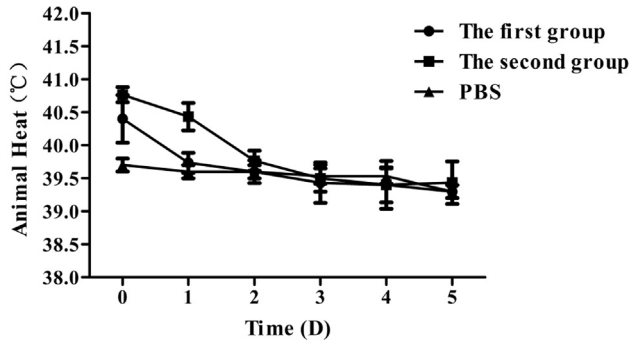

Fig. 1. The safety test for piglets infected with the Δ*SsPepO*/Δ*SsPspC* strain and PBS. Piglets were infected with 2×10^8 CFU (the first group) and 4×10^8 CFU (the second group) of Δ*SsPepO*/Δ*SsPspC*. The body temperature of piglets was recorded daily.

3.2. Seroconversion of piglets immunized with ΔSspepO/ΔSspspC

Immunization of piglets with Δ*SspepO*/Δ*SspspC* elicited a marked antibody response (Fig. 2A). The live vaccine-elicited IgG (IgG1, IgG2a) titers were significantly higher than those in the PBS control and blank control (Fig. 2B). IgG2a titers predominated over IgG1 titers, suggesting a bias towards a Th1-type immune response induced by Δ*SspepO*/Δ*SspspC* ($P < 0.01$; Fig. 2B).

3.3. Piglet whole-blood bacterial killing assay

We investigated whether immunization with Δ*SspepO*/Δ*SspspC* induced the production of a bactericidal or an opsonizing antibody against live SC19 in vitro. Antiserum against Δ*SsPepO*/Δ*SsPspC* and inactive vaccine (positive control) significantly inhibited the growth of *S. suis* 2 compared with the negative and blank control groups ($P < 0.001$; Fig. 3). There was a 55% reduction in *S.suis* 2 viability after incubation with sera of immune piglets, suggesting that the antibodies induced by Δ*SspepO*/Δ*SspspC* and the inactive

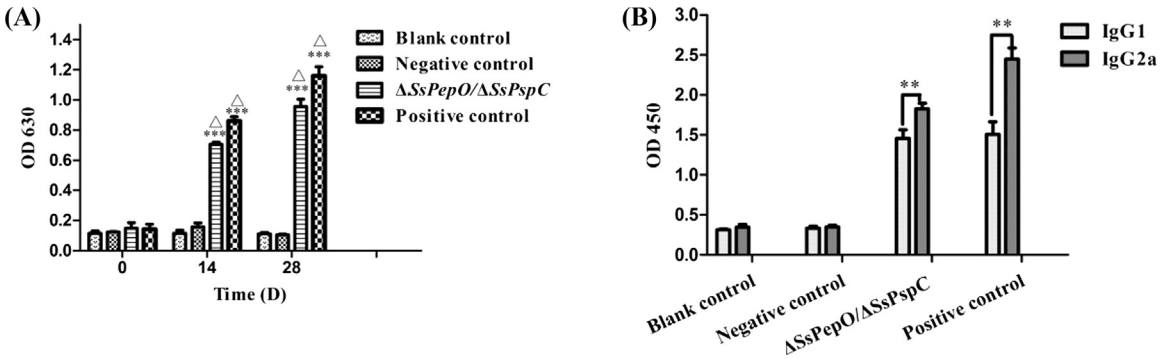

Fig. 2. Seroconversion of piglets immunized with the Δ*SspepO*/Δ*SspspC* double-mutant strain. (A) Pig serum IgG titers against Δ*SspepO*/Δ*SspspC* were examined via an enzyme-linked immunosorbent assay (ELISA). (B) Effect of the Δ*SspepO*/Δ*SspspC* on IgG1 and IgG2a titers in piglets determined via ELISA. The results are expressed as the mean specific absorbance ± SD values. **P < 0.01; ***P < 0.001; △, versus the blank control and negative control. Negative control were injected intramusculary with PBS. Blank control as a blank check. Positive control were vaccinated commercial *S.suis* 2 and 7 inactive vaccines (Keqian, Wuhan, Hubei, China).

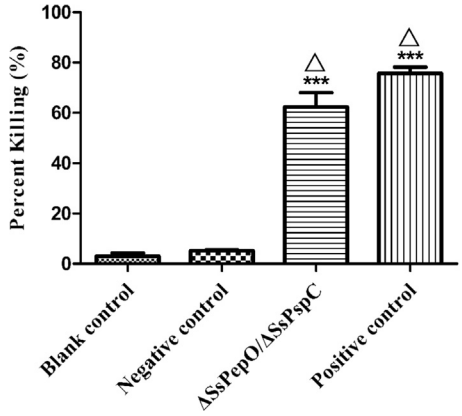

Fig. 3. Whole-blood bacterial killing activity of specific antibodies elicited in piglets via challenge with Δ*SsPepO*/Δ*SsPspC*. The results are expressed as the percentage bacterial killing activity in accordance with the percentage reduced bacterial growth in the immunized serum. Results were considered positive in cases of bacterial killing > 50% in at least one test. Results are expressed in pg/mL and represent the mean ± SD values of three independent assays. ***P < 0.001. △, versus the blank control and negative control. Negative control were injected intramusculary with PBS. Blank control as a blank check. Positive control were vaccinated commercial *S.suis* 2 and 7 inactive vaccines (Keqian, Wuhan, Hubei, China).

vaccine (positive control) potentially conferred immunoprotection against the *S. suis* 2 and 7 challenges.

3.4. Challenge of piglets with the serotype 2 and serotype 7

The live Δ*SspepO*/Δ*SspspC* strain vaccination trial was conducted in this study to evaluate the protective efficacy against *S. suis* serotypes 2 and 7 of the same clonal complex. The Δ*SspepO*/Δ*SspspC* strain markedly protected against morbidity following the challenge with serotypes 2 and 7, compared to those in the PBS control and blank control group (Table 1, Fig. 4A, and Fig. 4B). Two of five vaccinated piglets developed a fever, reduced food intake, and other clinical signs of disease or fibrinosuppurative lesions (Table 1 and Table 2). The challenge strain was isolated from at least one internal organ of all but one diseased piglets, always being associated with fibrinosuppurative inflammations. Isolation of the challenge strain from seven different tissues was achieved for one of five animals of the PBS control and blank control groups; however, none of those from the Δ*SspepO*/Δ*SspspC* and inactive vaccine (positive control)-immunized group. For one of five healthy piglets from the Δ*SspepO*/Δ*SspspC*-immunized group, the challenge strain was detected in the lungs but not in other tissues (Table 3). In general, pathohistological, clinical, and

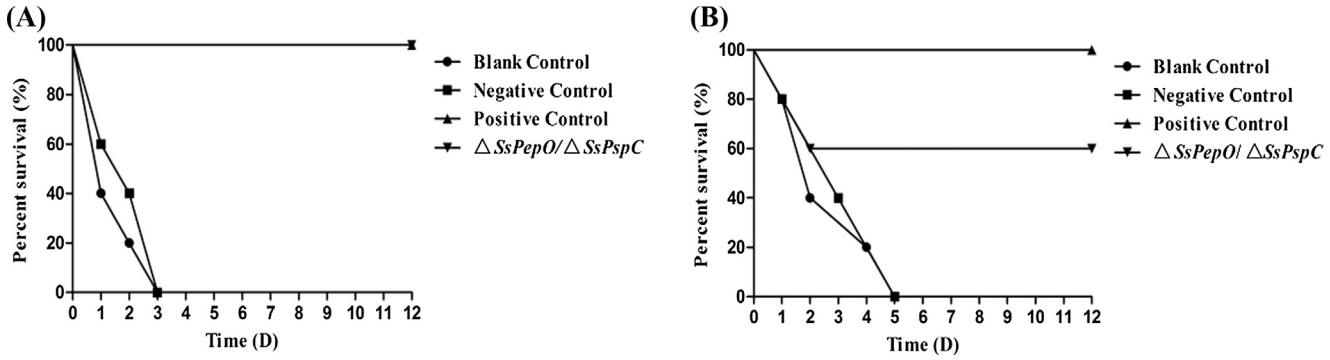

Fig. 4. Immunoprotective effects mediated by Δ*SsPepO*/Δ*SsPspC* challenge of piglets with serotypes 2 and 7. (A) Survival of mice immunized with the double-mutant Δ*SsPepO*/Δ*SsPspC*, inactive vaccine (positive control), and PBS (negative control) after infection with *Streptococcus suis* 2. (B) Survival of mice immunized with the double-mutant Δ*SsPepO*/Δ*SsPspC*, inactive vaccine (positive control), and PBS (negative control) following infection with *S. suis* 7. *S. suis* 2 was isolated from the brain of a diseased piglet collected in Sichuan outbreak in China in 2005 (Accession NO. MNPY00000000 in GenBank). *S. suis* 7 is a typical China strain isolated from a diseased pig. Negative control were injected intramusculary with PBS. Blank control as a blank check. Positive control were vaccinated commercial *S.suis* 2 and 7 inactive vaccines (Keqian, Wuhan, Hubei, China).

Table 2
Scoring of fibrinosuppurative lesions of piglets infected with *Streptococcus suis* strain 2 or 7 after immunization with Δ*SsPepO*/Δ*SsPspC*.

S.suis challenge serotype and immunization antigen	No. of piglets	No. of piglets with condition and score[a] /Total no. of piglets													
		Meningitis and/ or Chorioiditis			Pleuritis or Peritonitis			Synovialitis			Splenitis[b] or hepatitis			Pneumonia		
		5	3	1	4	2	1	4	2	1	4	2	1	4	2	1
Serotype 2																
Δ*SsPepO*/Δ*SsPspC*	5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	1/5
positive control	5	0/5	0/5	2/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	2/5	1/5
negative control	5	2/5	3/5	0/5	1/5	0/5	1/5	3/5	2/5	0/5	2/5	3/5	0/5	4/5	0/5	1/5
blank control	5	3/5	1/5	1/5	1/5	0/5	1/5	3/5	1/5	1/5	1/5	4/5	0/5	5/5	0/5	0/5
Serotype 7																
Δ*SsPepO*/Δ*SsPspC*	5	1/5	1/5	0/5	1/5	0/5	1/5	1/5	1/5	0/5	2/5	0/5	0/5	2/5	1/5	1/5
positive control	5	0/5	0/5	2/5	0/5	1/5	1/5	0/5	1/5	0/5	0/5	1/5	1/5	0/5	2/5	1/5
negative control	5	3/5	2/5	0/5	1/5	0/5	2/5	4/5[c]	1/5	0/5	3/5	2/5	0/5	3/5	2/5	0/5
blank control	5	1/5	2/5	2/5	1/5	0/5	1/5	3/5[c]	1/5	1/5	4/5	1/5	0/5	5/5	0/5	0/5

[a] Scores of 4 and 5 indicate moderate to severe diffuse or multifocal fibrinosuppurative inflammations. Scores of 2 and 3 indicate mild focal fibrinosuppurative inflammation.
[b] Neutrophil accumulation of the splenic red pulp.
[c] For one animal in each group, a score of 4 was assigned owing to high numbers of neutrophils in the smear of the joint fluid.

Table 3
Reisolation of the *Streptococcus suis* challenge strain from pigs immunized with Δ*SsPepO*/Δ*SsPspC* and then reinfected.

S.suis challenge serotype and immunization antigen	No. of piglets	*S.suis* challenge		No. of piglets with indicated site of the *S.suis* challenge strain[a] isolation /total no. of piglets						
		Route of administration[b]	CFU	Tonsils	Lung[c]	Serosa[d]	Spleen	Liver	CSF[e]	Joint fluid[f]
Serotype 2										
Δ*SsPepO*/Δ*SsPspC*	5	i.v.	10^6	0/5	1/5	0/5	0/5	0/5	0/5	0/5
positive control	5	i.v.	10^6	0/5	1/5	0/5	0/5	0/5	0/5	0/5
negative control	5	i.v.	10^6	3/5	5/5	2/5	2/5	1/5	2/5	3/5
blank control	5	i.v.	10^6	2/5	5/5	1/5	2/5	2/5	3/5	3/5
Serotype 7										
Δ*SsPepO*/Δ*SsPspC*	5	i.v.	1.5×10^{10}	1/5	3/5	1/5	2/5	0/5	1/5	2/5
positive control	5	i.v.	1.5×10^{10}	0/5	1/5	0/5	0/5	0/5	0/5	0/5
negative control	5	i.v.	1.5×10^{10}	2/5	5/5	1/5	1/5	2/5	3/5	3/5
blank control	5	i.v.	1.5×10^{10}	1/5	5/5	2/5	1/5	2/5	2/5	2/5

[a] Challenge strains were identified as described in Materials and Methods.
[b] i.v., intravenous.
[c] One cranial lobe was assessed.
[d] Pleural, peritoneal, or pericardial cavity.
[e] CSF, cerebrospinal fluid.
[f] One tarsal puncture was assessed in each animal. In cases of lameness, additional joint punctures were screened.

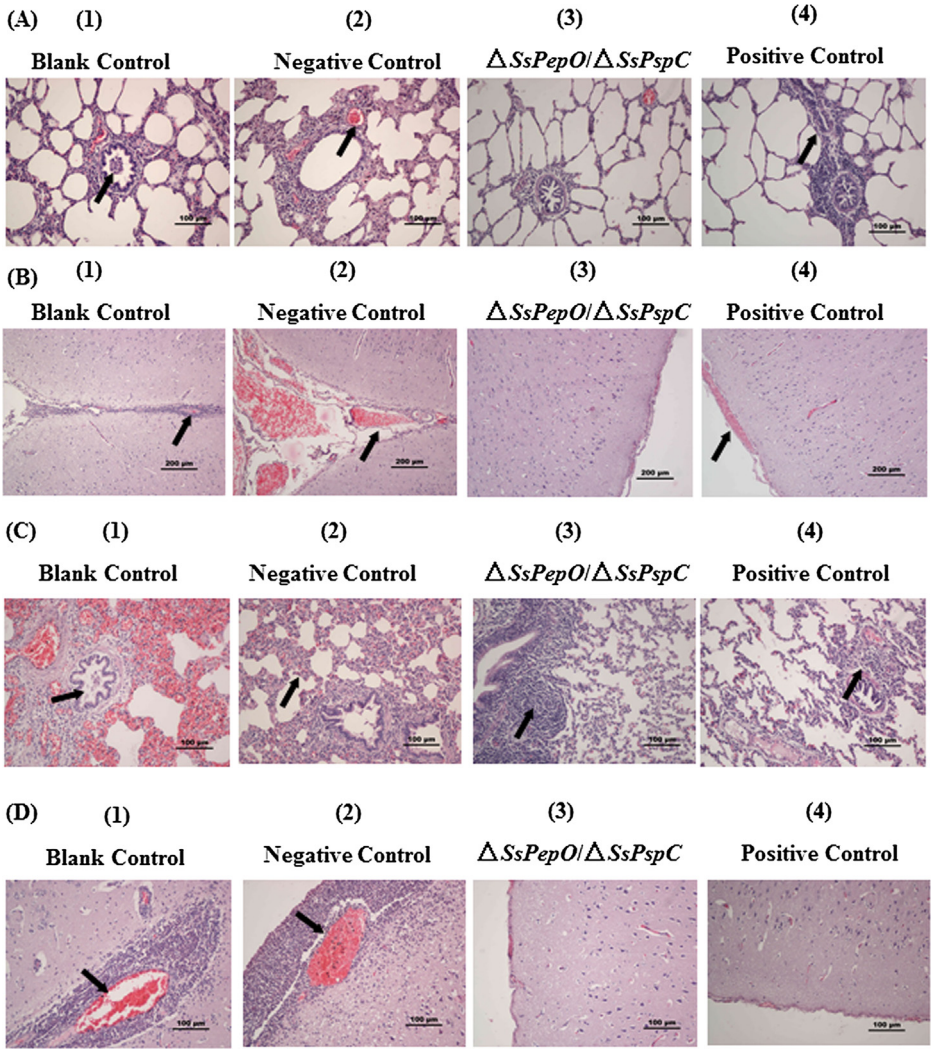

Fig. 5. Histopathological analysis of the lung and brain tissues from piglets immunized with ΔSsPepO/ΔSsPspC and the inactive vaccine (positive control) challenged with *S. suis* serotypes 2 and 7. (A) and (C) Lung sections from the blank control group and the negative control group displayed mild hyperemia, alveolar space and pulmonary interstitial slight leakage, alveolar septum thickening, serous effusion, and monocyte and lymphocyte infiltration, whereas sections from the Δ*SsPepO*/Δ*SsPspC*-immunized group exhibited only minor pathological changes. (B) and (D) Brain sections from the blank and negative control groups exhibited severely thickened, meningeal hemorrhage, a mass of inflammatory cell infiltration, and dissolved nerve cells. No prominent changes were observed in the double-mutant Δ*SsPepO*/Δ*SsPspC*. Negative control sera from PBS-inoculated animals. *S. suis* 2 was isolated from the brain of a diseased piglet collected in Sichuan outbreak in China in 2005 (Accession NO. MNPY00000000 in GenBank). *S. suis* 7 is a typical China strain isolated from a diseased pig. Negative control were injected intramusculary with PBS. Blank control as a blank check. Positive control were vaccinated commercial *S.suis* 2 and 7 inactive vaccines (Keqian, Wuhan, Hubei, China).

bacteriological screening indicated protective immunity against serotypes 2 and 7 in the Δ*SspepO*/Δ*SspspC* strain and inactive vaccine (positive control)-immunized group, but not in the PBS control and blank control groups.

Histopathological analysis

Histopathological analysis was carried out to examine the pathological changes in the brain and lungs of the infected pigs. Histopathological analysis revealed mild hyperemia, alveolar space and pulmonary interstitial slight leakage, alveolar septum thickening, serous effusion, and monocyte and lymphocyte infiltration in the lung tissues in the PBS control and blank control groups, compared with the Δ*SspepO*/Δ*SspspC*- and inactive vaccine (positive control)-immunized groups against serotypes 2 (Fig. 5A) and 7 (Fig. 5C). The meninges of pigs in the negative and blank control groups were severely thickened and meningeal hemorrhage, a mass of inflammatory cells infiltration, and dissolved neurons were observed, while the meninges of Δ*SspepO*/Δ*SspspC* and inactive vaccine (positive control)-immunized groups showed no obvious changes upon challenge with serotypes 2 (Fig. 5B) and 7 (Fig. 5D).

4. Discussion

For swine, a safe and effective vaccine for *S. suis* infections is still not available, notwithstanding numerous trial vaccines or patents [20,21,23]. Effective control of infections by different serotypes in pigs is also deterred by low levels of cross-protection, particularly when inactivated or subunit vaccines are used [13,22,23]. In this study, we evaluated *S. suis* vaccine candidates against two most important porcine pathogens in China, a highly virulent homologous serotype 2 strain and a heterologous serotype 7 strain. We previously reported that the Δ*SspepO*/Δ*SspspC* strain effectively protected mice against virulent strain *S. suis* 2 [13].

The piglets were intramuscularly injected with approximately 4×10^8 CFU of Δ*SspepO*/Δ*SspspC*; antibodies were produced a week after immunization and their titers, especially IgG2a, were significantly higher after booster immunization. Whole-blood bacterial killing assays indicated that antiserum against Δ*SspepO*/Δ*SspspC* exerted germicidal and phagocytic effects. Fourteen days after the second boost, piglets were challenged with virulent *S. suis* serotypes 2 and 7. Survival of piglets immunized with Δ*SspepO*/Δ*SspspC* against *S. suis* serotypes 2 and 7 were 100% and 60%, respectively. Compared to the negative and blank control groups, pathological changes and lung injury index of immune group were obviously lighter. Moreover, no obvious pathological changes were observed in the immunized group after the challenge.

This study is the first, to our knowledge, to demonstrate that Δ*SspepO*/Δ*SspspC* can induce immunoprotection against serotypes 2 and 7 strains. Therefore, future studies are required to investigate the mechanism underlying the Δ*SspepO*/Δ*SspspC*-induced immunity would yield essential information for the development of an inexpensive vaccine against *S. suis* infections. Furthermore, studies are required to establish diagnostic methods of *Streptococcus suis*-related diseases. Such advancements will facilitate the prevention and control of severe invasive diseases.

5. Conflict of interest statement

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled.

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