Immunogenicity and protective efficacy of recombinant *Haemophilus parasuis* SH0165 putative outer membrane proteins

Shulin Fu, Minmin Zhang, Juan Xu, Jiwen Ou, Yan Wang, Huazhen Liu, Jinlin Liu, Huan Chun, Weicheng Bei

**A R T I C L E   I N F O**

Article history:
Received 3 June 2012
Received in revised form 30 October 2012
Accepted 1 November 2012
Available online xxx

Keywords:
Recombinant proteins
*Haemophilus parasuis* SH0165
Outer membrane proteins
Immune responses
Protection

**A B S T R A C T**

*Haemophilus parasuis* (H. parasuis), the causative agent of swine polyarthritis, polyarthritis, and meningitis, is one of the most important bacterial diseases of pigs worldwide. Little vaccines currently exist that have a significant effect on infections with all pathogenic serovars of *H. parasuis*. *H. parasuis* putative outer membrane proteins (OMPs) are potentially essential components of the causative microorganism. The genomic sequence of *H. parasuis* serovar 5 strain SH0165 was completed in our laboratory, which allows us to target OMPs for the development of recombinant vaccines. In this study, we focused on 10 putative OMPs and all the putative OMPs were cloned, expressed and purified as HIS fusion proteins. Primary screening for immunoprotective potential was performed in mice challenged with an LD50 challenge. Out of these 10 OMPs three fusion proteins rGAPDH, rOpaA, and rHPS-0675 were found to be protective in a mouse model of *H. parasuis* infection. We further evaluated the immune responses and protective efficacy of rGAPDH, rOpaA, and rHPS-0675 in pig models. All three proteins elicited humoral antibody responses and conferred different levels of protection against challenge with a lethal dose of *H. parasuis* SH0165 in pig models. In addition, the antisera against the three individual proteins and the synergistic protein efficiently inhibited bacterial growth in a whole blood assay. The data demonstrated that the three proteins showed high value individually and the combination of rGAPDH, rOpaA, and rHPS-0675 offered the best protection. Our results indicate that rGAPDH, rOpaA, and rHPS-0675 induced protection against *H. parasuis* SH0165 infection, which may facilitate the development of a multi-component vaccine.

**1. Introduction**

*Haemophilus parasuis*, is the causative agent of Glässer’s disease in swine. There are currently no effective vaccines available that cover protection against all pathogenic serovars of *H. parasuis* [1]. So far 15 *H. parasuis* serovars have been described, but up to 25% of the isolates in some countries cannot be typed [2,3]. There have been no comprehensive studies of the correlation between serovar and pathogenicity of organism in conventional pigs [4]. To date, only a few virulence factors have been reported in relation to the pathogenicity of Glässer’s disease, but it is known that a lipooligosacharide of *H. parasuis* is responsible for endotoxic shock and the side effects [2,5]. The presence of a capsule is frequently correlated with virulence [6], and specific outer membrane profiles [7,8] and fimbrae [9] have been linked to the virulence of this microorganism. The currently commercially available inactivated vaccine confers protection against homologous challenge [10], but further research is needed to develop a new generation of vaccine that can stimulate long-term immunity and provide cross-protection against infections by several *H. parasuis* serovars.

The conventional approach to vaccine development depends on the identification of protective antigens for use as subunit vaccines [11]. A study has shown that outer membrane components contain good protective antigens [12]. Various *H. parasuis* outer membrane proteins (OMP), such as OmpP1, OmpP2, and OmpP5, have been cloned, and PaA, OmpP2, D15, and HPS-06257 have strong potential as vaccine candidates [13]. Although the results of protection experiments in mice with some OMPs have yielded promising results [13], a combination of key antigens and/or epitopes is likely to be more efficacious against multiple *H. parasuis* serovars, especially in outbred animals.

© 2012 Elsevier Ltd. All rights reserved.

Please cite this article in press as: Fu S, et al. Immunogenicity and protective efficacy of recombinant *Haemophilus parasuis* SH0165 putative outer membrane proteins. Vaccine (2012), http://dx.doi.org/10.1016/j.vaccine.2012.11.003
The sequencing of *H. parasuis* SH0165 has been completed by our group [14]. The current knowledge of the genomic sequence of pathogenic bacteria has facilitated recent vaccine development [11]. Because OMPs are the primary bacterial components that interact with host cells, targeting OMPs for the development of recombinant vaccines is of value.

In the current study, we focused on 10 putative OMPs which were predicted by SignalP 2.0 software. They were cloned, expressed, and purified as HIS fusion proteins, and initially screened for protective efficacy in a mouse model of infection by *H. parasuis*. The rGAPDH, rOapA, and rHPS-0675 which had good immunoprotection were re-evaluated separately or mixed as one group, as well as in combination in a piglet model.

2. Materials and methods

2.1. Bacterial strains, media, and plasmids

The *H. parasuis* SH0165 used in this study which is a highly virulent strain of serovar 5 was isolated from lung of a commercial pig with hemorrhagic pneumonia, fibrinous polyserositis, arthritis and meningitis. The SH0165 was grown in Tryptic soy broth (TSB; Difco Laboratories, USA) supplemented with 1 mol/L NAD (Sigma, USA) and 10% newborn calf serum (Gibco, USA). The highly virulent serovars of the 15 reference strains are serovar 1, 5, 10, 12, 13, 14 and the moderately virulent serovars are serovar 2, 4, 15 and the non-virulent serovars are serovar 3, 6, 7, 8, 9 and 11 [15]. *Escherichia coli* was grown in Luria-Bertani (LB) broth or agar (Difco) at 37 °C in the presence of 25 mg/mL kanamycin (Sigma, USA) when necessary.

2.2. Purification of recombinant proteins

Primers for high-fidelity PCR amplification of putative OMPs were designed and given in supplementary table 1. The insert fragment was sub-cloned into the pET-28a expression vector.

Supplementary material related to this article found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2012.11.003.

*E. coli* BL21 (DE3) (OD<sub>600</sub> = 0.6) were induced with 1 mM IPTG (Ameresco, USA). A pilot experiment was conducted and the molecular mass of the expressed recombinant protein was assessed by SDS–PAGE. Mass cultivation was performed in 1 L of LB broth with kanamycin (25 mg/mL). The proteins were purified, as previously described with some minor modifications [16]. Briefly, the cell pellet was suspended in 25 mL of buffer solution (50 mM Tris–HCl at pH 8.0, 500 mM NaCl and 1 mM PMSF) and disrupted in a French pressure cell. After centrifugation at 12,000 rpm for 15 min at 4 °C, the supernatant was mixed with 10 mL of Ni<sup>2+</sup>–NTA agarose (Qiagen, Germany) in a column. Proteins not bound to Ni-NTA agarose were removed using 150 mL binding buffer (50 mM Tris–HCl, pH 8.0, 500 mM NaCl, and 5 mM imidazole). The target protein was eluted by 10 mL elution buffer (50 mM Tris–HCl, pH 8.0, 500 mM NaCl, and 500 mM imidazole) and stored at −20 °C for further study.

2.3. Protection of 10 putative OMPs in mice

This study was carried out in strict accordance with the recommendations in the China Regulations for the Administration of Experimental Animals 1988 and the Hubei Regulations for the Administration of Affairs Concerning Experimental Animals 2005. The protocol was approved by China Hubei Province Science and Technology Department (Permit Number: SYXK(ER) 2010-0029). The animals were euthanized at the end of the experiments or when moribund during the experiment.

Prior to immunization, the proteins were mixed with Marcol 52 adjuvant (ESSO). Mice were immunized subcutaneously with recombinant proteins (200 μL, 50 μg), PBS (200 μL) emulsified in the same adjuvant as a negative control, and PBS only as a blank control. Subsequent booster immunizations were given on day 14 using the same antigen. Immunized mice were challenged intraperitoneally with 5LD<sub>50</sub> (LD<sub>50</sub> = 1.2 × 10<sup>6</sup>) of 6.0 × 10<sup>6</sup> *H. parasuis* SH0165. All mice were monitored for 14 days post-challenge, and sacrificed on day 28 when blood was collected by cardiac puncture.

2.4. Immunization and challenge experiments in piglets

The immunogenicity and protective efficiency experiments were carried out in the piglet model, as previously described with some modifications [13]. The piglets used in this study were sero-negative for *H. parasuis* by INGEZIM Haemophilus 11.HPS.K1 (INGEZIM, Spain). Seventy-eight piglets (30 days old) were randomly assigned to 6 groups of 13 each. Each purified recombinant protein (600 μg in 0.8 mL) was mixed with a 1.5-fold volume of Marcol 52 adjuvant, and used to immunize piglets in groups 1–3. The rGAPDH, rOapA, and rHPS-0675 (200 μg each) were mixed and used in combination in group 4. Piglets in group 5 were immunized with 0.8 mL of PBS emulsified in the same adjuvant and served as a negative control. Piglets in group 6 were inoculated with PBS only and used as a blank control. All groups were immunized by the intramuscular injection route on day 0. Subsequent booster immunizations were given on day 14 using the same antigen.

Blood samples were obtained by pre-caval vein bleeding on day 14 after the booster immunization for immunologic assays. Then all piglets were challenged with a lethal dose of 1.5 × 10<sup>10</sup>CFU (log-phase) of *H. parasuis* SH0165 in 2 mL of PBS by intranasal inoculation. All piglets were monitored for 14 days post-challenge, and morbidity and mortality were recorded. On day 14 post-challenge, all surviving piglets were euthanized. The lung, heart, and brain tissues were removed from each euthanized piglet for re-isolating bacteria.

2.5. Determination of antibody titers

Serum from immunized piglets was examined by enzyme linked immunosorbent assay (ELISA) for IgG titers, as described previously [13]. Briefly, 96-well microtiter plates were coated with purified rGAPDH (0.97 μg), or rOapA (1.12 μg), or rHPS-0675 (0.84 μg) or combination proteins (2.12 μg) and diluted in sodium carbonate buffer (pH 9.6) at 4 °C overnight. The plates were saturated with 150 μL of 0.5% skim milk for 1 h at 37 °C, then rinsed with washing buffer. Serially diluted pig sera (initially 1:100) were added and incubated for 30 min at 37 °C. Anti-porcine IgG-HRP (Southern Biotech, USA), diluted 1:5000 in washing buffer, was used as the secondary antibody and incubated for 30 min at 37 °C. For measuring IgG subclass, coated plates were subsequently incubated with dilutions of pig sera for 1 h at 37 °C. The wells were washed three times and incubated with 100 μL of mouse anti-pig IgG1-HRP (AbD Serotec, UK) or IgG2a-HRP (AbD Serotec, UK) diluted 1:5000 in PBS–Tween-20. The plates were washed 3 times, then the color was developed by adding 100 μL of the activated substrate solution (sodium citrate buffer containing 1 mg/mL of 3,3,5,5-tetramethylbenzidine and 0.03% H<sub>2</sub>O<sub>2</sub>) and incubating in the dark for 10 min. The reaction was stopped by adding 50 μL of 0.25% hydrofluoric acid to each well. The plates were read with a plate reader (Bio-Tek Instruments) at an absorbance of 630 nm. Antibody titer was calculated as the serum dilution that gave an OD630 of 0.3 above that of the preimmune sera.

Please cite this article in press as: Fu S, et al. Immunogenicity and protective efficacy of recombinant *Haemophilus parasuis* SH0165 putative outer membrane proteins. Vaccine (2012), http://dx.doi.org/10.1016/j.vaccine.2012.11.003
2.6. Lymphocyte proliferation

Lymphoproliferation assays were performed, as previously described with minor modifications [17]. Three piglets from each group were sacrificed on the 14th day after the last immunization, and the spleens were isolated as described previously [18]. Briefly, spleens were harvested aseptically and processed by gentle disruption with sterile stainless steel sieve and glass pestle and suspension of splenocytes in RPMI incomplete medium (Gibco, USA). Cell suspensions were centrifuged for 10 min at 200 × g. Erythrocytes were lysed by treatment with 0.84% ammonium sulfate for 10 min on ice. Then the cells were washed three times with Hank’s Balanced Salt Solution (HBSS) (Hyclone, USA), resuspended in complete RPMI medium (Gibco, USA). Two hundred microliters of cells were cultured (1 × 10^5 cells/mL) in 96-well culture plates. The cells were stimulated with the recombinant proteins and concanavalin A (5 ng/well) (WAKO, Japan) in vitro and incubated for 72 h at 37 °C in a 5% CO₂ incubator. Lymphoproliferation assays were performed using MTS reagent with a cell proliferation kit (Promega, USA), as per the manufacturer’s instructions. The lymphocytes were incubated in 96-well culture plates with MTS reagent for 4 h, and the absorbance was measured at 490 nm using an ELISA reader.

2.7. Determination of cytokines by ELISA

The supernatants from cell cultures were harvested after 72 h and stored at −80 °C. The levels of IL-2, IL-4, and IFN-γ were determined in culture supernatants using an ELISA kit (R&D, America), as per the manufacturer’s instructions.

2.8. Whole blood killing bacteria assay

The whole blood killing bacteria assay was determined, as previously described with minor modifications [13,19]. The bacterial cultures were thrice-washed with sterilized and diluted with PBS. A 10-μL dilution containing 10^6 bacterial cells was mixed with 190 μL of each inactivated serum sample to be tested which had been diluted 50% by sterilized PBS while the control contained the PBS only. The dilution was first incubated for 30 min at 37 °C and placed on ice. Then, non-immune, heparinized (10U/mL) piglet whole blood (100 μL) was added and the mixture was incubated at 37 °C with shaking for 1 h. The bacteria were plated on TSA containing 10% newborn calf serum and 1 μM/mL of NAD. The bacteria colonies were counted after 24 h. The results were expressed as percent killing, according to the following formula: (CFU after 1 h of growth with control serum) – (CFU after 1 h of growth with immune serum)/CFU after 1 h of growth with control serum × 100%. The results were determined from three independent assays.

2.9. Histopathology

The lungs and brains from immunized and non-immunized piglets were fixed by immersion in 10% neutral-buffered formalin embedded in paraffin, and then 4-μm-wide tissue sections were stained with H&E according to a standard protocol and examined under light microscopy.

2.10. Statistical analysis

The experimental data were expressed as mean±SD. The difference among two groups was analyzed using Student’s t-test and the difference among three groups or above was analyzed using the ANOVA and survival analysis was used the Logrank test.

### Table 1

<table>
<thead>
<tr>
<th>No.</th>
<th>Group</th>
<th>No. of surviving animals/total no. of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>rcdB</td>
<td>3/8 (37.5%)</td>
</tr>
<tr>
<td>2</td>
<td>rGAPDH</td>
<td>6/8 (75%)</td>
</tr>
<tr>
<td>3</td>
<td>rldG</td>
<td>1/8 (12.5%)</td>
</tr>
<tr>
<td>4</td>
<td>rOapA</td>
<td>7/8 (87.5%)</td>
</tr>
<tr>
<td>5</td>
<td>rOmpP1</td>
<td>1/8 (12.5%)</td>
</tr>
<tr>
<td>6</td>
<td>rHPS-0220</td>
<td>3/8 (37.5%)</td>
</tr>
<tr>
<td>7</td>
<td>rHPS-0675</td>
<td>6/8 (75%)</td>
</tr>
<tr>
<td>8</td>
<td>rHPS-1406</td>
<td>3/8 (37.5%)</td>
</tr>
<tr>
<td>9</td>
<td>rHPS-1834</td>
<td>3/8 (37.5%)</td>
</tr>
<tr>
<td>10</td>
<td>rHPS-2037</td>
<td>4/8 (50%)</td>
</tr>
<tr>
<td>11</td>
<td>Negative control</td>
<td>0/8 (0%)</td>
</tr>
<tr>
<td>12</td>
<td>Blank control</td>
<td>0/8 (0%)</td>
</tr>
</tbody>
</table>

p values of <0.05 were considered significant. *p < 0.05; **p < 0.01 and ***p < 0.001.

3. Results

3.1. Expression and purification of putative OMPs as HIS fusion proteins

The 10 possible OMPs all cloned and expressed as HIS fusion proteins (supplementary table 1). Each purified recombinant protein migrated as a single band during SDS–PAGE (Supplemental Fig. S1). And we investigated the distribution of 10 genes in all 15 serovars reference strains. The results showed that 10 genes are widely present in all reference strains (Supplemental Fig. S2).

Supplementary material related to this article found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2012.11.003.

Supplementary material related to this article found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2012.11.003.

3.2. Effect of protection of 10 putative recombinant proteins in mice

Table 1 depicts the protective efficacy of 10 proteins against lethal challenge by SH0165. The results showed that rGAPDH, rOapA, and rHPS-0675 provided 75%, 87.5%, and 75% protection, respectively, compared with the negative control group (adjuvant) and the blank control (PBS) after challenging with 5LD₅₀ of H. parasuis SH0165 (p < 0.05). The other recombinant proteins did not afford significant immune protection (Table 1) (survival rate was ≤50%). Therefore, the purpose of our subsequent work was to further evaluate the immunogenicity and protective efficacy of rGAPDH, rOapA, and rHPS-0675 alone and in combination in a piglet model.

3.3. Antibody responses to three recombinant proteins in piglets

The results presented in Fig. 1A clearly demonstrated that significant IgG antibody levels were detected against each antigen after immunization compared with the adjuvant or PBS groups (p < 0.01).

To reveal the type of immune response, the IgG1 and IgG2a subclasses were further determined. The results demonstrated that the levels of antibody isotypes (IgG1, IgG2a) were significantly higher in the rGAPDH, rOapA, rHPS-0675 and the combination protein (rGAPDH–OapA–HPS–0675) immunized groups compared to the blank control group and negative control group (IgG1, p < 0.0001; IgG2a, p < 0.0001) (Fig. 1B). Additionally, IgG2a responses predominated over IgG1 responses (p < 0.01) (Fig. 1B).

Please cite this article in press as: Fu S, et al. Immunogenicity and protective efficacy of recombinant Haemophilus parasuis SH0165 putative outer membrane proteins. Vaccine (2012), http://dx.doi.org/10.1016/j.vaccine.2012.11.003
3.4. Cellular immune response

As shown in Fig. 2A, a significant proliferative T-cell immune response was noted in the four groups of piglets immunized with three recombinant proteins (p < 0.001). Similarly, a strong T cell proliferative response was determined to concanavalin A (Con A) as a positive control (p < 0.001). However, the negative and blank control groups did not elicit a proliferative T-cell response (Fig. 2A).

The levels of IFN-γ and IL-2 from supernatants of splenocytes stimulated with recombinant protein were significantly higher in the piglets vaccinated with recombinant proteins when compared with the negative and blank control groups (Fig. 2B, p < 0.001; Fig. 2C, p < 0.01). However, there was no rise in IL-4 levels (data not shown). The results indicated that immunization of piglets with rGAPDH, rOapA, and rHPS-0675 triggered a Th1-type immune response.

3.5. Piglet whole blood killing bacteria assay

The survival of H. parasuis SH0165 was significantly attenuated in the whole blood of pigs immunized with recombinant proteins. To determine whether or not the proteins elicit antibody that is bactericidal and/or opsonic, the growth of H. parasuis SH0165 in non-immune pig blood treated with anti-protein and control sera (non-immune pig serum) was assessed. While H. parasuis SH0165 treated with non-immune pig serum grew well in non-immune pig blood, growth of H. parasuis SH0165 treated with protein-immune pig serum was clearly reduced (p < 0.001) (data not shown).
The piglet whole blood against rGAPDH, rHPS-0675, or rGAPDH-OapA-HPS-0675 significantly inhibited *H. parasuis* growth *in vitro* (>50%) (*p* < 0.001), but the piglet whole blood against the rOapA inhibition ratio was lower than others (Fig. 3). The inhibition ratio of the piglet whole blood against rGAPDH-OapA-HPS-0675 was higher than the other immunized groups (rGAPDH, *p* = 0.0149; rOapA, *p* = 0.0055; rHPS-0675, *p* = 0.0010) (Fig. 3).

The results of bactericidal activity of anti-protein serum showed that the serum against rGAPDH, rHPS-0675, or rOapA could inhibit the 15 reference strains growth *in vitro* compared with the negative control (*p* < 0.001) (Fig. S3).

Supplemental material related to this article found, in the online version, at [http://dx.doi.org/10.1016/j.vaccine.2012.11.003](http://dx.doi.org/10.1016/j.vaccine.2012.11.003).

### 3.6. Protective efficacy in pigs

The results of immunization and challenge experiments showed that rGAPDH, rOapA, and rHPS-0675 provided 70%, 50%, and 70% protection, respectively, compared with the negative and blank control groups (*p* < 0.05) (Fig. 4). The morbidity of all pigs in the negative and blank control groups was 100% within 48 hour and all piglets in the negative and blank control groups died within 4 days after being challenged with *H. parasuis* SH0165, but the rGAPDH-, rOapA-, and rHPS-0675-immunized groups had a higher effect within the observation days (*p* < 0.05) (Fig. 4).

Compared with the individual recombinant proteins, immunization with the combined protein (rGAPDH-OapA-HPS-0675) provided 80% protection against a lethal dose *H. parasuis* SH0165 challenge (*p* < 0.05) (Fig. 4). No bacteria were isolated from the piglets that survived on day 14 post-challenge.

### 3.7. Histopathologic analysis

All of the piglets which received adjuvant (negative control group) and blank control group developed severe lung and brain tissue damage, while all survival pigs from immunization groups did not appear significant pathological damage. The lung parenchyma and pleura were edematous, with massive proliferation of fibroblasts (Fig. 5B3). The bronchioles were filled with cellular exudates comprised of neutrophils (Fig. 5B3). The brain parenchyma was congested with marked edema (Fig. 5A3). The moribund pigs from immunization groups presented fibrosus pneumonia and meningitis symptoms (data not shown). However, only minor pathological changes were observed in lung tissues and brain of pigs immunization with the recombinant protein (Fig. 5A4 and B4). These findings suggested that immunization with recombinant protein prevents pathological changes following challenge infection with *H. parasuis* and confirm that the recombinant protein induces immunoprotection against infection of *H. parasuis* in pig.

### 4. Discussion

*H. parasuis* has recently re-emerged as one of the major causes of nursery mortality in pigs [20]. The pathogenicity of *H. parasuis* in piglets depends on the serovar. In our study, we used the Chinese local isolate, SH0165 (serovar 5) strain, which is a high virulent strain as infection strain and it is possible in according with the actual situation of infection. As far, no commercial vaccines with a significant effect on disease control against all serovars strains are available. Many recombinant vaccine candidates based on OMPs in *H. parasuis*, such as VtaA, PalA and native outer membrane proteins, are protein-based vaccines that have been evaluated for potential use [10,12,13]. Considering the wide variety of serovars of *H. parasuis* and the host specificity, it is tempting to screen more protecive antigens to develop a multi-component vaccine against heterologous infections. In our work, the results showed that the 10 genes widely existed in the 15 reference serovars strains and the 3 putative outer membrane proteins (GAPDH, HPS-0675, OapA) encoded by the genes produce immune protection against serovar 5 of *H. parasuis*. Furthermore, another three protective antigens (SmpA, YgIM, FOG) were screened in our group in the *H. parasuis* SH0165 strain using the same method in a mouse model infection [21]. And in this study at the same time we have evaluated the protective potential of recombinant proteins using a piglet model. The piglet is an ideal model of Glässer’s disease, and also allows us to study the protective efficacy against heterologous challenge. Although only 3 antigens in this study were screened and there are other studies with OMP as vaccines for *H. parasuis*, our results pave the way for targeting putative OMPs for the development of a recombinant vaccine.
GAPDH is a glycolytic enzyme and participates in the generation of bacterial energy, which is essential for growth in the absence of neoglucogenic substrates [22,23]. Research has shown that the surface-displayed GAPDH of *A. hydrophila* can effectively protect turbot from infections with *A. hydrophila* and *V. anguillarum* [24]. Opacity-associated protein A (OapA) is involved in the transparent clone phenotype of *H. influenzae* [25], and has a role in *H. influenzae* binding to human conjunctival epithelial cells [26]. The relevant IgG antibody subclass and Th type are critical for protection against a particular disease. The IgG1 and IgG2a subclasses have been determined to be markers of T helper 2 (Th2) and Th1 immune responses, respectively [27]. In this study, we found that the proteins induced significant humoral immune responses. The blood killing bacteria assay showed that antiserum against proteins significantly inhibited SH0165 growth. These findings suggest that antibody-mediated immune responses are likely to play an important role in immunoprotection.

Some previous studies showed that serum-resistance is a virulence mechanism in *H. parasuis* causing systemic disease in swine and non-virulent strains were sensitive to the bactericidal effect of the serum while virulent strains were mainly resistant [28], but there are existing difference between our experimental results and theirs. In our study, either non-virulent strains or virulent strains could be killed by the antiserum with different degrees, but non-virulent strains did not show more sensitive to the antiserum than the virulent strains. We speculate that the difference of serum-resistance may be caused by different epitopes of the proteins.

Together, our data showed that a combined vaccine (rGAPDH-OapA-HPS-0675) of *H. parasuis* was able to provide effective protection against *H. parasuis* serovar 5 infection. Further research is needed to understand the protective mechanisms which will eventually lead to the development of a multi-component vaccine in piglets.

Acknowledgements

This research is supported by grants from The National Basic Research Program (also called 973 Program) (No. 2012CB518805), National Nature Science Foundation of China (Nos. 30970109 and 31172352), National Programs for High Technology Research and Development of China (No. 2011AA10A210), the Hubei Province Technology Program (No. 2011BB0082) and the Foundation for Innovative Research Groups of the National Natural Science Foundation of China (No. 31121004).

References


Please cite this article in press as: Fu S, et al. Immunogenicity and protective efficacy of recombinant *Haemophilus parasuis* SH0165 putative outer membrane proteins. Vaccine (2012). http://dx.doi.org/10.1016/j.vaccine.2012.11.003


