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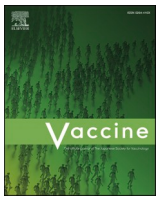

Sow vaccination with a novel recombinant protein vaccine protects piglets against *Streptococcus suis* infection

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ABSTRACT

Streptococcus suis is a severe zoonotic pathogen affecting weaned piglets. No commercial vaccine that provides protection against *S. suis* is available. A prototype vaccine, tentatively called VASIP (Vaccine Against *Streptococcus suis* Infection in Pigs), composed of five recombinant fusion proteins, encompassing 23 different protein domains, was used in this study. Pregnant sows were vaccinated on three occasions, at 68, 47 and 19 days prior to farrowing, resulting in high antibody levels, both in sera and in colostrum. Antibodies were transferred to the litter via colostrum. The litters from VASIP-vaccinated and placebo-vaccinated sows were challenged intravenously with *S. suis* at four or seven weeks of age in two different arms of the study. Body temperature and clinical signs (demeanour, behavioural CNS, and mobility) of infection showed that piglets from vaccinated sows were significantly protected against *S. suis* infection in the 4-week-old group and that the incidence of severe clinical signs was lower in the 7-week-old group compared with piglets from placebo sows. The study demonstrates the feasibility of vaccinating sows, rather than piglets, using recombinant fusion proteins to maximise protection against *S. suis* during the period in which they are most at risk of disease.

1. Introduction

Streptococcus suis is an important endemic swine pathogen found worldwide, which causes meningitis, septicemia, arthritis, and endocarditis in weaned piglets between 4 and 10 weeks of age [1]. Mortality rates can be as high as 20 % in some farms where co-infection with other pathogens may influence the severity of *S. suis* infection [2]. *S. suis* naturally colonises the mucosal surfaces of adult pigs, including the upper respiratory tract of the sow, without causing disease [3,4], and may be transferred to piglets in their first few days of life. The onset of disease in piglets coincides with a decline in naturally occurring maternal protective antibodies that were acquired from colostrum, providing a challenge for conventional vaccine approaches that rely on the vaccination of piglets.

S. suis is a zoonotic pathogen and may be transferred to humans by direct contact, particularly in countries with high density of pigs [5–7]. Several serotypes of *S. suis* exist, based on different capsular polysaccharides [8], with varying levels of pathogenicity and worldwide distribution [9]. Serotype 2 strains are recognised as the most prevalent cause of disease in both pigs and humans [10].

An extensive use of antibiotics in the pig industry as growth promoters [11] is believed to have been responsible for the emergence of antimicrobial resistance in *S. suis* [12,13]. There is a high risk of such resistance being transferred to other bacterial species [13]. However, phasing out antibiotics, other than for therapeutic use, requires the introduction of new measures to prevent *S. suis* disease. Vaccination, in combination with improved management, is an obvious strategy and the development of an effective vaccine against *S. suis* infections is highly desired. There are no commercial and widely used vaccines against *S. suis* on the market, but extensive research is going on in the area

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[14,15]. Autogenous vaccines based on herd-specific isolates of *S. suis*, which are killed and prepared for vaccine use, are used by some farmers. However, trials with autogenous vaccines have provided inconclusive results regarding both efficacy and safety [16], and the duration of antibodies transferred from vaccinated sows to piglets via colostrum may not be of sufficient duration to provide protection for piglets post-weaning [17–19]. The use of recombinant proteins as vaccines has been investigated in various models, including murine models [20–27], a rabbit model [28], and in the pig [29–35]. However, comparisons across these studies are difficult due to variations in challenge models, clinical scoring methods, and the vaccination strategies used [36].

In this study, we used a recombinant fusion protein vaccine comprised of 23 different protein fragments, thereby maximizing the number of bacterial targets towards addressing the diversity of pathogenic strains. Furthermore, to provide protection during the critical period immediately post-weaning, we vaccinated sows and measured the passive transfer of immunity provided via colostrum. Significant protective effects were demonstrated at four weeks after birth, highlighting the potential of this approach.

2. Materials and methods

2.1. Study design and animals

Eight pregnant sows were examined by a veterinarian to confirm that they were in good health on arrival to the facilities at Moredun Scientific. They were randomly allocated to groups 1 or 2. **Group 1**: After seven days of acclimatization, four sows were vaccinated via intramuscular injection with VASIP. **Group 2**: Four sows were given an adjuvant-only placebo vaccine as a control. Groups 1 and 2 were vaccinated with VASIP and placebo, respectively, 68 days before the expected farrowing (day 0 defined as the final day of farrowing; Fig. 1). A second and third dose of VASIP, or the placebo vaccine, was administered on days −47 and − 19 before farrowing on days −4 to 0. After farrowing, all piglets were helped to access teats and encouraged to consume as much colostrum as possible. **Group 3 and 5**: On day 23, 5 + 5 piglets from each of the 4 vaccinated sows were randomly allocated to group 3 (challenge at 4 weeks) and group 5 (challenge at 7 weeks) (n = 20 + 20). **Groups 4 and 6**: On day 23, 5 + 5 piglets from each of the 4 placebo-vaccinated control sows were randomly allocated to group 4 (challenge at 4 weeks) and group 6 (challenge at 7 weeks)(n = 20 + 20). Piglets in groups 3 and 4 were challenged with *S. suis* on day 28, at four weeks of age, and piglets in groups 5 and 6 were challenged on day 51, when they were seven weeks of age. Clinical observations of the piglets were conducted prior to the challenge, 4 h post-challenge, and then twice daily for 14 days following the challenge. In addition, further welfare observations were carried out as required.

2.2. Vaccine

The vaccine used in this study, VASIP, was comprised of 100 μg of each of five recombinant fusion proteins, A, B, C, D, and E, and 100 μg of

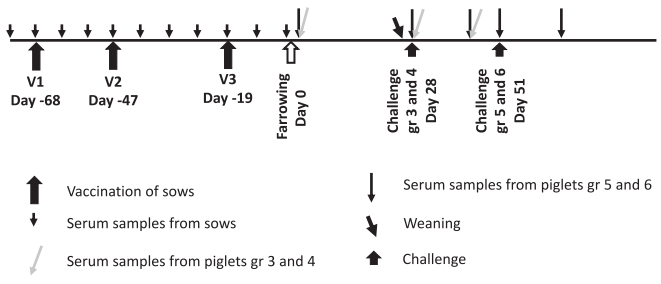

Fig. 1. Study design and time points for vaccination, sampling, farrowing and challenge.

Matrix-V adjuvant (Novavax, Gaithersburg, Md., U·S) in a volume of 1 ml. Each dose was given intramuscularly to the pregnant sows. The five recombinant fusion proteins used encompass 23 different domains of *S. suis* proteins, mainly localised on the bacterial surface. Details of these fragments and the rationale for their inclusion are given in Supplementary Table S1 and sequences in Supplementary Table S2. Supplementary Fig. S2 shows design of the fusions and lengths in amino acids.

2.3. Construction of gene fusions

Gene fragments were codon optimised and synthesised by GeneScript, individually or in some cases together. Fragments were amplified by PCR, so as to add suitable restriction sites. Fragments were then cleaved at these restriction sites and ligated together to obtain the correct reading frame. As intermediate vectors, pUC57 and pGex6P-1 were used. This procedure was done stepwise to add one fragment (or fragments) at each step to obtain each entire gene fusion. Final gene fusions were introduced into vector BmKny, cleaved by *Bam*HI and *Xho*I, and transformed into host strain *E. coli* BL21. Correct sequences in selected transformants were verified by sequencing. BmKny is an IPTG inducible expression vector, encoding kanamycin resistance for plasmid selection. For antigen E (3PCS), pGex6P-1 was used for expression as a GST-tagged fusion.

2.4. Induction of protein expression

E. coli BL21 cells containing the different constructs were grown at 37 °C in the presence of kanamycin to OD_{600nm} 0.5–1. IPTG was added to 50 μg/ml and the induction phase was carried out overnight with shaking at 25 °C.

2.5. Protein purification

Antigen A: The induced *E. coli* cells were centrifuged and resuspended in a solution containing 100 mM NaCl, 20 mM Tris pH 7.4 and 0.05 % Tween 20. Thereafter lysozyme was added to 20 μg/ml and the cells were repeatedly frozen/thawed until lysis. The lysate was sonicated and the debris was removed by centrifugation. The clarified lysate was sterile filtered (0.45 μm filter) and frozen in aliquots. Eighty ml clarified lysate was thawed and 2.4 g Allantoin was added to bind endotoxin. The Allantoin crystals with bound endotoxins were then removed by centrifugation. The treated lysate was bound to Q-sepharose in 100 mM NaCl, 20 mM Tris pH 7.4 and 0.05 % Tween 20. Washing was performed in 150 mM NaCl and 20 mM Tris pH 7.4. The elution was done in two steps, first in 250 mM NaCl and 20 mM Tris pH 7.4 and then in 300 mM NaCl and 20 mM Tris pH 7.4. The eluted protein was dialysed against 20 mM Tris-HCl pH 7.5 and 100 mM NaCl.

Antigen B: Harvesting and lysis was performed as for Antigen A with the exception thatwhen the cells were lysed, the solution was boiled for 15 min, cooled to room temperature and the cell debris was removed by centrifugation. The clarified lysate was sterile filtered (0.45 μm filter) and frozen in aliquots until purification of the protein was performed by ion exchange chromatography (Q Sepharose). Binding was performed in 50 mM NaCl and 20 mM Tris pH 7.4 and the elution was done in 300 mM NaCl and 20 mM Tris pH 7.4. The eluted protein was dialysed against 20 mM Tris-HCl pH 7.5 and 100 mM NaCl.

Antigen C: Harvesting, lysis, boiling and adsorption to Q Sepharose was done as described for Antigen B. Elution was done with 200 mM NaCl 20 mM Tris pH 7.4. Dialysis was.

performed in 10 mM Tris-HCl pH 7.4.

Antigen D: The same procedure as for Antigen C was used, except that elution from Q Sepharose was with 200 mM and 250 mM NaCl and 20 mM Tris pH 7.4. The eluted fractions were then pooled.

Antigen E: The same procedure for harvesting, lysis and clarification as for Antigen A was used. For affinity purification, glutathione Sepharose was used. Prescission protease was used to cleave Antigen E

from the GST-tag. Dialysis was performed in 20 mM Tris-HCl pH 7.4 and 100 mM NaCl.

Finally, all proteins were sterile filtered using a 0.20 μm filter and frozen until use.

2.6. Serological analysis

Sera were collected from sows on days −75, −68, −61, −54, −47, −40, −33, −26, −19, −13, and at the time of farrowing on days −4 to 0. Sera were collected from piglets in groups 3 and 4 on days 1, 28 and 42 and from piglets in groups 5 and 6 on days 1, 28, 42, 51 and 65 (or at the time of euthanasia if this was earlier). Blood was collected in non-heparinised tubes and clotted blood samples were centrifuged at 2000x*g* for 20 min. Antibodies against the combined antigens in VASIP were analyzed by ELISA as follows: Microtiter 96-well plates (Nunc) were coated with 100 μl of VASIP (each protein at 4 μg/ml, in phosphate buffered saline (PBS)) overnight at room temperature, then blocked for one hour at 37 °C with the addition of 100 μl of 2 % bovine serum albumin (BSA, Sigma, Art # A4503-100G) in PBS. After washing with PBS supplemented with 0.05 % Tween20 (PBST), 100 μl sera in PBST were added in a 2-fold serial dilution. Samples were incubated for two hours at 37 °C and washed with PBST, followed by the addition of 100 μl anti-pig-IgG antibodies conjugated with HRP (Sigma, Art # A5670-1ML) diluted 10,000 x in PBST. After one hour of incubation at 37 °C, the plates were washed with PBST. The detection of bound anti-pig antibodies was performed with SIGMAFAST™ OPD (Sigma, Art # P9187), according to the manufacturer's protocol, and the absorbance at 492 nm measured using a spectrophotometer. Serum titers were expressed as the log of the dilution required to obtain an absorbance of 1.5, which is at the linear part of the titration curve. Antibody levels against each of the five fusion proteins in VASIP were measured individually using the same procedure.

Whey was prepared from colostrum samples by adding approximately 20 μl rennet (Langdales, Lot: PL211B1, E: Jun22) to 1 ml of colostrum. The suspension was thoroughly mixed and incubated at +37 °C (± 2 °C) for 1 to 1.5 h. Each sample was then centrifuged at approximately 16,000 x*g* for 3 min, and the whey fraction was collected and analyzed for antibody levels using the same procedure as for sera.

2.7. Challenge with S. suis

The *S. suis* isolate (23/P0278/02/11) of serotype 2 (recovered from a three-months old piglet with neurological signs in England during 2011) was grown overnight at 37 °C on 5 % sheep blood agar plates and transferred to 3 ml vegetable peptone broth (VPB) supplemented with 3 % (*v*/v) horse serum in bijoux bottles to a turbidity of 1.5 McFarland units (McF) (density measured using a Densitometer (BioMerieux, Marcy-l'Étoile, France)). Each 3 ml volume was then added to 97 ml of pre-warmed supplemented VPB and the culture was incubated for 4 (± 0.5) hours at +37 °C (± 2 °C) on an orbital shaker set at 150 rpm. After incubation, the turbidity of the culture was recorded (target was between 3.0 and 4.0 McF). 10 ml of the culture was diluted in 90 ml of supplemented VPB to obtain a target concentration of 5×10^7 cfu/ml. On day 28, each piglet in groups 3 and 4, and on day 51, each piglet in groups 5 and 6 was challenged by the administration of 2 ml of 5×10^7 cfu/ml *S. suis* into the jugular vein.

2.8. Clinical observations

Piglets were weighed on days 23, 28, 35 and 42 (all groups), as well as on days 51 and 65 (groups 5 and 6). Clinical observations included assessments of demeanour, behavioural/central nervous system changes, mobility and rectal temperature (°C) according to a scoring system shown in Supplementary Table S3. Clinical observations were performed twice daily for 14 days following challenge. The piglets were euthanased 14 days post-challenge, or earlier on welfare grounds, using a lethal injection of Pentobarbitone Sodium BP (Vet) 20 % *w/v* by the i.v. or i.p. route. Subsequently, post-mortem examinations were performed to check for signs of infection.

2.9. Statistics

Fisher's exact test was used for comparing incidence of an observation and the Mann-Whitney test was used to analyze time to a particular observation and for comparison of clinical scoring. Antibody levels and body weights were compared using the Students *t*-test. A Log rank test was used for the "survival plot" in Fig. 6. A difference was considered significant if the *p*-value was below 0.05.

2.10. Ethical considerations

All animal procedures complied with the UK Animal (Scientific Procedures) Act 1986. Work was conducted under Home Office License Number PFA7E7AD6 (Aug 17th 2021). Animals showing unacceptable reactions were treated or euthanased as required to alleviate distress.

3. Results

3.1. Antibody responses

Antibody levels against the combined five fusion proteins in VASIP increased significantly in vaccinated sows compared with those that received the placebo vaccine, with $p \leq 0.001$ for each day starting two weeks after the first vaccination (Fig. 2). A slight booster effect in the antibody response was observed after the second vaccination (V2), but no further increase was noted after the third vaccination (V3). Similarly, antibody levels against the five individual fusion proteins in VASIP increased significantly in sows vaccinated with VASIP compared to those vaccinated with the placebo, with $p < 0.006$ from two weeks after the first vaccination (Fig. S1).

Whey was prepared from colostrum collected from two of the four sows vaccinated with VASIP and three of the placebo-vaccinated sows. There was a significant difference in antibody titers measured in colostrum between sows vaccinated with VASIP relative to placebo-vaccinated sows ($p = 0.012$). Antibody levels in colostrum were comparable to those measured in the sera from sows in groups 1 and 2, reflecting their vaccination status (Fig. 2).

One day post-birth, serum antibody levels in piglets from sows vaccinated with VASIP were comparable to those in the sera and colostrum samples obtained from the vaccinated sows. The antibody responses to the components of VASIP in sera from piglets of placebo-vaccinated sows was equivalent to those measured in these sows. However, the piglets had significantly lower levels of antibodies compared to those from VASIP-vaccinated sows and their piglets. Although the antibodies transferred to the piglets waned over time, levels in piglets from sows vaccinated with VASIP consistently remained above those of piglets from placebo-vaccinated sows ($p \leq 0.004$).

3.2. Clinical observations

The clinical scoring consisted of demeanour, behaviour/CNS, mobility and rectal temperature (Supplementary Table 3). Average clinical scores for each observation time point are shown in Fig. 3 for groups 3 and 4 (challenge at 4 weeks) (*p*-values for demeanour, behaviour/CNS, mobility, and TCS were 0.005, 0.011, 0.00001, and 0.0001, respectively), and Fig. 4 for groups 5 and 6 (challenge at 7 weeks) (not significant). The number of animals that were euthanased prematurely before the study end point in groups 3, 4, 5, and 6 were 9, 12, 12 and 15, respectively.

The time to develop a demeanour score of 1 was significantly longer in the four-week study in piglets in group 3 (from VASIP-vaccinated sows) compared with those in group 4 (from placebo vaccinated sows)

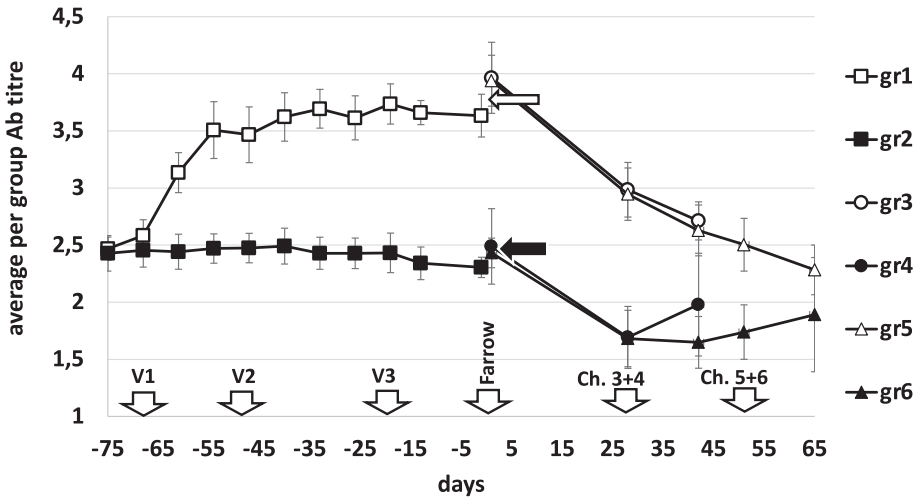

Fig. 2. Antibody responses in sera against all antigens in VASIP in vaccinated ($n = 4$) and placebo-vaccinated sows (n = 4) (open and closed squares, respectively). Arrows show antibodies in whey (open and closed from vaccinated and placebo-vaccinated, respectively). Antibodies transferred from VASIP-vaccinated sows to the litter in groups 3 (from VASIP vaccinated sows and challenged at 4 weeks)($n = 20$) and 5 (from VASIP vaccinated sows and challenged at 7 weeks) (n = 20) are shown by open circles and triangles, respectively. Antibodies transferred from placebo-vaccinated sows to the litter in groups 4 (from placebo vaccinated sows and challenged at 4 weeks) (n = 20) and 6 (from placebo vaccinated sows and challenged at 7 weeks) (n = 20) are shown by closed circles and triangles, respectively. Mean and SD are shown.

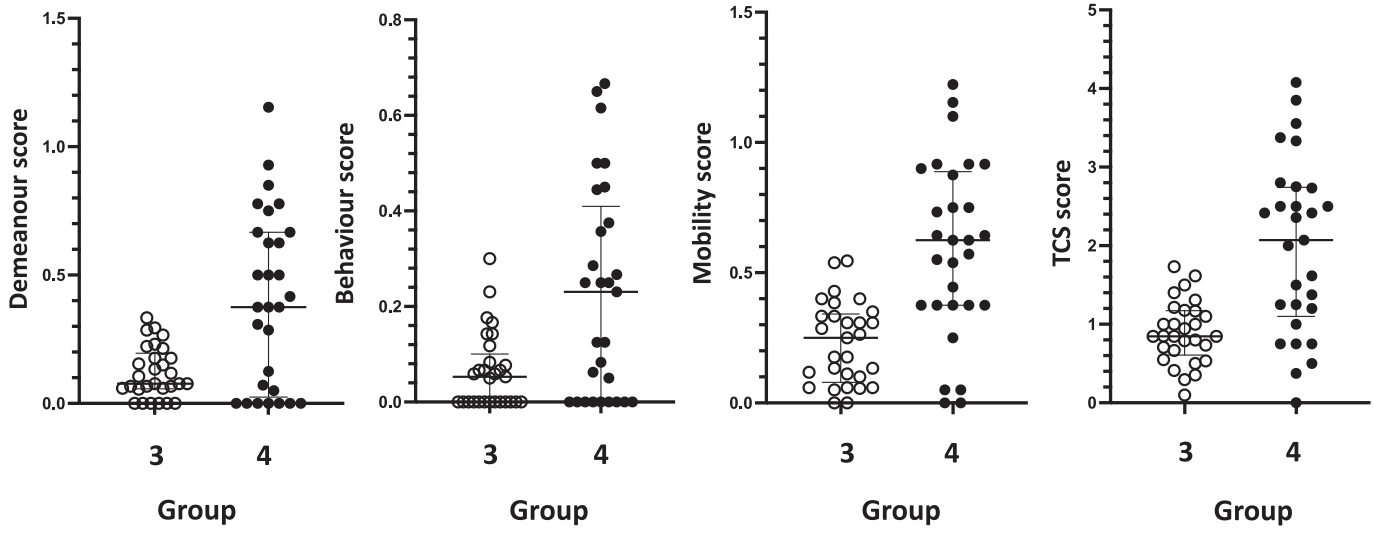

Fig. 3. Average clinical scoring (demeanour, behaviour/CNS, mobility and TCS) at each observation time for groups 3 and 4. The median and interquartile range for groups 3 and 4 across observation timepoints are shown.

($p = 0.00008$). The incidence of developing a demeanour score of 1 was lower in group 3 than in group 4 (15 and 20 respectively) ($p = 0.047$). There were 9 piglets in group 3 and 16 piglets in group 4 developing a demeanour score of 2 or above ($p = 0.048$). In the seven-week study, groups 5 (from VASIP-vaccinated sows) and 6 (from placebo vaccinated sows) also differed in that severe demeanour with a score of 3 was found in two piglets in group 5 and nine piglets in group 6 ($p = 0.03$).

The time to develop a behaviour/CNS score of 1 was significantly longer in piglets in group 3 compared to group 4 ($p = 0.015$). The incidence of developing a behaviour/CNS score of 1 or above was lower in group 3 than in group 4 (11 and 20, resp.) ($p = 0.0012$). In piglets challenged at 7 weeks of age, the onset of the development of a behaviour/CNS score of 1 or above was also significantly longer in piglets from vaccinated sows ($p = 0.008$). There was a tendency of lower incidence of any behaviour/CNS signs in group 5 compared with group 6 (9 and 16 resp.) ($p = 0.11$). There were four piglets in Group 5 and ten in Group 6 that developed a severe behaviour/CNS score of 2 or 3 ($p = 0.09$).

The time to develop a mobility score of 2 or 3 was significantly longer in group 3 compared to group 4 ($p = 0.017$). The incidence of having a mobility score of 2 or 3 was lower in group 3 than in group 4 (8 and 17 resp.)($p = 0.008$). In piglets challenged at 7 weeks of age, the onset of a mobility score was not significantly different between the groups. However, only three piglets in group 5 had a score of 2 or 3 compared with 10 in group 6 ($p = 0.04$).

Total clinical score (TCS) (adding scoring from rectal temperature, demeanour, behaviour/CNS and mobility with maximum score of 12) further demonstrated the protective effect of antibodies from vaccinated sows. Group 3 and 4 had 9 and 19 piglets, respectively, reaching a TCS of 5 or above ($p = 0.0012$). The average number of observation points (observations made twice daily) to reach a TCS of 5 were 20.7 and 8.4 for groups 3 and 4, respectively ($p = 0.00046$). The incidence of reaching a more severe TCS of 7 was lower in group 5 compared with group 6 (8 and 16 respectively) ($p = 0.023$) although the time to reach a

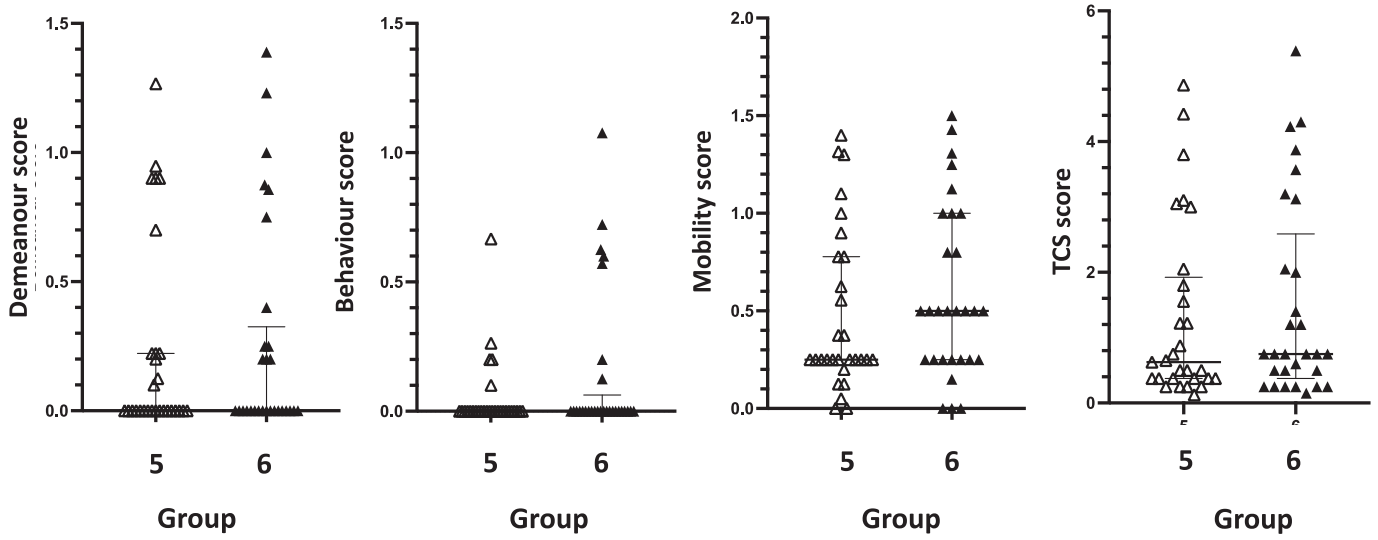

Fig. 4. Average clinical scoring (demeanour, behaviour/CNS, mobility and TCS) at each observation time for groups 5 and 6. The median and interquartile range for groups 5 and 6 across observation timepoints are shown.

TCS of 7 did not differ between these groups.

Fig. 5 shows rectal temperature over time for groups 3, 4, 5, and 6. For diseased animals that were euthanased for welfare reasons, the last observation was carried forward. Fig. 6 shows a "survival" plot for groups 3 and 4 of the number of observations before piglets reached a rectal temperature of ≥40.5 °C or were euthanased on welfare grounds ($p = 0.00003$, Log rank test).

3.3. Body weight

The average weight gain during the challenge period was higher in piglets from VASIP-vaccinated sows compared to those from placebo-vaccinated sows. Piglets from VASIP-vaccinated sows in group 3 had an average weight gain of 30.9 %, (from 8.1 to 10.6 kg) compared to 10.1 % (from 8.9 to 9.8 kg) in piglets from group 4, which were from placebo-vaccinated sows ($p = 0.016$). The average weight gain during the challenge period of piglets in groups 5 and 6 were 22.0 % (from 16.8 to 20.5 kg) and 8.5 %, (from 17.6 to 19.1 kg), respectively ($p = 0.18$). The average birth weight for all piglets was 1.59 kg.

4. Discussion

Vaccination of sows with recombinant proteins derived from *Streptococcus suis* conferred significant protection to the litter against experimentally induced infection with *S. suis* at four weeks of age. Piglets in the seven-week-old group showed a significant lower incidence of severe TCS. In pigs, antibodies from the sow do not cross the placenta to reach the fetus. Instead, immune protection for a newborn piglet relies on antibodies obtained from colostrum. Vaccination of young piglets, although immune competent from a very early age, is far more cumbersome than vaccination of the sow. This strategy of vaccinating the sow has been adopted for some commercially available vaccines, thereby making them more user-friendly than those intended for piglets. The use of a recombinant protein to vaccinate sows, thereby protecting the litter, has recently been described [37].

The longevity of colostrum-derived protective antibodies in piglets

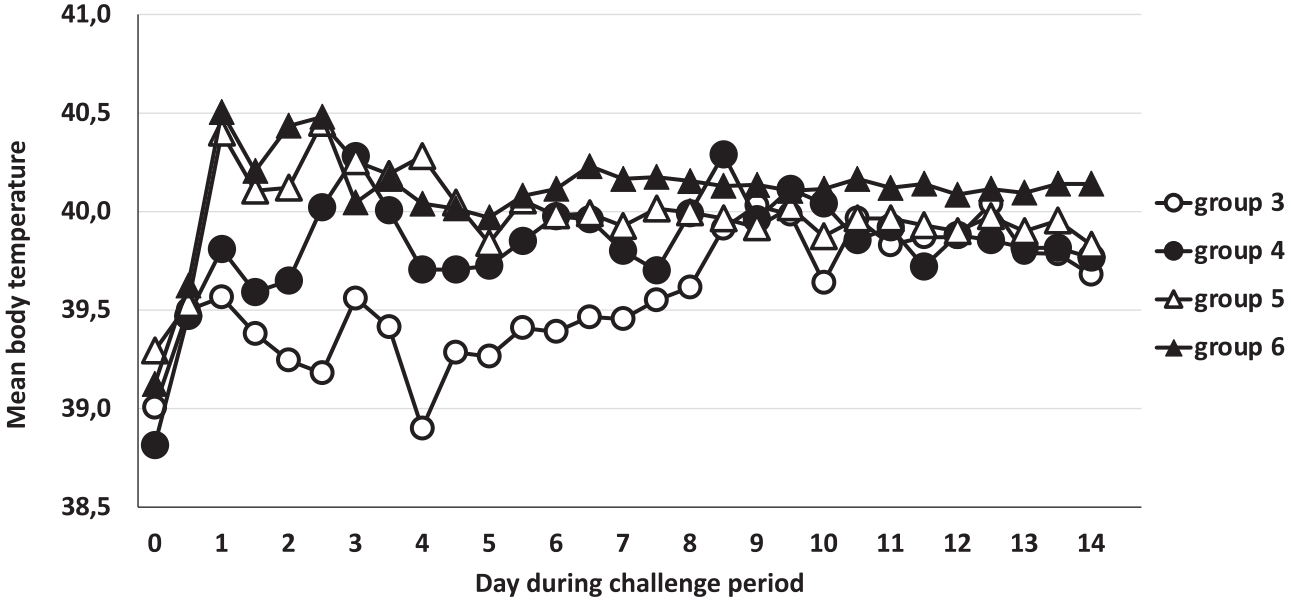

Fig. 5. Rectal temperature (mean values) of piglets. The last observation was carried forward for euthanased animals.

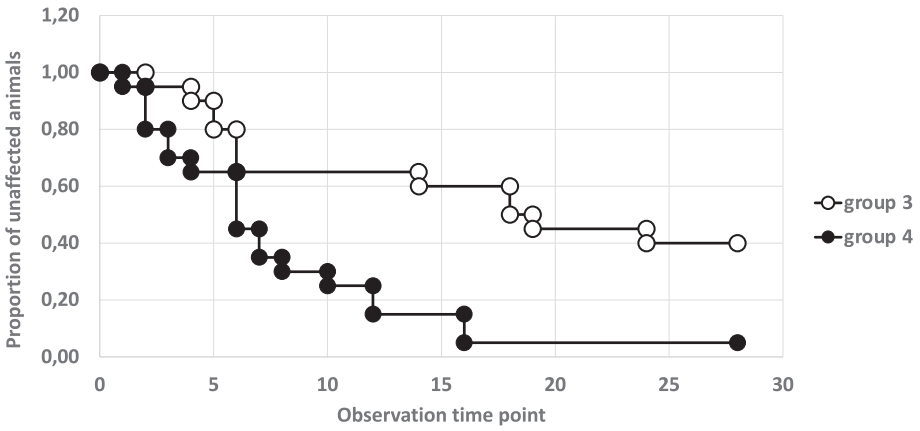

Fig. 6. Survival plot with the endpoint defined as affected by reaching a rectal temperature of 40.5 °C or euthanasia on welfare grounds. Observations were done twice daily; time point 28 thus means end of day 14.

from VASIP-vaccinated sows extended over at least four weeks showing a significant level of protection. At seven weeks of age protection was much less pronounced; antibodies led to reduced incidence of severe clinical signs (mobility score of 2–3, demeanour score of 3 and TCS of 7). The half-life of the maternally derived antibodies was approximately 9 to 11 days, as shown in Fig. 2. In a previous study on colostrum derived antibodies in piglets, we found similar rates of waning (Flock et al., unpublished). It should be noted that antibody levels in piglets (groups 4 and 6) from placebo-vaccinated sows were higher at birth than at time of challenge, with a decline that exhibited a similar half-life to that observed in piglets from VASIP-vaccinated sows, albeit starting from a much lower level. The antibody levels in placebo-vaccinated sows and their piglets are specific and result from the colonization of sows with *S. suis*. This natural antibody level is presumably sufficient to provide partial protection to newborn piglets before weaning, but its decline renders piglets from placebo-vaccinated sows, and in populations of piglets in the field, susceptible to *S. suis* infection by the time of weaning at three to four weeks of age.

The 23 domains of *S. suis* antigens used in the VASIP vaccine are fused together and produced as five recombinant proteins. The objective of including such a large number of domains is to target as many important *S. suis* proteins as possible and to maximise the level of cross-protection conferred to piglets against the diverse array of *S. suis* strains that cause disease on farms across the world. Furthermore, it is fair to expect that the multitude of antigens enhances the protective efficacy of the vaccine. These domains are derived from cell surface proteins, and antibodies against them may be opsonic and would thereby facilitate clearance of *S. suis* by the immune system, and block the biological functions of these proteins, which are known, or predicted, to be important to the virulence of *S. suis*. It was recently demonstrated that pig antibodies against recombinant Ide_{Ssuis} have a bactericidal effect in vitro and led to slightly prolonged onset of infection in challenged piglets [35]. Two regions of Ide_{Ssuis} are included in VASIP. The strategy employed here of using several recombinant antigens was used also by Liu et al. [27] who used 8 antigens. A good protection was obtained in that study in a murine model and antibodies were opsonic. Protocols for the purification of the proteins used in VASIP were developed to be as simple as possible, with a single chromatographic step, thereby being suitable for large-scale manufacturing. This recombinant fusion protein approach has also been utilised in the vaccine Strangvac, which contains eight important protein domains required for the virulence of *Streptococcus equi*. Strangvac has demonstrated high efficacy in clinical trials using a high challenge dose [38], and has shown great promise for the prevention of strangles in the field since its launch in Europe in 2022 [39].

The experimental infection model used in this study is severe and involved the administration of 10^8 colony forming units of a virulent strain of *S. suis* serotype 2 (ST1) via intravenous injection to induce systemic disease. Another widely used model of infection has been described [40] where the nasal mucosal surface was pre-treated with acetic acid, to improve the infection rate, followed by intranasal challenge. However, this approach may damage the molecular composition of the mucosal surface, including immunoglobulins. Of the piglets from placebo-vaccinated sows (n = 40) (groups 4 and 6), only one piglet remained free from clinical signs of infection (TCS exceeding a value of 3), resulting in an overall infection rate of 97.5 % (39 of 40 piglets from placebo-vaccinated sows). Combining data for piglets in groups 3 and 5 (n = 40), from vaccinated sows, there were 9 piglets free from clinical signs of infection, resulting in an infection rate of 77.5 % (31 of 40 piglets, p = 0.014, compared with groups 4 and 6 combined). The high infection rate aimed at in this study is required to minimize the number of animals included in the study, while allowing for the evaluation of protective effects in accordance with the principles of the 3Rs. However, infection in the field is likely to involve far fewer bacteria to which piglets are first exposed at an earlier age when the acquired antibody responses are higher. Therefore, it is reasonable to assume that better levels of efficacy will be achieved with the VASIP vaccine in the field than under the severe challenge conditions used in this study.

In the experimental setting used here, piglets were assisted to get equal amounts of colostrum to minimize variability between individuals since this was a controlled proof-of-principle study. However, in a natural situation intake of colostrum could vary significantly between animals and protection may differ substantially in field scenarios, where colostrum intake cannot be controlled or monitored.

The choice of adjuvant has been shown to influence the protection provided by autogenous vaccines [41]. Furthermore, immune response against autogenous vaccines also depends on the manufacturing [19]. Increased antibody response, induced by autogenous vaccination, improved maternal antibody transfer but did not extend beyond 5 weeks measured by opsonophagocytosis [19]. Future studies will address this aspect to optimise the potency of antibody responses induced by VASIP, enhancing their transfer to piglets to prolong protective immunity and maximise the cross-protective effects against diverse strains of *S. suis*.

In conclusion, a vaccine composed of recombinant bacterial surface proteins administered to pregnant sows conferred protection to piglets against experimentally induced *S. suis* infection.

CRediT authorship contribution statement

Sara Frosth: Writing – review & editing, Visualization, Validation, Investigation, Formal analysis, Data curation. **David Reddick:** Writing – review & editing, Validation, Project administration, Methodology, Investigation, Data curation. **Francesco Righetti:** Writing – review & editing, Methodology, Formal analysis. **Joakim Bjerketorp:** Writing –

review & editing, Investigation. **Karin Jacobsson:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Birgitta Henriques-Normark:** Writing – review & editing, Methodology, Formal analysis. **Magdalena Jacobson:** Writing – review & editing, Formal analysis. **Bengt Guss:** Writing – review & editing, Methodology, Funding acquisition, Formal analysis. **Tim Wood:** Writing – review & editing, Visualization, Formal analysis, Data curation. **Lars Frykberg:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Jan-Ingmar Flock:** Writing – review & editing, Writing – original draft, Visualization, Funding acquisition, Formal analysis, Data curation. **Andrew Waller:** Writing – review & editing, Validation, Project administration, Funding acquisition, Formal analysis, Data curation.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: This work was sponsored by Intervacc AB. J-IF is a board member of Intervacc AB and ASW is the CSO. The following authors are stakeholders of Intervacc AB: J-IF, ASW, BG, SF, JB and LF. The following authors are the authors for a patent (WO2023203238-A1) owned by Intervacc AB relevant for the vaccine described and used in this study: SF, KJ, JB, LF, BG, J-IF, ASW.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2025.127077.

Data availability

Data will be made available on request.

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