RESEARCH REPORT
SWINE HEALTH

Title: Efficacy of a *Streptococcus suis* vaccine for the prevention of *Streptococcus suis* infection. – **NPB# 00-131**

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ABSTRACT

A 2.0 kilobase-pair *EcoRI* DNA fragment of *Streptococcus suis* type 2, was previously cloned, sequenced and partially characterized. Coupled in vitro transcription and translation experiment, and western blotting using polyclonal antibody raised against whole-cell of *S. suis* type 2 revealed that the DNA region encodes a protein with an electrophoretic mobility of 38-kilodalton which appeared to be cell wall associated. The protein was overexpressed, purified and evaluated for the biological activity with respect to protection. Two groups of five pigs were vaccinated either with VAC-38, containing the purified recombinant 38-kilodalton protein derived from *Streptococcus suis* serotype 2 strain 1933, or with a placebo vaccine. On the day of challenge three of the 5 pigs that received the VAC-38 showed strong antibody titer against the protein as determined by western blotting whereas the remaining two had low titers. No antibody to the protein was detected pre- and post-vaccination in the placebo (control) groups. After challenge, the placebo vaccinated pigs developed severe clinical signs characterized by lameness involving several joints, a depressed appearance, high temperatures and /or neurological signs. Three of the pigs died 48 hours post challenge resulting in 60% mortality. The other two pigs fully recovered over time. One of the five pigs that gave low antibody titer in the vaccinated group suffered minor arthritis and slightly elevated temperatures but fully recovered with time whereas the three pigs that gave high antibody titer and another pig that gave a low antibody titer showed no clinical signs of disease resulting in zero percent mortality. This preliminary result suggest that the recombinant 38-kDa protein of *S. suis* could be a good candidate for the development of a recombinant subunit vaccine for the prevention of *S. suis* infection in pigs. The polymerase chain reaction (PCR) and western blot techniques showed that the gene encoding the 38 kDa protein is conserved and expressed among the *S. suis* strains tested encompassing several serotypes, suggesting that the vaccine may offer cross protection across capsular types.

INTRODUCTION

Streptococcus suis is a gram-positive, hemolytic and facultatively anaerobic coccus. Strains of this organism are divided into serotypes according to polysaccharide capsular antigens. Thirty five capsular serotypes (types 1 to 34 and 1/2) have been identified. Of the 35 serotypes, type 2 is the most frequently isolated serotype from pigs with disease. Strains belonging to other serotypes such as 1/2, 1, 7, 9, and 14 can also cause disease. *S. suis* outbreaks in pigs has been recognized worldwide. The disease conditions caused by *S. suis* type 2 include arthritis, meningitis, pneumonia, septicemia, endocarditis, encephalitis, polyserositis, abortions, and abscesses. In humans, *S. suis* type 2 can cause meningitis which can result in permanent deafness and septicemia (3). Given that the production of pork products represents an annual turnover of 50 million animals and 30 billion dollars per year in the United States losses of 1% of any given herd would represent a 300 million dollar loss per year, on a national scale, due to *S. suis* infection. Because the use of antibiotics in feed and drinking water has not successfully controlled the disease, and vaccination against the disease has been largely unsuccessful (3), more effective means of prevention and control are needed. Although antibiotic compounds are provided in the diets of weaned pigs to combat pathogens, including *S. suis,* the routine use of these substances has become increasingly controversial. Concerns about drug residues in processed meat, fears of increased antibiotic resistance in animal and human pathogens, and the possibility of increased regulation or withdrawal of these products from the market has prompted some segments of the meat industry to seek other avenues of control such as the use of new and second-generation vaccines. Moreover, prophylactic medication is not recommended due to the likelihood of selecting for resistant strains of *S. suis* within the herd. Better strategies for control and elimination of this pathogen would result in substantially increased productivity and profitability for swine farmers, and presumably lower consumer costs.

Identification, characterization and functional analysis of several proteins and their evaluation as a means for subunit vaccine development have been the subjects of considerable research in recent years. In addition, research on the genes encoding such proteins provides a good understanding at the molecular level, of events involved in protection. This is an important consideration because little is known about the virulence factors and protective antigens of *S. suis*.

One commonly followed route to make a vaccine against a bacterial disease is the production and testing of whole cell vaccine preparation (bacterin). This method has been done for *S. suis,* however the vaccine is serotype specific as a result do not confer cross protection across capsular types. Given the fact that at present on the basis of polysaccharide capsule 35 different *S. suis* serotypes exist and genetic heterogeneity has been shown to exist between and within serotypes (2), a serotype-independent and serologically cross-reactive protective antigen would be a preferred basis for vaccine. Thus, identification of an antigenic factor shared among isolates irrespective of serotype may be helpful in the development of a subunit vaccine that will cross protect against strains.

OBJECTIVE

To test the usefulness of a recombinant 38-kilodalton antigen of *S. suis* in the development of a subunit vaccine for the prevention of *S. suis* infection in pigs.

PROCEDURES

Bacterial strains. *S. suis* type 2 strain 1933 is a virulent isolate recovered from a pig with meningitis. It was obtained from Dr. M. M. Chengappa, Kansas State University, Manhattan. Other *S. suis* isolates were also obtained from the same source.

Cloning. A 2.0 kb *EcoRI* DNA fragment encoding the 38-kDa *S. suis* protein was previously cloned, sequenced and partially characterized (1).

Purification of the recombinant 38 kDa protein. The 2.0 kb fragment was fused to pBAD-Myc-His overexpression vector. The gene was then overexpressed and purified by affinity chromatography using the Xpress purification system for 6xHis-Tagged proteins (Invitrogen, Carlsbad, CA). The quantity of the protein was measured by the method of Lowery using bovine serum albumin (BSA) as standard.

Animals. The pigs were purchased from Auburn University Swine Facility, Auburn, Alabama at four weeks of age. Pigs from this farm have no history of *S. suis* infection.

Antibodies against the 38-kDa protein: Prior to the first dose of vaccine, 2 weeks after the first dose of vaccine and two weeks after the second dose just before challenge, blood samples were taken from each pig and the sera were tested by western blotting for antibodies against the 38-kDa protein.

For Western blotting, purified protein was separated by sodium dodecyle sulfate polyacrylamide gel electrophoresis(SDS-PAGE) and transferred to a nitrocellulose membrane. Following transfer, the membrane was cut into strip. The strips were then exposed to a 1:100 or 1:500 dilution of pig serum, followed by a 1:1000 dilution of alkaline phosphatase-conjugated affinity purified anti-swine IgG (Rockland Immunochemicals, Gilbertsville, PA). Blots were developed with a 5-bromo-4chloro-indolyl phosphate-nitoblue tetrazolium salt mixture.

Adujavants: Fruend complete and incomplete adjuvants were used.

Vaccine preparation: Five hundred microliters containing approximately 100 μg of the protein was mixed with equal volume of Fruend complete adjuvant for the primary vaccination of each animal. Two weeks after the initial vaccination, pigs received a booster of the same vaccine preparations by the same route this time emulsified 1:1 with Fruend's incomplete adjuvant. For the preparation of the placebo-vaccines the antigen solutions were replaced by a physiological saline.

Vaccination: Ten four-week-old pigs, identified by numbered ear tags were divided into two groups of five and vaccinated intramuscularly at two injection sites in the neck with 1 ml of VAC-38 containing the 38-kDa based vaccine (pigs 43, 44, 45, 46, 47) or placebo (pigs 92, 93, 94, 95, 96). Two weeks after the first dose the pigs were boosted with the same dose of vaccines by the same route.

Challenge: Two weeks after the booster dose the pigs were challenged intravenously in the ear vein with 1.5 X 10^6 cfu of an overnight culture of the homologous *S. suis* serotype 2 strain 1933. The pigs were monitored twice daily for the following clinical signs of disease: fever, lameness, inappetence, nervous system disorders and depression. For animal welfare reasons, pigs that were moribound or showed nervous signs were killed by an intravenous injection of Beuthanasia, and exsanguinated. Lesions resulting from the injections of the vaccines were recorded.

The experiments were approved by the ethical committee of the School of Veterinary Medicine, Tuskegee University, Tuskegee, AL in accordance with AVMA guidelines.

Polymerase chain reaction (PCR). Oligonucleotide primers were designed by using the 38-kDa protein gene sequence data (1). The sequences of the primers were 5'-ATGCCACGGATTACCTTCCC-3' and 5'-CCGTCTCCTTAATGATCCGC-3'. The primer pair was designed to amplify a 253 bp fragment from *S. suis* DNA. Amplification reactions were performed in a total volume of 50 µl containing 10 mM Tris-HCL (pH 8.3); 1.5 mM $MgCl_2$; 50 mM KCl; 0.001% gelatin; 200 µM of each deoxynucleoside Triphosphate (dATP, dCTP, dGTP, dTTP); 1 µM of each primer; 0.5 U of Taq polymerase (Perkin Elmer Corp., Norwalk, CT) and 100 ng of DNA template. PCR was performed in a Perkin Elmer 2400 thermocycler, comprising 5 min of preincubation at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C. Final extension was performed for 7 min at 72°C. The negative control was a reaction mixture containing all reagents but no DNA template. The PCR products were visualized by electrophoresis on a 0.8% agarose gel following standard procedures.

RESULTS:

Purification of the recombinant 38-kDa protein: To produce a sufficient quantity of the protein for vaccination, the DNA fragment encoding the protein was overexpressed and purified. The purified protein gave a prominent 38-kDa band (Fig. 1). No evidence of contaminating proteins was observed indicating that the 38-kDa protein was purified to homogeneity.

Antibodies against the purified protein. Sera obtained from all of the experimental pigs did not react with the 38-kDa protein at the start of the experiment (day zero). The lack of reactivity with the protein indicated that the pigs had not been previously exposed to the organism. In group 1 (vaccinated group), two weeks post vaccination two of the five pigs (pigs 44 & 47) showed easily detectable antibodies against the protein while three pigs (pigs 43, 45 & 46) gave low antibody responses. Two weeks after the booster dose (day 28) three of the five pigs (pig 44, 45 & 47) mounted strong antibody responses to the protein while 2 of the animals mounted low antibody response to the protein. No antibody reactivity with the protein was detected throughout this period in pig sera obtained from the placebo group (Table 1; Figure 2). These results indicated that the protein is immunogenic in pigs and that the level of antibody production is dependent on the particular animal.

Protective value of the recombinant protein: Three of the five pigs vaccinated with the placebo vaccine died two to four days after the challenge as a result of the infection or had to be killed for animal welfare reasons. The remaining two suffered temporary illness but recovered with time (Table 2). In this group, specific clinical signs of disease, such as lameness, nervous signs and incoordination were frequently recorded. Non-specific clinical signs of disease, such as depression and lack of appetite were also observed. The pigs body temperatures and leucocyte counts were also increased. In contrast, the formulated vaccine containing the 38-kDa protein (VAC-38) conferred complete protection against the homologous challenge (Table 2). Only one of the pigs in this group showed temporary clinical signs of arthritis, and depression. These preliminary findings indicated that the *S. suis* recombinant 38-kDa protein could be a good candidate for the development of a recombinant subunit vaccine for the prevention of *S. suis* infection in pigs. Lesions were observed at the vaccination site in all pigs.

PCR. The PCR assay was used to determine the extent of conservation of the 38-kDa gene. The primer amplified the DNA of all strains tested encompassing serotypes 1 through 19 and 1/2 (Figure 3) suggesting that the gene may be conserved across

capsular types. Western blot analysis with polyclonal antibody raised against the recombinant protein revealed that the gene is expressed by all strains tested (data not shown) suggesting that the vaccine may confer cross protection across capsular types.

Figure 1 . Overexpression and purification of the 38-kDa recombinant protein. An SDS-10% polyacrylamide gel stained with coomassie brilliant blue R-250 is shown. Lanes: M, rainbow molecular size marker in kilodaltons; 1, whole-cell lysate uninduced with arabinose; whole-cell lysate induced with arabinose; and 3 , purified protein.

Table 1. Antibody reactivity of sera from individual pigs with the 38-kDa protein at four weeks post vaccination as determined by western blot analysis.

Animal ID	Day Zero	Day 14	Day 28	Comment
43	□	±	+	Vaccination group
44	-	+ +	+ + + +	Vaccination group
45	-	±	+ + +	Vaccination group
46	-	±	+	Vaccination group
47	-	+ +	+ + + +	Vaccination group
92	-	-	-	Placebo group
93	-	-	-	Placebo group
94	-	-	-	Placebo group
95	-	-	-	Placebo group
96	-	-	-	Placebo group

+ + + + very strong reactivity
+ + + strong reactivity
± weak reactivity
- no reactivity

Figure 2. Strips showing immunoblot analysis of pig sera four weeks post-vaccination and their apparent reactivities against the purified recombinant 38-kDa protein. Lanes: M, molecular size marker in kilodaltons; 1, pig 93; 2, pig 94; 3, pig 43; 4, pig 44; 5, pig 45; 6, pig 46; and 7, pig 47.

Table 2: Clinical signs score and mortality rate of pigs immunized with the recombinant 38-kilodalton protein-based vaccine following *S. suis* type 2 challenge.

Group Number	Treatment	No. sick/ No. injected	No. dead/ No. injected	% Mortalitty
1	Placebo	5/5	3/5	60
2.	38-kDa protein based vaccine (VAC-38)	1/5	0/5	0

Figure 3. Example of ethidium bromide-stained agarose gel after electrophoresis of PCR products with primers designed from the nucleotide sequence of the gene encoding the 38-kDa protein. DNA from isolates of *S. suis* type 2 were used as the template. Size of the PCR product is indicated by number on the left. Lanes: M, ΦX174 DNA molecular weight marker (Promega); 1-23, *S. suis* type 2; 24, negative control.

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