Preliminary Assessment of a *Haemophilus parasuis* Bacterin for use in Specific Pathogen Free Swine

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ABSTRACT

A whole cell formalin killed trivalent *Haemophilus parasuis* bacterin was tested for efficacy in four week old, weaned specific pathogen free pigs challenged under laboratory conditions. The vaccine contained three field strains of *H. parasuis* selected from confirmed cases of Glasser's disease. Two different formulations were evaluated in separate trials. In trial 1, ten pigs received 5 mL of bacterin subcutaneously in the neck, followed by a second 5 mL dose two weeks later. Another ten pigs served as nonvaccinated controls. One week after the second dose, all pigs were subjected to an aerosol challenge containing the strains of *H. parasuis* present in the vaccine. In trial 2, a broth rather than a saline based vaccine was prepared, and tested as in trial 1. In both trials, the vaccinated pigs remained healthy postchallenge, while eight of nine (Trial 1) and eight of ten (Trial 2) nonvaccinated pigs succumbed to Glasser's disease.

RÉSUMÉ

Cette étude consistait à déterminer l'efficacité d'un vaccin préparé avec trois souches *d'Haemophilus parasuis*, isolées de cas cliniques de la maladie de Glasser, et inactivé avec de la formaline. Les auteurs utilisèrent à cette fin des porcelets exempts d'organismes pathogènes spécifiques et âgés de quatre semaines. Dans une première expérience, dix porcelets recurent 5 mL du vaccin précité, dans le tissu sous-cutané du cou, à deux reprises et à deux semaines d'intervalle, alors que dix autres servirent de témoins. Une semaine après la deuxième injection de vaccin, tous ces porcelets subirent une infection de défi, au moyen d'aérosols des trois souches vaccinales d'*H. parasuis*. Dans la deuxième expérience, les auteurs utilisèrent un vaccin à base de bouillon de cultures plutôt que de solution saline et ils l'évaluèrent comme dans la première expérience. Dans ces deux expériences, les porcelets vaccinés résistèrent à l'infection de défi, alors que huit des neuf témoins de la première et huit des dix de la deuxième succombèrent à la maladie de Glasser.

INTRODUCTION

The development of strategies to reduce the economic impact of disease in swine herds has been a priority of the pork industry for many years. Certain swine diseases that cannot be eliminated once they enter a herd and that are difficult to detect in their subclinical state pose a unique problem. To ensure that pigs are free of certain specific pathogens (SPF) they must be raised as gnotobiotes. After microbial adaptation they can be used to establish swine herds that are strictly isolated from conventional swine.

In Ontario, SPF herds provide a source of breeding stock free of a number of economically important pathogens. When SPF pigs are mixed with conventional animals, they are exposed to *Haemophilus parasuis*, a normal component of the nasal flora of conventional swine. This initial infection with *H. parasuis* can result in a fatal fulminating polyserositis referred to as Glasser's disease in the SPF animals, while the conventional animals remain unaffected (1,2). Animals that survive the initial infection may exhibit poor growth rates as a result of the effects of chronic disease (1). The availability of a vaccine against Glasser's disease would eliminate one of the most important disease problems which occurs when SPF pigs are mixed with conventional animals.

In 1981 a vaccine, Glassinord®, was marketed in Denmark and was claimed to provide complete protection for SPF pigs against Glasser's disease (3). However, this vaccine was unavailable to Canadian swine producers due to government regulations restricting the importation of biologicals. This paper describes the preliminary evaluation of a formalin killed whole-cell *H. parasuis* bacterin developed at the Ontario Veterinary College.

MATERIALS AND METHODS

VACCINES

Two types of vaccines were prepared from three strains of *H. parasuis* selected from confirmed field cases of Glasser's disease and used in two different trials. These strains were

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nicotinamide adenine dinucleotide (NAD) requiring, nonhemolytic α fucosidase positive, urease negative and mannitol negative. For the first trial they were grown on chocolate agar plates supplemented with 0.01% NAD (seven plates for each of the three strains) in 5% CO_2 at 37°C. After 18 h incubation the bacteria from 21 plates were harvested in 100 mL phosphate buffered saline (PBS) and a sample was removed for counting. The final suspension contained approximately 10^9 colony forming units (CFU) per mL as determined by plate count. After the addition of 0.2 mL 37% formalin, the suspension was reincubated for 24 h. Following incubation the bacterial suspension failed to grow on chocolate agar supplemented with 0.01% NAD or to cause any abnormal conditions after injection into four *H. parasuis* negative test pigs. Aluminum hydroxide was added as adjuvant.

In trial 2, the vaccine was prepared by inoculating the three bacterial strains listed above into a flask containing 200 mL of tryptophan yeast extract broth plus 0.01% NAD which was preheated to 37°C. It was then incubated for 8 h. Formalin was added to a concentration of 0.2% and the culture incubated for another 24 h and tested for sterility and lack of toxicity in the same manner as for trial 1, with negative result. Aluminum hydroxide was added to the formalin killed broth suspension as an adjuvant.

EXPERIMENTAL ANIMALS AND SELECTION

Specific pathogen free pigs weaned at four weeks were selected from the University of Guelph Arkell Research Station swine herd. This herd was reestablished in 1981, with cesarean derived stock and has been maintained in strict isolation since that time. The pigs were negative for *H. parasuis* as determined by culture of nasal swabs as described previously (4). The number of animals selected for the vaccine trial was based on the expected incidence of disease as a result of experimental inoculation (5) and expected vaccine efficacy (6,7,8).

EXPERIMENTAL PROTOCOL

The guidelines in the "Guide to the Care and Use of Experimental Animals" of the Canadian Council on Animal Care were followed. For each trial 20 animals four weeks of age were divided into two groups with ten pigs in each. Pigs from four different litters were equally distributed between the two groups. Each pig was weighed and identified upon entry into the trial. The pigs were housed in the grower barn at the Arkell Swine Research Station. One group was immunized and the other group served as non-immunized controls. Each pig of the immunized group received 5 mL of bacterin divided into two doses of 2.5 mL each and injected subcutaneously behind each ear. A second 5 mL dose was administered in the same manner two weeks later. The control group received no injections. One week after the second dose, both groups were moved to an experimental isolation unit where they were immediately challenged with an aerosol containing *H. parasuis* and subsequently observed. In trial 2 only, pigs were weighed at weekly intervals prior to challenge to evaluate possible effects of vaccination on growth rate.

Affected animals were euthanized by intravenous injection of barbiturate when they became recumbent and necropsies were performed. Swabs containing material from the abdominal, pericardial, pleural, and joint cavities, and brain, were plated onto chocolate agar supplemented with a streak of 1% NAD. Survivors were sacrificed four weeks after the challenge with *H. parasuis*. Postmortem examinations and bacteriology were performed as for affected animals. Pericardial and joint samples were submitted for mycoplasma culture for all pigs.

CHALLENGE PROCEDURE

The aerosol challenge of *H. parasuis* was prepared as follows:

Chocolate agar plates supplemented with 0.01% NAD were inoculated with the vaccine strains and incubated for 18 h in 5% CO_2 at 37°C. The bacteria were harvested in 50 mL PBS which contained 1 x 10^{10} CFU/mL, as determined by plate count.

Groups of ten pigs (five vaccinates and five controls) were confined in a 1 m wide by 1.8 m long and 1 m high sealed metal container. A DeVilbiss nebulizer (DeVilbiss Model 65, Somerset, Pennsylvania) was attached to a port in the top of the container and then set for maximum output (3.33 mL/min). Aerosol droplets as small as 1 μm containing *H. parasuis* were generated by the nebulizer. The nebulizer was operated for 15 min and a thick fog was generated inside the chamber. An Anderson Air Sampler (Anderson Samplers Consulting Service (Model 0640), 1074 Ash Avenue, Provo, Utah) was connected to the container by a port in the roof. The air was sampled for 1 min following the completion of the nebulization procedure and greater than 7 x 10^3 CFU/mL were found to be present. Plate counts were also performed on the sample remaining in the nebulization compartment of the Devilbiss nebulizer and this suspension contained 2 x 10^8 CFU/mL. Following challenge the pigs from trial 1 and trial 2 were housed in separate isolation rooms and observed twice daily for clinical signs of Glasser's disease. Rectal temperatures were recorded once daily.

RESULTS

TRIAL 1

The only adverse effects of vaccination were small swellings which developed at the injection sites of the vaccinated pigs. One of the nonvaccinates developed an umbilical abscess and died prior to nebulization.

The vaccinated pigs maintained normal temperatures, appetites and activity until they were sacrificed four weeks after aerosol challenge. Necropsy and organ culture for *H. parasuis* were negative for pathological lesions and microbial growth. All nine nonvaccinated pigs showed clinical signs within three to five days postchallenge. Initially, the affected animals were depressed and anorectic. In several hours this progressed to reluctance to rise and finally inability to stand. To minimize unnecessary discomfort, they were sacrificed at this stage. The necropsy and bacteriological findings are presented in Table I. All pericardial and joint samples were negative for mycoplasma.

TRIAL 2

Except for a small swelling at the injection site no other adverse effects

TABLE I. Response of Clinically Affected Nonvaccinated Pigs to Challenge with *H. parasuis* — Trial 1

Pig	Necropsy Findings					Bacteriology				
Number	Br	Peri	Abd	Jt	Pl	Br	Peri	Abd	Jt	Pl
61	-	+	+	++	-	+	-	-	-	+
62	-	+	+	++	-	-	-	-	+	-
63	-	+	+	+	-	+	-	-	-	+
64	-	-	-	-	-	-	+	-	+	-
65	-	-	-	-	-	+	+	-	+	-
66	-	-	++	-	++	-	-	+	+	+
67	-	-	-	-	-	-	-	+	-	-
68	-	++	++	-	++	-	+	+	-	+
69	-	-	-	-	-	-	-	+	-	-

Abbreviations: Br = brain; Peri = pericardium; Abd = abdomen; Jt = joint; Pl = pleura; + = mild lesion or bacteria present; ++ = moderate lesion; - = no lesion or bacteria present

of vaccination were observed. No significant difference in weight gain was observed between vaccinates and nonvaccinates in the three weeks before challenge. One vaccinated pig was euthanized after it developed a sterile abscess within the right elbow joint one week after initial vaccination.

Six of the ten nonvaccinates succumbed to Glasser's disease, while the vaccinated pigs remained healthy as in trial 1 (Table II).

DISCUSSION

In this study both the broth and saline based *H. parasuis* bacterins were effective in providing protection against experimentally produced Glasser's disease using challenge strains homologous to those contained in the vaccine. It was not possible to compare the efficacies of broth versus saline based bacterins due to the low number of animals involved in each trial and because both preparations provided protection to all of the vaccinated animals.

One of the first reports of the impact of Glasser's disease on SPF animals came from Denmark, where it was observed that up to 50% of the SPF animals placed in a conventional facility showed clinical signs of Glasser's disease (9). This problem was alleviated by the use of a *H. parasuis* bacterin administered to the SPF pigs prior to exposure to conventional pigs (9). The method for preparation of the vaccine tested in our trials is a modification of that used by Nielsen (9). However, it differs from the latter in that it contains *H. parasuis* strains isolated from diseased pigs in Ontario. The occurrence of disease after mixing of SPF and conventional pigs is unpredictable (10,11,12). Alternatives for prevention such as prophylactic administration of antibiotics have not been found to significantly reduce morbidity or mortality due to *H. parasuis* infection (1,13), or the beneficial effects have been variable and of short duration. The promising results found in this experiment suggest that vaccination may be the best way to prevent Glasser's disease in Canadian SPF animals.

In these trials, attempts were made to approximate field conditions as closely as possible. In order to mimic the environmental stress patterns associated with shipment and exposure to conventional pigs, the experimental animals were immediately exposed to nebulized *H. parasuis* upon arrival to the new facility from the University farm. In experimental inoculations of pigs with *H. parasuis*, a minimum dose of 10^9 viable organisms per mL delivered intratracheally or intraperitoneally has been reported to be necessary to produce Glasser's disease comparable to that observed during severe field outbreaks (14,15). In this experiment, the bacterial suspension used to generate the aerosol contained 10^9 CFU per mL; however, the actual number of organisms that each pig received was not measured. Although the heavy fog generated in the nebulization chamber presumably represents a larger dose than in natural exposure, protection was maintained in the vaccinated animals.

It has been established by serological methods that *H. parasuis* is a very heterogeneous species (16,17), yet, in the first three years of use of the Danish vaccine, no failures have been reported (3). Although this vaccine has not been tested for protection against all strains, the experience in Denmark suggests that cross-protection does occur since many strains may be present within as well as between farms (4,16). Further investigation is required to determine the degree of cross-protection between or among strains of *H. parasuis*.

This study shows that protection in SPF pigs can be achieved by vaccination with a *H. parasuis* bacterin containing strains homologous to those used in aerosol challenge. Continued investigation into the extent of cross-protection between strains would provide further information for use of the vaccine in the field.

TABLE II. Response of Nonvaccinated Pigs to Challenge with *Haemophilus parasuis*

Pig	Necropsy Findings					Bacteriology				
Number	Br	Peri	Abd	Jt	Pl	Br	Peri	Abd	Jt	Pl
85	-	-	+	+	-	-	-	-	-	+
86	-	+++	++	+	+++	-	+	+	+	+
87 NA	-	-	-	-	-	-	-	-	-	-
88	-	-	-	+	-	-	+	-	-	-
89 NA	-	-	-	-	-	-	-	-	-	-
90	-	-	+	-	-	-	+	-	-	-
91 NA	-	-	-	-	-	-	-	-	-	-
92 NA	-	-	-	-	-	-	-	-	-	-
93	-	+	-	++	-	+	+	+	-	-
94	-	+	-	++	-	-	-	-	-	+

Abbreviations: Br = brain; Peri = pericardium; Abd = abdomen; Jt = joint; Pl = pleura; + = mild lesion or bacteria present; ++ = moderate lesion; +++ = marked lesion; NA = not clinically affected; - = no bacteria or lesions present

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