Protection of pigs against challenge with virulent *Streptococcus suis* serotype 2 strains by a muramidase-released protein and extracellular factor vaccine

H. J. Wisselink, U. Vecht, N. Stockhofs-Zurwieden, H. E. Smith

The efficacy of a muramidase-released protein (MRP) and extracellular factor (EF) vaccine in preventing infection and disease in pigs challenged either with a homologous or a heterologous *Streptococcus suis* serotype 2 strain (MRP+EF+) was compared with the efficacy of a vaccine containing formalin-killed bacterin of *S suis* serotype 2 (MRP+EF). The enhancement of the immune response by different adjuvants (a water-in-oil emulsion [WO] and an aluminium hydroxide-based adjuvant [AH]) and their side effects were also studied. The MRP and EF were purified by affinity chromatography. Pigs were vaccinated twice at three weeks and six weeks of age and challenged intravenously with virulent *S suis* serotype 2 strains (MRP+EF+) at eight weeks of age. At challenge, the pigs vaccinated with MRP+EF/WO had high anti-MRP and anti-EF titres and were protected as effectively as pigs vaccinated with WO-formulated vaccines with bacterin. Eight of the nine pigs survived the challenge and almost no clinical signs of disease were observed. The titres obtained with the MRP+EF/AH vaccine were low and only two of the five pigs were protected. Pigs vaccinated with either MRP or EF were less well protected; three of the four pigs died after challenge but the clinical signs of disease were significantly less severe than those observed in the placebo-vaccinated pigs. The protective capacity of the bacterin/AH vaccine was very low, and the mortality among these pigs was as high as in the placebo-vaccinated pigs (80 per cent). Postmortem histological examination revealed meningitis, polyserositis and arthritis in the clinically affected pigs. The results demonstrate that a subunit vaccine containing both MRP and EF, formulated with the WO adjuvant, protected pigs against challenge with virulent *S suis* type 2 strains.

STREPTOCOCCAL meningitis, polyserositis and polyarthritis, is a severe, often fatal disease of young pigs at weaning, and is usually caused by *Streptococcus suis* serotype 2 (Vecht and others 1985, Higgins and others 1992, Reams and others 1994). *S suis* strains can be differentiated by serotype on the basis of their capsular polysaccharides. At present, 35 serotypes are known (Perch and others 1983, Gottschalk and others 1989, 1991, Higgins and others 1995). The virulence of most serotypes is unclear. Strains of serotypes 2 and 1 are considered to be the most virulent serotypes, but strains of some other serotypes, such as 7, 9 and 14 have also been associated with disease (Sihovenen and others 1988, Jacobs and others 1995, MacLennan and others 1996). The economic impact of *S suis* infections on the pig industry is substantial (Chengappa and others 1990), and the prophylactic use of antibiotics in food and drinking water has so far been unsuccessful in controlling the disease. Furthermore, antibiotics are becoming less effective because of an increase in resistance among *S suis* isolates and their use is unpopular because of the public awareness of antimicrobial residues (Aarestrup and others 1998).

Little is known about the protective antigens of *S suis*. Whole-cell vaccines with live avirulent or killed virulent *S suis* serotype 2 cells protected pigs against challenge with a strain of a homologous serotype (Holt and others 1988, 1990, Busque and others 1997). However, vaccination with whole cells did not induce protection against challenge with a strain of a heterologous serotype (Kebede and others 1990). Jacobs and others (1996) showed that a vaccine based on the purified 54 kDa suilysin protected against a homologous challenge, but the absence of this haemolysin from a substantial number of isolates obtained from diseased pigs limits the usefulness of this vaccine (Segers and others 1998).

In previous work, the authors identified two proteins, the 136 kDa muramidase-released protein (MRP) and the 110 kDa extracellular factor protein (EF), as markers of virulent *S suis* serotype 1 and 2 strains (Vecht and others 1991). The MRP is a membrane-associated protein and EF is an extracellular protein. Both are transported across the bacterial membrane and are major antigens recognised by convalescent sera of infected pigs (Vecht and others 1991). This paper describes the results of studies of the efficacy of MRP and EF vaccines in preventing infection and disease in pigs challenged with a homologous or a heterologous *S suis* serotype 2 strain (MRP+EF+), in comparison with the protective capacity of a vaccine containing formalin-killed bacterin of *S suis* serotype 2 (MRP+EF+).

**MATERIALS AND METHODS**

**Bacterial strains**

Strains 4005 (3) and 3881 (10) of *S suis* serotype 2, both belonging to the phenotype MRP+EF+, were used. Both strains were field isolates, isolated from pigs suffering from meningitis and were virulent for newborn germ-free pigs (Vecht and others 1992).

**Adjuvants**

Two adjuvants, a water-in-oil emulsion (Spectol; ID-Lelystad) (WO) and a 2 per cent aluminium hydroxide gel (Alhydrogel; Superfos Biosoector) (AH) were used.

**Antigens**

For the preparation of the vaccine, MRP and EF were purified by affinity chromatography. A two litre culture of strain 4005, grown in Todd Hewett broth (CM 189; Oxoid) was centrifuged at 4000 g for 15 minutes. The supernatant was cleared from remaining cells by filtration under air pressure through 0·2 µm filters (Millipore). Monoclonal antibodies MRP+, or EF2 (ID-Lelystad) were coupled separately to cyanogen bromide-activated Sepharose 4B in accordance with the instructions of the supplier (Pharmacia). After appropriate washing the bound proteins were eluted with glycine-hydrochloric acid buffer (0·1 M, pH 2·8), and the pH of the fractions was immediately increased to 7·0 with 1 M sodium hydroxide. The fractions were measured with the MRP or EF double antibody...
sandwich (DAS) ELISA for the detection of MRP and EF proteins as described by Vecht and others (1993). Fractions positive for MRP and EF were pooled and dialysed overnight against physiological saline. The purified proteins gave single bands in silver-stained sodium dodecyl sulphate-polyacrylamide gels. The quantities of proteins were measured with the bicinchoninic acid protein assay reagent (Pierce), using bovine serum albumin (BSA) as standard. Approximately 100 μg of MRP and 240 μg of EF were obtained per litre of culture.

For the preparation of the whole-cell vaccine, strain 4005 was incubated overnight at 37°C in 100 ml Todd-Hewitt broth. The culture contained about 1 × 10^10 colony-forming units (cfu)/ml. Fifty ml was centrifuged at 4000 g for 15 minutes. Pellets were washed twice in phosphate-buffered saline (PBS; 136-89 mM sodium chloride, 2-68 mM potassium chloride, 8-1 mM sodium hydrogen phosphate, 2-79 mM potassium hydrogen phosphate, pH 7-2) and resuspended in 2-5 ml PBS. To this suspension, 250 μl 3 per cent formalin was added, and it was maintained at 4°C overnight. The next day, the suspension was checked for the absence of live bacteria by plating on 6 per cent Columbia horse blood agar base (CM 331; Oxoid). To formalin, the cells were washed twice with physiological saline and resuspended in physiological saline to a final count of approximately 1 × 10^8 cells/ml.

**Vaccine preparation**

WO or AH was used as the adjuvant. For the preparation of emulsions in the WO adjuvant, four parts of the water phase containing the antigen were mixed with five parts of WO (Bokhout and others 1981).

For the vaccinations with the AH adjuvant, 1-25 mg metallic aluminium was used per dose, according to the manufacturer's instructions. The antigens and AH were stirred for four hours at 4°C. To control adsorption, 1 ml of the mixture was centrifuged in an Eppendorf centrifuge at 10,000 rpm for three minutes, and the supernatants were analysed for the absence of the antigens either by MRP and EF DAS ELISA (Vecht and others 1993) or spectrophotometrically by using an Ultrospec 3000 spectrophotometer (Pharmacia Biotech) at wavelengths of 250 to 650 nm.

For the preparation of the placebo-vaccines the antigen solutions were replaced by a physiological saline solution.

**Vaccination**

Fifty-five three-week-old pigs, crossbreeds of Yorkshire and Dutch landrace, were obtained from the specified pathogen-free herd of the tD-Lelystad for two experiments. In both experiments the pigs were allotted to six treatment groups each consisting of four or five pigs. The pigs were separated and housed in boxes at the animal facilities of the tD-Lelystad.

Priming vaccinations were given at the age of three weeks. Each dose contained 50 μg MRP, 50 μg EF or 1 × 10^8 formalin-killed whole cells, either separately or in combination, and they were administered intramuscularly, divided over two injection sites, in both upper hind legs. Three weeks later the pigs were boosted intramuscularly in the neck with the same dose of the vaccines without adjuvants.

**Challenge**

Two weeks after the second vaccination, the pigs were challenged intravenously in the ear vein with 1 × 10^8 cfu of the homologous S suis serotype 2 strain 4005 (experiment 1) or the heterologous S suis serotype 2 strain 3881 (experiment 2). The inocula were prepared as described by Vecchi and others (1992). The pigs were monitored twice daily for the following clinical signs of disease: fever, disorders of the nervous system, lameness, inappetence and depression. Blood samples were taken once a week before the challenge and three times a week after the challenge to monitor the immune response. White blood cells were counted with a semi-cell blood counter (Sysmex, model F 800; Charles Griffin Medical Systems). The number of neutrophils was calculated after a differential count of Giemsa-stained blood smears. For animal welfare reasons, pigs that were moribund or showed nervous signs were killed by an intravenous injection of pentobarbiturate, exsanguinated and examined postmortem. Tissue specimens taken from the central nervous system (CNS), serosa, and joints were examined bacteriologically and histologically as described by Vecht and others (1992). Lesions resulting from the injections of the vaccines were recorded.

The experiments were approved by the ethical committee of the Institute for Animal Science and Health in accordance with Dutch law on animal experiments.

**Antibodies against MRP and EF**

The sera were tested for antibodies against MRP and EF by an indirect ELISA. Each well of the polystyrene microtitre plates was coated for 18 hours at 37°C with 30 ng of MRP for the indirect MRP ELISA or with 25 ng of EF for the indirect EF ELISA. Pig sera in two-fold dilutions from 1:5 to 1:5120 in PBS containing 0-05 per cent Tween 80 were added and the plates were incubated for one hour at 37°C. Serum from a gnotobiotic pig which had survived an infection with the virulent S suis serotype 2 strain 4005 (MRP+EF+) was used as a positive control. As a conjugate, monoclonal antibody mouse anti-swine immunoglobulin G labelled with horseradish peroxidase (ID-Lelystad) was used. After incubation for one hour at 37°C, the substrate 5-aminosalicylic acid with hydrogen peroxide was added. After incubation for two hours at room temperature, the absorbance at 450 nm was read. Titres were expressed as the reciprocal of the log, of the highest dilution showing an absorbance of more than 50 per cent of the positive control.

**Statistical analysis**

Data concerning the mortality and lesions of the various groups were analysed simultaneously by the non-parametric Fisher-Freeman-Halton exact test. When there was overall significance, Fisher's exact test was used to make pair-wise comparisons between the various groups. In a similar manner, the antibody titres against MRP and EF, the clinical signs of disease, fever and the number of leucocytes were subjected to exact median tests for simultaneous comparisons, followed by exact pair-wise permutation tests. The last test was only used if there was overall significance. The significance level was set at 95 per cent.

**RESULTS**

**Antibody titres against MRP and EF**

All the pigs vaccinated with the MRP and EF vaccines with WO as adjuvant developed high anti-MRP and anti-EF titres (Table 1). At the time of challenge the average antibody titres against MRP ranged from 6208 to 23,170 and against EF from 7131 to 13,308. The titres obtained after vaccination with MRP+EF/AH were much lower; at the time of challenge, the average antibody titre against MRP was 388 and against EF it was 1522.

Pigs vaccinated with the bacterin vaccines developed low anti-MRP titres only. In the pigs vaccinated with bacterin/WO they started to develop after the booster administration to an average anti-MRP titre of 158 at the time of challenge. No anti-EF titres could be detected in this group either before or after challenge. In the bacterin/AH group, none of the pigs had anti-MRP or anti-EF titres at the time of challenge.

As expected, none of the pigs vaccinated with the placebo vaccines had developed antibodies against MRP or EF at the time of challenge.

**Protection**

Two weeks after the second vaccination, the pigs were challenged intravenously in the ear vein with 1 × 10^8 cfu of the
TABLE 1: Effect of vaccination with various Streptococcus suis serotype 2 vaccines on mean (± s.e.) log antibody titre against muramidase-released protein (MRP) and extracellular factor (EF) in pigs at the time of challenge

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Antigens</th>
<th>MRP</th>
<th>EF</th>
<th>MRP+EF</th>
<th>MRP+EF+bacterin</th>
<th>Placebo</th>
<th>Placebo No adjuvant</th>
<th>Antilog MRP</th>
<th>Antilog EF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MRP</td>
<td>13.8±(1.1)</td>
<td>14.263</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>EF</td>
<td>0</td>
<td>0</td>
<td>12.4±(1.3)</td>
<td>7131</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>MRP+EF</td>
<td>13.5±(0.9)</td>
<td>11.585</td>
<td>13.3±(0.8)</td>
<td>10,086</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>MRP+EF+bacterin</td>
<td>12.6±(1.7)</td>
<td>6208</td>
<td>13.1±(0.8)</td>
<td>8780</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Placebo No adjuvant</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>MRP</td>
<td>14.5±(1.3)</td>
<td>21.170</td>
<td>13.7±(1.8)</td>
<td>13,308</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>EF</td>
<td>0</td>
<td>0</td>
<td>12.4±(1.3)</td>
<td>7131</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>MRP+EF</td>
<td>13.5±(1.3)</td>
<td>23.170</td>
<td>13.7±(1.8)</td>
<td>13,308</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Placebo No adjuvant</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* In experiment 1, pigs were challenged with the homologous S suis serotype 2 strain 4005. (MRP+EF+), and in experiment 2 with the heterologous S suis serotype 2 strain 3881 (MRP+EF+). Significantly different from group placebo/WO, experiment 1. Significantly different from group placebo/WO, experiment 2. Significantly different from group placebo/AH, experiment 2. WO Water-in-oil emulsion, AH Aluminium hydroxide-based adjuvant

A homologous S suis serotype 2 strain 4005 (experiment 1) or the heterologous S suis serotype 2 strain 3881 (experiment 2). Seventeen of the 19 pigs vaccinated with the placebo vaccines died one to four days after the challenge as a result of the infection or had to be killed for animal welfare reasons (Table 2). In these groups, specific clinical signs of disease, such as lameness and nervous signs, were frequently recorded. Non-specific clinical signs of disease, such as depression, recumbency and lack of appetite were also frequently observed. The pigs' body temperatures and leucocyte counts were also increased. In contrast, the MRP-formulated vaccines containing both MRP and EF conferred a higher degree of protection. Compared with the placebo-vaccinated pigs, mortality was significantly (P<0.05) lower, and 11 of the 13 pigs survived the challenge, both with the homologous and with the heterologous serotype 2 strain. The clinical signs of disease and the increase in body temperatures were also significantly (P<0.05) reduced. Vaccines containing either MRP or EF were less protective than the vaccine containing both proteins (Table 2, experiment 1), and most of the pigs in these groups did not survive the challenge. However, compared with the pigs in the placebo-vaccinated groups, the pigs vaccinated with either MRP or EF showed significantly (P<0.05) fewer specific clinical signs of disease and had lower fever and lower leucocyte counts. The MRP+EF+AH vaccine conferred a low degree of protection; only three of the five pigs survived the challenge with the heterologous S suis serotype 2 strain, but, compared with the placebo-vaccinated pigs, the specific clinical signs of disease and the fever were significantly (P<0.05) lower (Table 2, experiment 2). Eight of the nine pigs vaccinated with WO-formulated vaccines containing bacterin were protected against a challenge with the homologous or heterologous serotype 2 strain; mortality in these groups was significantly (P<0.05) lower than in the placebo-vaccinated groups, and there were fewer specific or non-specific signs of disease (P<0.05) and a lower level of fever. In contrast, a bacterin/AH vaccine conferred less protection; four of the five pigs vaccinated with this vaccine died two to four days after challenge, and specific signs of disease were observed as often as in the placebo-vaccinated pigs.

Postmortem results confirmed the clinical findings. Histological examination revealed meningitis, polyserositis and arthritis in all 19 of the placebo-vaccinated pigs, in four of the five pigs vaccinated with bacterin/AH, and in three of the five vaccinated with EF/WO (Table 3). In contrast, the pigs vaccinated with MRP+EF/WO, bacterin/WO and MRP+EF+bacterin/WO had significantly (P<0.05) fewer lesions, and bacteria were isolated from the lesion sites less frequently. Lesions were observed at the injection site in the pigs of all groups, except for those vaccinated with the AH-formulated placebo vaccine. They ranged in severity from being less than 1 cm² in size and involving connective tissue only, to being more than 1 cm², with necrosis, microabscesses or granulomas (Table 3).

TABLE 2: Results of the vaccination of pigs with muramidase-released protein (MRP), extracellular factor (EF) and/or bacterin of Streptococcus suis serotype 2 followed by a homologous or heterologous S suis serotype 2 challenge

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Antigens</th>
<th>Adjuvant</th>
<th>Number of pigs</th>
<th>Number of pigs surviving challenge</th>
<th>Number of pigs</th>
<th>Mean number of days after challenge</th>
<th>Clinical signs of disease (%)</th>
<th>Fever index (%)</th>
<th>Leucocytosis index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MRP</td>
<td>WO</td>
<td>4</td>
<td>3</td>
<td>5.0</td>
<td>5.0</td>
<td>15*</td>
<td>21*</td>
<td>38*</td>
</tr>
<tr>
<td></td>
<td>EF</td>
<td>WO</td>
<td>4</td>
<td>3</td>
<td>2-6</td>
<td>36*</td>
<td>36*</td>
<td>43*</td>
<td>65*</td>
</tr>
<tr>
<td></td>
<td>MRP+EF</td>
<td>WO</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>16*</td>
<td>16*</td>
<td>61*</td>
<td>31*</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>WO</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>13*</td>
<td>17*</td>
<td>16*</td>
<td>52*</td>
</tr>
<tr>
<td></td>
<td>Placebo No adjuvant</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>13*</td>
<td>17*</td>
<td>16*</td>
<td>52*</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>MRP+EF</td>
<td>WO</td>
<td>5</td>
<td>0*</td>
<td>NA</td>
<td>46</td>
<td>84</td>
<td>77</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>MRP+EF</td>
<td>AH</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>37*</td>
<td>56</td>
<td>86</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>WO</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>31*</td>
<td>54</td>
<td>76</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>AH</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>34*</td>
<td>57</td>
<td>90</td>
<td></td>
</tr>
</tbody>
</table>

* In experiment 1, pigs were challenged with the homologous S suis serotype 2 strain 4005 (MRP+EF+). MRP and EF were purified from S suis serotype 2 strain 4005 (MRP+EF+). Bacterin was prepared from S suis serotype 2 strain 4005 (MRP+EF+) and experiment 2 with the heterologous S suis serotype 2 strain 3881 (MRP+EF+). The significant differences from group placebo/WO, experiment 1. \( \text{MRP+EF} \) significantly different from group placebo/WO, experiment 2. \( \text{MRP+EF} \) significantly different from group placebo/AH, experiment 2. NA Not applicable, WO Water-in-oil emulsion, AH Aluminium hydroxide-based adjuvant

The Veterinary Record, April 14, 2001 475
**DISCUSSION**

These results show that vaccines containing MRP+EF/WO protected pigs against challenge with either a homologous or heterologous *S suis* serotype 2 strain with the phenotype MRP+EF+. The MRP+EF/WO vaccine was as protective as a bacterin/wo vaccine. All but one of the pigs vaccinated with MRP+EF/WO survived the challenge and few specific clinical signs of disease were observed. The MRP/WO or EF/WO vaccines were much less protective; three of the four vaccinated pigs died after challenge. However, compared with the placebo-vaccinated pigs, the pigs vaccinated with MRP/WO or EF/WO showed significantly fewer clinical signs of disease. These data are in accordance with the results of Jacobs and others (1996) who described a vaccine which contained most of the extracellular antigens produced by an *S suis* serotype 2 strain, with EF being the most abundant protein. However, the vaccine only partially protected pigs against challenge with a virulent *S suis* type 2 strain.

The protection observed with the MRP and/or EF vaccines was associated with the levels of anti-MRP and anti-EF antibodies. The MRP+EF/WO vaccine induced high antibody titres and protected pigs effectively against challenge with either homologous or heterologous *S suis* serotype 2 strains. On the other hand, the vaccine with MRP+EF/AH induced lower antibody titres and the pigs were less well protected. In contrast, no association between anti-MRP and anti-EF titres and protection was observed with the bacterin vaccines. Pigs vaccinated with bacterin/wo had low antibody titres against MRP and EF but nevertheless appeared to be completely protected against challenge. It seems likely that other antigens than MRP and EF are responsible for this protection, for example capsular antigens, as has been suggested by Kebede and others (1990).

WO adjuvant was superior to AH adjuvant in its capacity to stimulate an immune response after vaccination with MRP and EF and to confer protection against challenge with virulent *S suis* serotype 2 strains. Similarly, Ripley (1983) showed that an oil-based adjuvant produced a significant antibody response with killed bacterins, whereas only a transient increase in antibodies was observed after vaccination with an AH-formulated vaccine. However, it appeared that both WO and AH adjuvants caused serious lesions at the injection sites, and a suitable alternative adjuvant or a refinement of WO is therefore desirable.

Holt and others (1990) found that a vaccine containing bacterin without an adjuvant protected as well as bacterin formulated either with Freund's incomplete adjuvant or with aluminium hydroxide gel as an adjuvant. However, the same experiments showed that the protective response to bacterin was stimulated when the size of the inoculum was increased from $10^6$ to $10^7$ killed cells. In this study, the bacterin contained only $10^7$ killed cells, and a strong potentiating adjuvant like WO seemed to be necessary to obtain protection.

Whole-cell vaccines are probably serotype-specific, because protection was achieved only against a strain of a homologous serotype, and the vaccines failed to protect against other serotypes (Kebede and others 1990, Foster and others 1994). Subunit vaccines based on proteins conserved among serotypes may be more useful in veterinary practice if they protect against challenge with strains of heterologous serotypes. Jacobs and others (1996) suggested that suilysin, a thiol-activated haemolysin from *S suis* serotype 2, could be such a cross-protection factor. Vaccination challenge experiments in pigs indicated that this vaccine protected against challenge with a homologous serotype. However, the haemolysin is absent from quite a number of strains of *S suis* isolated from diseased pigs in the field. This implies that other vaccine components will be necessary to provide protection against all field strains (Jacobs and others 1996, Segers and others 1998). A 52 kDa immunoglobulin-binding protein which has recently been shown to be identical to a 60 kDa heat-shock protein that is produced by various serotypes, could be another candidate for a subunit vaccine (Serhir and others 1995, Benkirane and others 1997, 1998). The protective value of this protein has not been tested.

In Europe, the USA and Australia, most of the *S suis* serotype 2 strains isolated from diseased pigs produce MRP and EF (Mwaniki and others 1994, Galina and others 1996, Wisselink and others 2000). In these countries an MRP+EF vaccine could therefore be of great value. However, most of the *S suis* serotype 2 strains isolated from diseased pigs in Canada appeared to be MRP and EF negative (Gottschalk and others 1998). Apparently, Canadian strains differ from strains isolated in other countries. The proteins MRP and/or EF are not only produced by serotype 2 strains. High percentages of European *S suis* serotype 1, 1/2 and 14 strains, isolated from tissues associated with *S suis* infections such as brain, serosa, joint, heart and other organs of diseased pigs, expressed MRP

### Table 3: Lesions observed in pigs after vaccination with muramidase-released protein (MRP), extracellular factor (EF) and/or bacterin of *Streptococcus suis* serotype 2 followed by a homologous or heterologous *S suis* serotype 2 challenge

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Antigens</th>
<th>Adjuvant</th>
<th>Number of pigs</th>
<th>Number of pigs with lesions</th>
<th>Number of pigs with lesions at site of injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MRP</td>
<td>WO</td>
<td>4</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>EF</td>
<td>WO</td>
<td>4</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>MRP+EF</td>
<td>WO</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>MRP+EF+bacterin</td>
<td>WO</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>WO</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>No adjuvant</td>
<td>5</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

* In experiment 1, pigs were challenged with the homologous *S suis* serotype 2 strain 3881 (MRP+EF+), and in experiment 2 with the heterologous *S suis* serotype 2 strain 3881 (MRP+EF+).

1 Meningitis was characterised by inflammation of cerebrum, cerebellum, pons, mesencephalon and medulla oblongata.

2 Polyserositis was characterised by inflammation of peri- and epicardium, thoracic pleura and peritoneum.

3 Polynarthrosis was characterised by inflammation of cartil, tarsal, knee, elbow, shoulder and hip joints.

4 Total number of pigs with lesions; some pigs developed more than one lesion.

5 Significantly different from group placebo/wo, experiment 1.

6 Significantly different from group placebo/wo, experiment 2.

**WO** Water-in-oil emulsion, **AH** Aluminium hydroxide-based adjuvant.
and EF, and more than 80 per cent of the S suis type 9 strains produced an MRP+ protein, a high molecular weight variant of the 136 kDa MRP (Wisselink and others 2000). In addition to serotype 2 strains, strains of serotypes 1, 1/2, 7, 9 and 14 are frequently isolated from diseased pigs. Further work is needed to determine whether MRP and EF are involved in the protection of pigs infected with strains of other serotypes producing MRP and/or EF.

ACKNOWLEDGEMENTS

This work was partly supported by Vétosphin Biotechnologie, Lure, France.

References


Abstract

Measurements of postoperative pain in cats

THREE groups of six cats were subjected to either tenectomy or onychectomy, or to no surgical intervention. Manual palpation and simple descriptive scales and visual analogue scales were then applied to assess and record the level of pain suffered by the cat, and the results were compared with measurements of the plasma concentrations of beta-endorphin and cortisol. Only the scores on the visual analogue scales and the responses of the cats to palpation differed significantly between the control group and the groups subjected to surgery; the physiological measurements failed to differentiate between them.

Protection of pigs against challenge with virulent *Streptococcus suis* serotype 2 strains by a muramidase-released protein and extracellular factor vaccine


*Veterinary Record* 2001 148: 473-477
doi: 10.1136/vr.148.15.473

Updated information and services can be found at:
http://veterinaryrecord.bmj.com/content/148/15/473

**References**

These include:

Article cited in:
http://veterinaryrecord.bmj.com/content/148/15/473#related-urls

**Email alerting service**

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

**Notes**

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/