The protection of fattening pigs against foot and mouth disease with an oil-adjuvanted vaccine: Studies on South American FMD virus strains

O. BASARAB*, O. UMEHARA and L.F. URIBE**

Summary: The performance of a trivalent oil-adjuvanted FMD vaccine formulated with O1 Campos, A24 Cruzeiro and C3 Indaial FMD antigens has been evaluated in fattening pigs in Brazil. As previously demonstrated, a single dose of this type of vaccine injected at the time of weaning induced a rapid onset of protection against challenge and a protracted immunity lasting at least for four months, i.e. the usual life span of fattening pigs. No general or local clinical signs occur after vaccination by the intramuscular or intraperitoneal routes. Some of the animals inoculated intramuscularly had residual tissue reactions at the time of slaughter but these could be dissected off the carcass with the loss of only a small (100 to 200 g) portion of muscle tissue. Intraperitoneal inoculation was not associated with residual lesions affecting the carcass.

INTRODUCTION

In the preceding communication (Basarab and Pay, 1982) the performance of a primary water-in-oil (w/o) emulsion FMD vaccine was discussed in terms of its potency, onset and duration of immunity, physical properties and potency-stability, routes of inoculation and residual local reactions. In this report we describe the results obtained in fattening pigs inoculated with trivalent w/o emulsion FMD vaccines (O1 Campos, A24 Cruzeiro and C3 Indaial) at the time of weaning. The purpose of this investigation was to evaluate the performance of the emulsion formulation using FMD virus antigens derived from strains prevalent in South America and the application of this vaccine under local conditions in Brazil.

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The intraperitoneal (i/p) route of inoculation was investigated with a view to circumventing the residual tissue reactions occasionally observed when the intramuscular (i/m) route is used to administer the vaccine.

**MATERIALS AND METHODS**

w/o vaccines were prepared from inactivated concentrated FMD antigens of the strains O1 Campos, A24 Cruzeiro and C3 Indaial as described before (Basarab and Pay, 1982).

Groups of Large White × Landrace cross weaners were inoculated with the oil emulsion vaccines using the dose volumes and routes of inoculation described in the « Results and Discussion » section.

Evaluation of the immune status induced by the vaccines was carried out by inoculation challenge in the bulb of the heel using homologous virus preparations of proven virulence for pigs as described previously (Basarab and Pay, 1982). The challenge viruses were titrated in 6-month-old pigs by the method of Burrows (1966). Serum samples were also taken at suitable intervals and tested for specific activity by the metabolic inhibition test (Martin and Chapman, 1961).

**RESULTS AND DISCUSSION**

In one experiment, groups of weaners were blood-sampled and inoculated i/m in the neck with either 0.4, 2 or 10 ml of trivalent w/o emulsion vaccine (1/5, 1 and 5 times the standard 2 ml dose respectively). Neither local nor general clinical signs were observed in any of these animals following inoculation. Challenge tests were carried out at 7, 21 and 126 days post-inoculation with the results shown in Table I.

The development of specific antibodies is shown in Figure 1. All the animals were tested for FMD antibodies at the start of the experiment and all animals undergoing challenge were tested for antibodies against all three virus types at the time of challenge. No explanation is available for the poor duration of immunity given by the type O component in the group of animals.

Some of the pigs of groups 1 to 4 were allowed to recover after challenge and kept till the end of the fattening period when they were killed at a slaughterhouse and the carcasses examined for residual tissue reactions in the muscles of the neck. Twenty-seven carcasses out of thirty-two passed meat inspection by the veterinary inspector of the abattoir; in some cases a small amount of muscle from the neck was dissected off to remove a minor, circumscribed lesion. In the remaining five carcasses the larger residual lesions required more extensive dissection. The lesions were largely as described by McKercher *et al.* (1971). Some droplets of emulsion could be found persisting
The graphs show the development of antibodies and their level over a period of 4 months after vaccination. Each point within the same curve represents a different group of animals. Each point within one group represents the mean antibody titre of all 30 pigs in that group against each one of the three viruses used in the experiment.
TABLE I
Results of challenge tests (first experiment)

<table>
<thead>
<tr>
<th>Time of inoculation before challenge</th>
<th>Groups and treatment</th>
<th>Challenge viruses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>O₁ (&gt;5)¹</td>
</tr>
<tr>
<td>7 days w/o O-A-C batch 1</td>
<td>(1) 1 x dose</td>
<td>7/10²</td>
</tr>
<tr>
<td></td>
<td>(2) 5 x dose</td>
<td>3/5</td>
</tr>
<tr>
<td>21 days</td>
<td>(3) 1 x dose</td>
<td>7/10</td>
</tr>
<tr>
<td></td>
<td>(4) 1/5 x dose</td>
<td>1/5</td>
</tr>
<tr>
<td></td>
<td>(1.3)³</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>(5) Controls</td>
<td>0/10</td>
</tr>
<tr>
<td>126 days</td>
<td>(6) 1 x dose</td>
<td>0/10</td>
</tr>
<tr>
<td></td>
<td>(7) Controls</td>
<td>0/5</td>
</tr>
</tbody>
</table>

¹ log₁₀ ID₅₀ (pig) challenged dose used.
² Protected/total.
³ PD₅₀ estimate.

in the draining lymph nodes of the inoculated area. It is noteworthy that the size and appearance of the lesions was not related to inoculum size, which varied from 0.4 ml in some animals up to 10 ml in others.

The residual reactions may depend on a number of factors, including the individual reactivity of the animals and the chance event of certain microorganisms capable of enhancing the tissue reaction being drawn deep from the surface of the skin into the tissues during inoculation.

The potency-stability of this experimental vaccine was tested after the emulsion had been stored for 12 months at 4°C since the previous challenge and 15 months from initial preparation. A second batch of w/o emulsion trivalent vaccine was tested at the same time, inoculating weanlings with 2 ml by either the i/m or the i/p route. The test also evaluated duration of immunity following i/p inoculation and the results are summarised in Table II.

Clearly, the first batch of w/o emulsion trivalent vaccine had retained its potency for the period of storage and it can be concluded that it has a shelf-life of at least 1 year.

The second batch of w/o emulsion trivalent vaccine was also potent and protected against challenge with all three strains at 21 dpi. This batch which was also used to inoculate piglets by the i/p route confirmed the previous finding about this route of inoculation (Basarab and Pay, 1982). Namely, protection of at least the same level and duration as that in animals inoculated i/m, was achieved. In addition, i/p inoculation has invariably been found to
be a simple and easy procedure involving no more effort than the i/m method. Field trials in Brazil have confirmed the latter view under practical conditions when vaccination of several hundreds of weaner pigs by the i/p route was carried out (Nicholls et al., 1982).

The i/p route was investigated in these experiments with a view to circumventing the possibility of carcass blemish which may follow inoculation of oil-adjuvanted vaccines by other routes. The results were most encouraging. Again, neither local nor general reactions were observed in any of the animals inoculated i/p in the experiments or in those pigs inoculated i/p in the field trials. Many of the latter were slaughtered at the end of the fattening period and no significant residual tissue reactions were found or carcasses condemned for this reason. For instance, out of a group of 99 pigs, only 7 had some very small nodules of 5 mm diameter on the parietal peritoneal membrane. These were possibly due to deposition of some vaccine along the track of the needle during penetration of the abdominal wall. The i/p route should therefore be considered as the route of choice for the inoculation of weanling pigs with mineral oil-adjuvanted preparations when the possibility of reactions affecting a proportion of the carcasses is an important factor. Residual reactions have also been observed in pigs following the use of double emulsion commercial vaccines (Garland, 1977). The i/m route would be suggested for use in breeding stock since residual lesions would not be an important aspect in these animals.

The oil emulsion FMD vaccines described give the required protection against the main FMD European and South American virus strains in fattening pigs. A small volume dose (2 ml) induces rapid onset of immunity which lasts for at least until the end of the fattening period (4 months post-weaning). Oil-adjuvanted vaccines usually induce an immune response that

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**TABLE II**

Results of challenge tests (second experiment)

<table>
<thead>
<tr>
<th>Time of inoculation before challenge</th>
<th>Groups and treatment</th>
<th>Challenge viruses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$O_1$ (&gt;5)$^1$</td>
</tr>
<tr>
<td>21 days</td>
<td>(1) w/o O-A-C$^2$ i/m</td>
<td>5/8</td>
</tr>
<tr>
<td>21 days</td>
<td>(2) w/o O-A-C$^3$ i/m</td>
<td>7/8</td>
</tr>
<tr>
<td>21 days</td>
<td>(3) w/o O-A-C$^3$ i/p</td>
<td>8/8</td>
</tr>
<tr>
<td></td>
<td>(4) Controls</td>
<td>0/5</td>
</tr>
<tr>
<td>120 days</td>
<td>(5) w/o O-A-C$^3$ i/p</td>
<td>5/8</td>
</tr>
<tr>
<td></td>
<td>(6) Controls</td>
<td>0/3</td>
</tr>
</tbody>
</table>

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1 log$^{10}$ ID$_{50}$ (pig) challenge dose used

2 w/o O-A-C vaccine, batch 1

after 12 months storage at 4°C

3 w/o O-A-C vaccine, batch 2

4 Protected/total
takes a relatively long time to reach a peak. The rapid onset of protective immunity induced by this vaccine is therefore a noteworthy and valuable property of the formulation for the control of the spread of FMD epizootics. The formulation is easy to inject on account of its low viscosity. It is physically stable and a shelf-life (potency-stability) of at least 1 year has been demonstrated. No general or local clinical signs are induced after inoculation by the i/m or i/p routes. The former may give rise to residual tissue reactions in a proportion of pigs which are observable at slaughter, but these may be avoided by the use of the i/p route. Data from field trials in Brazil (Nicholls et al., 1982) and experience from field use in Spain over a period of 6 years lend support to the laboratory results presented here.

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PROTECCIÓN ANTIAFTOSA DE CERDOS DE Ceba CON UNA VACUNA EN ADYUVANTE OLEOSO : ESTUDIO DE CEPAS SUDAMERICANAS DEL VIRUS AFTOSO. — O. Basarab, O. Umehara y L.F. Uribe.

Resumen : Se han evaluado los resultados conseguidos con una vacuna antiaftosa trivalente en adyuvante oleoso, que incluye los antígenos O¿ Campos, A24
Cruzeiro y C₃ Indaial, en cerdos de ceba de Brasil. Como se demostró anteriormente, una dosis única de este tipo de vacuna, administrada en el momento del destete, indujo rápidamente en los animales el establecimiento de la inmunidad contra la prueba virulenta; dura la inmunidad por lo menos cuatro meses, o sea la habitual duración de vida de los cerdos de ceba. No se manifiesta ningún síntoma clínico, general o local, después de la vacunación por las vías intramuscular o intraperitoneal. Algunos de los animales inmunizados por vía intramuscular presentaron en el momento del sacrificio reacciones tisulares residuales, pero pudieron cortarse las mismas de la canal con una pérdida mínima de tejido muscular (100 a 200 g). La inyección intraperitoneal no produjo lesiones residuales que afectaran a la canal.

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REFERENCES


