# FOOT-AND-MOUTH DISEASE OIL ADJUVANTED VACCINES FOR PIGS. III. IMMUNE RESPONSE OF VACCINES EMULSIFIED BY ULTRASONIC OR MECHANICAL EQUIPMENT[1]

*Ivo Gomes[2]; P. Augé de Mello[2]; A. Alonso Fernández[2]; Kleise de Freitas Costa[2]*

SUMMARY

The immune response and protection of pigs vaccinated intraperitoneally with double emulsion oil adjuvanted foot-and-mouth disease (FMD) vaccines were evaluated by antibody tests and challenge. One vaccine was prepared with ultrasonic equipment (Vaccine 1) and the other vaccine with a mixer-emulsifier (Vaccine 2). Both vaccines were also evaluated in a guinea pig $PD_{50}$ test. All systems used indicated that both vaccines were satisfactory but that Vaccine 2 was slightly superior to Vaccine 1.

It was also found that a mouse protection index (MPI) of 3.0 or a neutralization titer of 2.5 indicate a high degree of protection of the pigs. A low MPI not necessarily indicates lack of protection.

Of 22 pigs which developed lesions after exposure, 20 pigs became VIA antibody positive. However, only six of the 38 pigs without lesions developed VIA antibodies at 15 days post-exposure. This observation is noteworthy because a high percentage of vaccinated cattle which do not develop lesions after exposure to virus become VIA antibody positive (*9*). This difference might indicate a fundamental difference between subclinical FMD infection of cattle and pigs.

## INTRODUCTION

In an earlier paper (*2*) the successful intraperitoneal vaccination of young pigs with inactivated foot-and-mouth disease (FMD) double emulsion oil vaccine was reported. In a further study (*3*) using similar vaccines by the intraperitoneal route it was observed that a good correlation existed between antibody levels at 30 days post-vaccination (DPV) and challenge results. It was shown in those experiments that the challenge results were similar with needle or contact exposure.

The present experiments were done to compare double emulsion FMD vaccines prepared with ultrasonic[3] or mechanical[4] emulsification equipment. The results of this study also contribute towards the development of suitable potency assay methods for this type of FMD vaccine in pigs.

## MATERIALS AND METHODS

### Virus

The FMD virus strains used for vaccine production were $O_1$ Campos, A Bage, A Venceslau and $C_3$ Indaial.

All strains used for vaccines production were of bovine origin and had received a maximum of 7 passages in $BHK_{21}$ cells.

The $O_1$ Campos virus used for pigs inoculation was of the 11th bovine passages.

### Vaccines

The antigens used for the formulation of the vaccines were produced in the vaccine production pilot plant of this Center in roller bottles with $BHK_{21}$ clone 13 cells. Virus suspensions were inactivated with 0.001 M binary ethyleneimine (BEI) for 24 hours (*4*).

The characteristics of the antigens are listed in Table 1. Equal volumes of O, A and C antigen suspensions were used in the trivalent suspension

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[2] Pan American Foot-and-Mouth Disease Center, PAHO/WHO, Caixa Postal 589, 20000 Rio de Janeiro-RJ, Brazil.

[3] Minisonic, Ultrasonic Ltd. Otley Road Shipley, West Yorkshire, England.

[4] Silverson Machine (Sales) Ltd., London.

used to formulate the vaccine. The A component consisted of equal volumes of the A Bage and A Venceslau suspensions.

TABLE 1. *Characteristics of the antigens used for the formulation of the vaccines*

| Virus | Cell culture infectivity titer/ml | $CF_{50}$[a] titer |
|---|---|---|
| O1 Campos | $10^{6.6}$ | 1:24 |
| A Bage | $10^{7.6}$ | 1:28 |
| A Venceslau | $10^{7.4}$ | 1:26 |
| C3 Indaial | $10^{7.2}$ | 1:16 |

[a]CF = complement fixation test (4 $HCF_{50}$ as 90 min.).

*Vaccine 1*: was prepared in a Minisonic apparatus. First, 450 ml of a mixture of 9 parts mineral oil[5] and one part of mannide monooleate[6] were emulsified with 450 ml of the trivalent antigen suspension to form a water-in-oil type of suspension (primary emulsion).

This emulsion had similar characteristics as vaccines used in earlier experiments (*5*). The primary emulsion was re-emulsified with 900 ml PBS, pH 7.4 containing 2% polyoxyethylene 20 sorbitan monooleate[7] and merthiolate (final concentration 1:30.000 w/v) to form a water-in-oil-in-water type emulsion (double emulsion).

*Vaccine 2*: was prepared from an identical antigen suspension using a mechanical bench top mixer-emulsifier.

The primary emulsion consisted of 150 ml of the oily phase as described for Vaccine 1 and 150 ml of the trivalent antigen suspension.

For the double emulsion 300 ml of PBS pH 7.4 was added which contained 2% polyoxyethylene 20 sorbitan monooleate and merthiolate 1:30.000.

**Guinea pigs potency tests**

Dilutions of the vaccines were made in PBS pH 7.4 (1:3, 1:9 and 1:27). Six guinea pigs, 3-4 months old, weighing 550 ± 50 g each were inoculated by intramuscular route with 0.25 ml of the undiluted vaccines or with the dilutions.

After 30 days the guinea pigs were inoculated intradermalplantar in one foot pad and with $10^3$ generalizing doses 50% ($GD_{50}$) of the $O_1$ Campos strain. The lesions in the non-inoculated foot pads were observed at 5 days after inoculation. The estimation of the GP $PD_{50}$/0.25 ml of the vaccine prepared with the minisonic apparatus was 9 and for vaccine prepared by mechanical emulsification with the Silverson equipment was 27.

**Pig potency test**

Two months old recently weaned hybrid Humus-Seghers[8] pigs of approximately 20 kg were used in this experiment. Groups of 8 pigs were inoculated intraperitoneally with 3 ml of the vaccines or with dilutions similar to those used in the guinea pigs.

At 30 DPV vaccinated animals were placed in contact with two unvaccinated pigs inoculated intraplantar with $10^4$ mouse $ID_{50}$ of type $O_1$ Campos virus strain and with 3 unvaccinated contact pigs. The animals were examined on day 10 after the start of the exposure, when the only and final reading of the lesions was made.

**Antibodies**

Pigs were bled before vaccination at 30 DPV and 15 days after the start of virus exposure.

Antibody assay was done by the mouse protection test (*6*), microneutralization (*7*) and by agar gel double diffusion for VIA antibodies (*1*).

## RESULTS

The mean neutralization titers of the sera 30 DPV are listed in Table 2. It can be observed that the O and A antigens induced a better response

[5]Marcol 52 – Exxon Corporation, USA.

[6]Arlacel A – ICI America Inc., Atlas Chemicals Division.

[7]Tween 80 – ICI America Inc., Atlas Chemical Division.

[8]Humus Agrícola S/A. Via Armando Salles Oliveira, SP-322 km 356, Pitangueiras, SP, Brasil.

than the C antigen and that the titers observed with the vaccine emulsified with the Silverson equipment (Vaccine 2) are higher than those obtained with the Minisonic apparatus (Vaccine 1). Also, as a rule there is a very consistent antibody titer/dose response.

At challenge one of the inoculated donor pigs died. The other donor pig as well as 3 non-vaccinated contact pigs developed severe FMD. The individual responses of each of the vaccinated pigs for type $O_1$ virus are listed in Tables 3 and 4. Here, also, the graded decrease in the mouse protection indices (MPI) and microneutralization test (MNT) with diluting the vaccine can be observed, perhaps with the exception of the MPI for the 1:3 group of Vaccine 1. The same dose response can be noted for the development of foot lesions, with exception of the 1:3 group of Vaccine 2, in which 3 pigs had lesions on 1 or 2 feet.

The post-challenge boost of the MPI and MNT indicate that the pigs were well exposed to the challenge virus. It is noteworthy in this connection that a high portion of the pigs which did not develop lesions remained VIA antibody negative at 15 days post-challenge, even though several pigs got a boost of the neutralization titer or MPI.

The pigs with one or two feet affected did not suffer from the disease as did the unvaccinated control pigs or some of the more severely affected pigs in the 1:27 group of Vaccine 1. Pigs with such limited lesions continued to eat, their movements were not impaired and their lesions healed quickly.

Table 5 summarizes the challenge results of the pigs in relation to their antibody response. Of the 27 pigs with an MPI in the 0.0–0.9 range only 11 generalized. Thus, a low MPI of a vaccinated pig not necessarily indicates lack of protection. The 19 pigs with an MPI $\geqslant 3.0$ all were protected. Seven pigs with an MNT $\leqslant 1.4$ generalized and 31 of those with $\geqslant 2.5$ were protected.

The mean MPI and MNT for the pigs with lesions as 4 feet were $0.43 \pm 0.08$[9] and $1.54 \pm 0.16$ respectively. These values for the pigs without lesions were $3.02 \pm 0.35$ and $2.96 \pm 0.10$, respectively. The MPI and MNT of the pigs which had 1 or 2 feet affected were lower but not significantly different from those of the fully protected pigs (MPI $2.11 \pm 0.57$ and MNT $2.64 \pm 0.18$).

[9]Mean and standard error of the mean.

TABLE 2. *Mean serum microneutralization titers of pigs at 30 days after vaccination with dilution series of trivalent oil adjuvanted FMD vaccine*

| Vaccine dilution | Vaccine 1 (Minisonic) | | | Vaccine 2 (Silverson) | | |
|---|---|---|---|---|---|---|
| | Virus | | | Virus | | |
| | O | A | C | O | A | C |
| 1:1 | $3.2 \pm 0.25$[a] | $3.3 \pm 0.22$ | $2.7 \pm 0.45$ | $\geqslant 3.5$ | $\geqslant 3.5$ | $3.3 \pm 0.22$ |
| 1:3 | $2.5 \pm 0.54$ | $2.2 \pm 0.30$ | $2.0 \pm 0.25$ | $3.3 \pm 0.22$ | $2.9 \pm 0.37$ | $2.8 \pm 0.49$ |
| 1:9 | $1.7 \pm 0.45$ | $1.7 \pm 0.53$ | $1.6 \pm 0.59$ | $3.0 \pm 0.39$ | $3.0 \pm 0.36$ | $2.6 \pm 0.39$ |
| 1:27 | $\leqslant 1.2$ | $\leqslant 1.5$ | $\leqslant 1.2$ | $2.2 \pm 0.31$ | $2.2 \pm 0.25$ | $1.9 \pm 0.39$ |

[a]Mean and standard deviation.

TABLE 3. *Response of pigs vaccinated with double emulsion FMD oil vaccine No. 1 (Minisonic) and exposure to FMD virus type $O_1$*

| Vaccine dilution | Serum No. | 30 days post-vaccination | | | 15 days post-exposure | | |
|---|---|---|---|---|---|---|---|
| | | MPI | MNT | Lesions | MPI | MNT | VIA |
| 1/1 | 1 | ⩾ 5.3 | 3.2 | Neg. | ⩾ 5.5 | 3.3 | – |
| | 2 | ⩾ 4.3 | 2.9 | Neg. | ⩾ 5.5 | 3.3 | – |
| | 3 | ⩾ 5.3 | 3.0 | Neg. | 4.8 | 4.1 | – |
| | 4 | 2.6 | 3.5 | Neg. | ⩾ 5.5 | 2.9 | – |
| | 5 | 2.5 | 3.0 | Neg. | 2.1 | 2.3 | – |
| | 6 | ⩾ 5.3 | 3.6 | Neg. | ⩾ 5.5 | 3.2 | – |
| | 7 | 4.9 | 3.2 | Neg. | ⩾ 5.5 | 4.1 | – |
| | 8 | ⩾ 5.3 | 3.2 | Neg. | ⩾ 5.5 | 2.9 | – |
| 1/3 | 9 | 0.8 | 2.9 | Neg. | ⩾ 4.5 | 2.9 | – |
| | 10 | 1.0 | 2.9 | Neg. | ⩾ 4.5 | 3.3 | – |
| | 11 | 0.8 | 2.3 | Neg. | ⩾ 4.5 | 2.2 | – |
| | 12 | 0.0 | 1.7 | Neg. | ⩾ 4.5 | 2.9 | + |
| | 13 | 0.8 | 3.2 | Neg. | ⩾ 4.5 | 2.6 | – |
| | 14 | 0.5 | 2.6 | 4 F | ⩾ 5.5 | ⩾ 3.5 | + |
| | 15 | 0.7 | 2.9 | Neg. | *a* | *a* | *a* |
| | 16 | 0.0 | 1.8 | Neg. | ⩾ 4.5 | ⩾ 3.5 | + |
| 1/9 | 17 | 0.4 | 1.7 | 4 F | ⩾ 5.4 | 2.9 | + |
| | 18 | 0.4 | 2.3 | Neg. | ⩾ 5.4 | 3.3 | – |
| | 19 | 0.7 | ⩽ 1.2 | 4 F | ⩾ 5.4 | 3.3 | + |
| | 20 | 0.9 | 2.0 | 2 F | ⩾ 5.4 | 3.3 | + |
| | 21 | 0.7 | 1.8 | 4 F | ⩾ 5.4 | 3.2 | + |
| | 22 | 0.4 | ⩽ 1.2 | 4 F | ⩾ 5.4 | ⩾ 3.6 | + |
| | 23 | 1.3 | 2.4 | 2 F | ⩾ 5.4 | ⩾ 3.6 | – |
| | 24 | 0.4 | 1.5 | Neg. | ⩾ 4.4 | 3.3 | – |
| 1/27 | 25 | 0.4 | ⩽ 1.2 | 4 F | 5.4 | 2.7 | + |
| | 26 | 0.4 | ⩽ 1.2 | 4 F | *b* | *b* | *b* |
| | 27 | 0.4 | 1.7 | Neg. | 5.4 | ⩾ 3.5 | – |
| | 28 | 0.0 | ⩽ 1.2 | 4 F | 5.4 | 3.2 | + |
| | 29 | 0.2 | ⩽ 1.2 | 4 F | 5.4 | ⩾ 3.5 | + |
| | 30 | 0.2 | ⩽ 1.2 | 4 F | *b* | *b* | *b* |

*a* = Died after exposure to virus, not related to FMD.
*b* = Died after exposure to virus, likely due to FMD.
\+ = Positive.
– = Negative.
MPI = Mouse protection indices.
MNT = Microneutralization test.
F = Feet.

TABLE 4. *Response of pigs vaccinated with double emulsion FMD oil vaccine No. 2 (Silverson) and exposure to FMD virus type $O_1$*

| Vaccine dilution | Serum No. | 30 days post-vaccination | | | 15 days post-exposure | | |
|---|---|---|---|---|---|---|---|
| | | MPI | MNT | Lesions | MPI | MNT | VIA |
| 1/1 | 36 | ⩾ 5.3 | ⩾ 3.5 | Neg. | ⩾ 4.9 | ⩾ 3.5 | – |
| | 37 | ⩾ 5.3 | ⩾ 3.5 | Neg. | ⩾ 4.9 | 3.5 | – |
| | 38 | ⩾ 5.3 | ⩾ 3.6 | Neg. | ⩾ 4.9 | ⩾ 4.2 | – |
| | 39 | ⩾ 5.3 | ⩾ 3.5 | Neg. | ⩾ 4.9 | ⩾ 3.5 | – |
| | 40 | ⩾ 5.3 | ⩾ 3.5 | Neg. | ⩾ 4.9 | 2.9 | – |
| | 41 | ⩾ 5.3 | ⩾ 3.6 | Neg. | ⩾ 4.9 | 2.7 | – |
| | 42 | ⩾ 5.3 | 3.2 | Neg. | ⩾ 4.9 | ⩾ 3.5 | – |
| 1/3 | 43 | 4.4 | 3.0 | Neg. | ⩾ 4.9 | ⩾ 3.5 | + |
| | 44 | ⩾ 5.3 | 3.3 | Neg. | ⩾ 4.9 | ⩾ 3.6 | + |
| | 45 | 2.0 | 3.2 | 1 F | ⩾ 4.9 | ⩾ 3.6 | – |
| | 46 | ⩾ 5.3 | ⩾ 3.5 | 2 F | ⩾ 4.9 | ⩾ 4.5 | + |
| | 47 | 3.8 | 3.3 | 2 F | ⩾ 4.9 | ⩾ 4.8 | + |
| | 48 | ⩾ 5.2 | ⩾ 3.8 | Neg. | ⩾ 4.9 | ⩾ 4.8 | – |
| | 49 | ⩾ 5.2 | ⩾ 3.6 | Neg. | ⩾ 4.9 | 2.9 | – |
| | 50 | 1.0 | 3.2 | Neg. | 3.9 | 3.2 | – |
| 1/9 | 51 | 1.5 | 3.0 | Neg. | 4.4 | 3.5 | + |
| | 52 | ⩽ 0.8 | 3.0 | Neg. | ⩾ 4.8 | 3.8 | + |
| | 53 | 2.9 | ⩾ 3.6 | Neg. | ⩾ 5.8 | 3.2 | – |
| | 54 | 0.9 | 2.6 | Neg. | ⩾ 4.8 | ⩾ 3.6 | – |
| | 55 | ⩾ 5.2 | 3.2 | Neg. | ⩾ 5.8 | ⩾ 3.9 | – |
| | 56 | 1.5 | 2.4 | 1 F | ⩾ 5.8 | 2.6 | + |
| | 57 | 1.8 | 3.2 | Neg. | ⩾ 4.8 | ⩾ 3.6 | – |
| | 58 | ⩾ 5.2 | 3.3 | Neg. | ⩾ 5.8 | 3.5 | – |
| 1/27 | 59 | 3.4 | 2.3 | 2 F | ⩾ 5.8 | 3.3 | + |
| | 60 | 0.4 | 1.8 | Neg. | ⩾ 4.8 | 2.9 | – |
| | 61 | 0.4 | 1.8 | Neg. | ⩾ 4.8 | 3.2 | – |
| | 62 | 0.2 | 2.1 | 2 F | ⩾ 5.8 | ⩾ 3.5 | + |
| | 63 | 0.9 | 2.4 | 4 F | ⩾ 5.8 | ⩾ 3.6 | + |
| | 64 | 0.6 | 2.6 | 1 F | ⩾ 5.8 | ⩾ 3.5 | + |

+ = Positive.
– = Negative.

TABLE 5. *Foot lesions of pigs challenged with $O_1$ type FMD virus 30 days after vaccination with dilution series of oil adjuvanted FMD vaccine*

| Tests | Number of pigs | Number of feet affected | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 |
| Mouse protection index | | | | | | |
| 0.0 – 0.9 | 27 | 13 | 1 | 2 | – | 11 |
| 1.0 – 1.9 | 6 | 4 | 1 | 1 | – | – |
| 2.0 – 2.9 | 7 | 3 | 1 | 3 | – | – |
| ⩾3.0 | 19 | 19 | – | – | – | – |
| Total | 59 | 39 | 3 | 6 | 0 | 11 |
| Microneutralization titer | | | | | | |
| ⩽1.4 | 7 | – | – | – | – | 7 |
| 1.5 – 1.9 | 8 | 6 | – | – | – | 2 |
| 2.0 – 2.4 | 8 | 2 | 1 | 4 | – | 1 |
| 2.5 | 36 | 31 | 2 | 2 | – | 1 |
| Total | 59 | 39 | 3 | 6 | 0 | 11 |

## DISCUSSION

Both vaccines gave excellent protection of pigs against severe contact challenge. However, the vaccine prepared with the Silverson equipment was slightly superior in performance. Further tests are needed to show if the difference between the two emulsification techniques is consistent.

It is encouraging that the GP $PD_{50}$, the neutralization test, the mouse protection test and the challenge test used to estimate the potency of the vaccines all gave this same indication.

It is important to note the efficiency of the contact exposure used in this experiment. The vaccinated pigs, unvaccinated contact pigs and virus donors all were housed together. The unvaccinated contact pigs became severely ill and an antibody boost of all of the pigs with lower pre-challenge titers showed that all animals were well exposed to virus. All vaccinated pigs with MPI ⩾ 3.0 and 31 out of 36 with MNT ⩾ 2.5 were fully protected.

In several of the pigs with the higher antibody titers virus replication apparently was inhibited to the extent that VIA antibody formation could not be detected.

Of 22 pigs which developed lesions after exposure 20 pigs became VIA antibody positive. However, only six of the 38 pigs without lesions developed VIA antibodies at 15 days post-exposure. This observation is noteworthy because a high percentage of vaccinated cattle which do not develop lesions after exposure to virus become VIA antibody positive (*9*). This difference might indicate a fundamental difference between subclinical FMD infection of cattle and pigs.

One of the main problems which still remains to be resolved is the classification of pigs with lesions on one or two feet which remain perfectly healthy otherwise. Gomes (*8*) already noted that even convalescent pigs can develop local lesions when they are in a heavily contaminated environment. It is likely that pigs with only one or two affected feet have local lesions caused by the infectious skin abrasions, particularly, since according to their antibody responses those pigs more likely belong to the fully protected group than to the group of fully generalized pigs.

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