FOOT-AND-MOUTH DISEASE OIL ADJUVANTED VACCINES FOR PIGS. I. DOUBLE EMULSION VACCINE APPLIED BY DIFFERENT ROUTES

P. Augé de Mello[1]; *Ivo Gomes*[1]

SUMMARY

Satisfactory immune response was obtained up to 110 days post-vaccination (DPV) with subcutaneous, intramuscular and intraperitoneal inoculation of oil adjuvanted double emulsion vaccine containing unconcentrated and unpurified antigens. No significant differences in response were observed between the different routes of application when compared to primary emulsion vaccine applied subcutaneously. However, challenge at 110 DPV showed some problems in interpretation of results. Several pigs developed vesicular lesions despite high antibody titers. These lesions did not affect the general health of the animals. The double emulsion vaccine, IP application, produced no local reactions nor macroscopic alteration of the regional lymph nodes. For field use intraperitoneal vaccination with double emulsion appears preferable to primary emulsion.

INTRODUCTION

Several papers (3, 5, 8, 10, 12, 13, 14, 15, 16, 18, 21) reported good levels of protection obtained from primary emulsion oil vaccines in pigs. However, these vaccines were not always acceptable in the field because of tissue reactions at the point of inoculation and the regional lymph nodes (13, 18, 20).

Anderson *et al.* (1) and Mowat (17) confirmed that oil adjuvanted vaccine provided acceptable local tissue reactions and satisfactory immunity when prepared in double emulsion (11) with purified, concentrated antigens and applied in small volumes of 0.1 to 0.5 ml intradermally in the dorsal site of the ear. Anderson *et al.* (1) suggested that this double emulsion formulation administered in small doses produces less reaction than the primary emulsion, both at the point of the inoculation and in the regional lymph nodes. It should therefore be more acceptable for use in the field.

In the present study young pigs were inoculated with double emulsion oil adjuvanted vaccine using foot-and-mouth disease (FMD) antigens without concentration or purification. Observations were made of the lesions produced by this type of vaccine when applied by subcutaneous (SC), intramuscular (IM) and intraperitoneal (IP) routes.

MATERIALS AND METHODS

Vaccines

Primary oil emulsion vaccines were prepared as described (2) using mineral oil[2] and 10% of monooleate of manitol[3] emulsified with equal parts of a trivalent antigen suspension inactivated by binary ethylenimine (4). The double emulsion (11) was made from the primary emulsion by mechanical emulsification[4] of the primary emulsion with an equal volume of phosphate buffer saline for 0.04 M, pH 7.4 with 2% of polyoxyethylene 20 monooleate of sorbitan[5]. Table 1 lists the characteristics of the antigens.

Pigs

Recently weaned, 2-month old Landrace pigs of approximately 20 kg were used. Three groups of 10 pigs each were inoculated with the double emulsion vaccine: one group subcutaneously at

[1]Pan-American Foot-and-Mouth Disease Center, Caixa Postal 589, ZC-00, Rio de Janeiro, RJ, Brazil.

[2]Marcol 52, Exxon Corporation U.S.A.

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

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

[5]Tween 80, ICI America Inc. Atlas Chemicals Division.

the base of the ear; the second group intramuscularly in the side of the neck; and the third group intraperitoneally. A fourth group of 10 pigs was inoculated subcutaneously at the base of the ear with a primary emulsion vaccine. To ensure that all pigs received the same quantity of antigen and oil phase, 1.5 ml of the primary emulsion vaccine was inoculated and 3.0 ml of the double emulsion vaccine.

TABLE 1. *Characteristics of foot-and-mouth disease antigens* [a]

Antigens	Titers	
	$CCID_{50}$/ml	FC
O_1 Campos	7.5	1/14
A_{24} Cruzeiro	7.5	1/14
C_3 Resende	7.3	1/15

[a] 2 ℓ Roller bottles of BHK 21 C 13 cells.
$CCID_{50}$ = 50% infective cell culture doses.
CF = Complement fixation.

Antibody studies

Blood samples were collected from the pigs before vaccination and at 30, 60 and 90 DPV. The serum was tested by the microneutralization test (7) against the strains of FMD virus used in vaccine preparation. The 90 DPV sera were also tested by the mouse protection test (6) against O_1 Campos, the challenge strain.

Virus challenge

The animals were challenged at 110 DPV against strain O_1 Campos. Four unvaccinated pigs received intraplantar inoculation with $10^{4.6}$ mouse $LD_{50\%}$ of the O_1 Campos virus. These 4 pigs were placed in the same isolation unit with the vaccinated animals, along with 4 unvaccinated controls pigs.

The pigs were then examined daily for lesions until the 12th day, when the last observation was made.

Scoring criterion

Observed lesions were classified according to their location. One point was given for each affected leg plus one for snout or lip, giving a total of 5 possible points per animal.

RESULTS

The results of the microneutralization tests at 30, 60 and 90 DPV are summarized in Table 2. No significant differences appeared in the antibody levels of pigs vaccinated with primary or double emulsion vaccines. Also, no differences were observed in the immune response when the 3 vaccination application routes were compared.

Table 3 shows the results of clinical observations of the pigs at 110 DPV against FMD virus O_1 Campos. Most of the vaccinated pigs presented some type of vesicular lesion, even if only superficial. The animals did not appear to suffer and moved about easily. Thus there was no economic loss due to the disease. Evaluation by individual scores and group averages showed marked differences with the control group since two pigs in the inoculated groups and two in the contact groups died of FMD infection. The survivors in the control groups all had maximum scores.

In most cases no correlation existed between antibody levels and observed lesions. Eighteen of the 38 animals challenged showed some type of vesicular lesion, despite mouse protection indices (MPI) $\geqslant$2.5. Only 3 of 35 were negative with MPI $\geqslant$4.0. In the microtiter test 27 of the 38 pigs showed lesions and had neutralization titers $\geqslant$2.0, while the negative animals had titers $\geqslant$2.4. However, 8 animals with the same titers generalized.

The post-mortem examination carried out 30-40 days after challenge showed that pigs inoculated with primary emulsion oil vaccine had unacceptable tissue reaction at the point of inoculation.

There were also oil emulsion deposits in the regional lymph nodes. The double emulsion vaccine inoculated subcutaneously or intramuscularly produced acceptable local lesions at the point of inoculation. No macroscopic deposits of oil emulsion were observed in the regional lymph nodes. It is noteworthy that the double emulsion vaccine inoculated intraperitoneally produced no tissue reaction nor macroscopic deposits in the mesenteric lymph nodes.

TABLE 2. *Mean of microneutralization titers of sera obtained from pigs vaccinated with oil adjuvanted vaccine*

Inoculation route	Emulsion type	Virus type	Days post-vaccination			
			0	30	60	90
SC	Primary	O_1	<1.0	2.19 ± 0.18	2.06 ± 0.32	2.15 ± 0.28
		A_{24}	<1.0	2.52 ± 0.26	2.15 ± 0.31	1.95 ± 0.35
		C_3	<1.0	2.18 ± 0.32	1.97 ± 0.32	2.21 ± 0.40
	Double	O_1	<1.0	2.44 ± 0.32	2.14 ± 0.35	2.10 ± 0.35
		A_{24}	<1.0	2.39 ± 0.40	1.97 ± 0.38	1.94 ± 0.40
		C_3	<1.0	2.19 ± 0.29	1.97 ± 0.41	2.15 ± 0.27
IM	Double	O_1	<1.0	2.42 ± 0.35[a]	2.04 ± 0.52	2.35 ± 0.38
		A_{24}	<1.0	2.61 ± 0.33	2.46 ± 0.57	2.13 ± 0.50
		C_3	<1.0	2.34 ± 0.28	2.08 ± 0.52	2.47 ± 0.40
IP	Double	O_1	<1.0	2.54 ± 0.36	2.03 ± 0.19	2.16 ± 0.38[a]
		A_{24}	<1.0	2.78 ± 0.29	2.46 ± 0.25	2.25 ± 0.38
		C_3	<1.0	2.53 ± 0.43	2.34 ± 0.30	2.38 ± 0.42

[a] Mean of 9 pigs (1 died).

SC = Subcutaneous. IM = Intramuscular. IP = Intraperitoneal.

TABLE 3. *Individual clinical reactions of pigs at 110 days post-vaccination with exposure by contact FMDV strain O_1 Campos*

Inoculation route	Emulsion Type	Antibody evaluation			Lesions	Score mean
		Animal No.	MN	MPI		
SC	Primary	1	2.4	4.4	1P	2.3
		2	2.1	1.3	4P	
		3	1.8	0.3	4P	
		4	2.1	4.0	2P, L	
		5	1.7	1.2	2P, S	
		6	2.3	4.1	1P	
		7	2.0	2.3	4P	
		8	2.6	> 4.6	Neg.	
		9	2.3	3.7	2P	
		10	2.4	2.0	2P	
	Double	11	1.5	0.3	4P, S	3.1
		12	2.3	2.2	3P	
		13	2.0	0.5	4P, S	
		14	2.3	> 4.0	2P	
		15	2.3	1.5	1P, S	
		16	2.1	0.4	3P	
		17	2.4	4.0	Neg.	
		18	2.7	3.0	4P	
		19	1.8	1.0	2P	
		20	1.8	0.5	4P, S, L	
IM	Double	21	2.4	3.9	1P	2.0
		22	2.1	1.4	2P	
		23	2.0	> 3.6	2P	
		24	2.9	> 4.6	1P	
		25	2.4	5.0	2P	
		26	2.9	> 3.6	4P	
		27	2.0	1.9	4P	
		28	2.0	0.5	2P	
		29	2.7	> 4.0	Neg.	
IP	Double	30	1.8	1.4	4P	2.1
		31	2.6	4.0	3P	
		32	2.3	> 3.6	3P	
		33	1.8	0.9	4P	
		34	2.3	2.0	2P	
		35	2.3	1.6	1P	
		36	...	> 4.6	1P	
		37	2.7	> 4.6	2P	
		38	1.7	0.6	1P	
Control			<1.0	...	4P, S, L[a]	5

[a] All animals had generalized lesions and 4 died after infection.

MN = Microneutralization. MPI = Mouse protection indices.

P = Pad. S = Snout. L = Lip.

... = not done.

DISCUSSION

The experiment was limited to 110 DPV since this period was considered essential to the pig's protection, in the productive period between weaning and slaughter, not counting breeder stocks. The experiment was thus designed to approximate as closely as possible commercial pig breeding conditions.

Protection results were not entirely satisfactory since the majority of vaccinated pigs presented some vesicular lesion after challenge by contact at 110 DPV; however, the practical value of the vaccination was evident. The lesions did not affect the overall health of the animals, and they continued to feed normally. The control animals however were severely infected: 2 of the contacts and 2 of the inoculated animals died, while the survivors suffered a precipitate weight loss, became cachectic and did not recover.

The experiment pointed to some problems which must be considered in future vaccine experiments using pigs. As noted by other authors (19), pigs with high antibody titers had some vesicular lesions. Gomes (9) also observed that pigs with high antibody levels could develop lesions when reexposed to homologous virus, thus indicating that the lesions are probably produced by local virus replication.

In this experiment the hind feet were twice as often affected as the front feet. Gomes (9) suggested that excessive examination of the animals produces microtraumas, small skin abrasions, which permit introduction of virus from the environment. We believe that many of the lesions observed were actually caused by the daily examination of the pigs. It should also be noted that lesions observed in the first few days were still easily visible at the 12th day. Thus any lesion appearing within the first few days would not be overlooked if only one reading was made at 10 or 12 days.

Analysis of antibody levels showed that the IP route gave an immune response similar to those obtained by the SC and IM routes, although the IP route produces neither local reactions nor macroscopic changes in the lymph nodes. This result confirmed the preliminary observation made by Augé de Mello (unpublished data) which indicated that this formulation does not produce a local reaction nor a macroscopic alteration of the regional lymph nodes. It should be mentioned that primary emulsion vaccine applied by the IP route produces small, easily visible deposits in the mesenteric lymph nodes as well as in the abdomen. Therefore for pigs FMD vaccine in the form of double emulsion and use of IP seem to be more promising than primary emulsion. These results indicate the need for further experiments using only double emulsion vaccine inoculated by the IP route.

REFERENCES

1. ANDERSON, E.C.; MASTERS, R.C.; MOWAT, G.N. Immune response of pigs to inactivated foot-and-mouth disease vaccines. Response to emulsion vaccines. *Res. vet. Sci. 12:* 242-350, 1971.
2. AUGÉ DE MELLO, P.; ASTUDILLO, V.; GOMES, I.; CAMPOS GARCIA, J.T. Aplicación en el campo de vacuna antiaftosa oleosa e inactivada: vacunación y revacunación de bovinos jóvenes. (Field application of inactivated oil adjuvanted foot-and-mouth disease virus vaccine: vaccination and revaccination of young cattle). *Bltn Centro Panamericano Fiebre Aftosa 19-20:* 31-38, 39-47, 1975.
3. BACHRACH, H.L.; McKERCHER, P.D. Immunology of foot-and-mouth disease in swine: Experimental inactivated virus vaccine. *J. Am. vet. Med. Ass. 160* (4): 521-526, 1972.
4. BAHNEMANN, H.G.; AUGÉ DE MELLO, P.; ABARACON, D.; GOMES I. Immunogenecity in cattle of foot-and-mouth disease vaccines inactivated with binary ethylenimine. *Bull. Off. int. Epiz. 81* (11-12): 1335-1343, 1974.
5. CAPORALE, G.; ROSSI, G.A.; PELLICCIONI, A. Vaccination of swine against foot-and-mouth disease (FMD) with hydroxylamine and acetylethyleneimine inactivated virus. *Vet. ital. 20:* 137-147, 1969.
6. CUNHA, R.G.; BAPTISTA Jr., J.A.; SERRÃO, U.M.; TORTURELLA, I. El uso de los ratones lactantes en la evaluación de los anticuerpos contra el virus de la fiebre aftosa y su significación inmunológica. *Gac. vet.*, B. Aires *19* (110): 243-267, 1957.
7. FERREIRA, Maria Elma V. Prueba de microneutralización para estudios de anticuerpos de la fiebre aftosa. (Microtiter neutralization test for the study

of foot-and-mouth disease antibodies). *Bltn Centro Panamericano Fiebre Aftosa 21-22:* 17-20, 21-24, 1976.

8. GRAVES, J.H.; McKERCHER, P.D.; FARRIS, H. E.; COWAN, K.M. Early response of cattle and swine to inactivated foot-and-mouth disease virus. *Res. vet. Sci. 9:* 35-40, 1968.
9. GOMES, I. Fiebre aftosa: reacción de cerdos convalecientes a la exposición de virus homólogo. (Foot-and-mouth disease: reaction of convalescent pigs to homologous virus exposure). *Bltn Centro Panamericano Fiebre Aftosa 26:* 15-18, 19-22, 1977.
10. GIRAUD, M.; GUILLOTEAU, B.; PERROT, A.; DEBROCK, C.; PRUNET, P. Recherches sur l'activité d'un nouveau vaccin anti-aphteux chez le porc. *Bull. Off. int. Epiz. 71* (3-4): 285-306, 1969.
11. HERBERT, W.T. Multiple emulsions - A new form of mineral-oil antigen adjuvant. *Lancet 16:* 771, 1965.
12. McKERCHER, P.D.; GIORDANO, A.R. Foot-and-mouth disease in swine. I. The immune response of swine to chemically-treated and non-treated foot-and-mouth disease virus. *Arch. ges. Virusforsch. 20:* 39-53, 1967.
13. McKERCHER, P.D.; GAILIUNAS, P. Response of swine to inactivated foot-and-mouth disease vaccine. Duration of immunity and local tissue reaction. *Arch. ges. Virusforsch. 28* (2): 165-176, 1969.
14. McKERCHER, P.D.; FARRIS, Jr. H.E. Foot-and-Mouth Disease in Swine: Response to inactivated vaccines. *Arch. ges. Virusforsch. 22* (3-4): 451-461, 1967.
15. McKERCHER, P.D. Response to swine to oil adjuvant vaccine. *International Symposium on Foot-and-Mouth Disease 8:* 151-160, 1967.
16. MORGAN, D.O.; McKERCHER, P.D.; BACHRACH, H.L. Quantitation of the antigenicity and immunogenicity of purified foot-and-mouth disease virus vaccine for swine and steers. *Appl. Microbiol. 20* (5): 770-774, 1970.
17. MOWAT, G.N. Quantities of purified antigen required to immunize swine against foot-and-mouth disease. *Bull. Off. int. Epiz. 77* (5-6): 887-897, 1972.
18. UNITED STATES DEPARTMENT OF AGRICULTURE AND PAN-AMERICAN HEALTH ORGANIZATION. Vacunas contra la fiebre aftosa. II. Estudios sobre la duración de la inmunidad en bovinos y porcinos. (Foot-and-mouth disease vaccines. II. Studies on duration of immunity in cattle and pigs). *Bltn Centro Panamericano Fiebre Aftosa 19-20:* 17-23, 24-30, 1975.
19. WITTMANN, G.; BAUER, K.; MUSSGAY, M. Experiments on vaccination of pigs with ethyl-ethyleneimine (EEI) diethylaminoethyl dextran (DEAE-D) foot-and-mouth disease vaccines. Influence of route of inoculation and dose of antigen on the duration of immunity. *Arch. ges. Virusforsch. 36:* 251-264, 1972.
20. WITTMANN, G.; BAUER, K. Local reactions after infecting pigs with foot-and-mouth disease vaccine containing Freund's adjuvant. *Berl. Münch tierärztl. Wschr. 82* (1): 2-4, 1969.
21. WITTMANN, G.; BAUER, K.; MUSSGAY, M. Essais de vaccination de porcs avec des vaccins à base de virus aphteux inactivé. I. Essais avec du virus O inactivé par l'hydroxylamine, le formol, la chaleur et le pH. *Bull. Off. int. Epiz. 71* (3-4): 351-379, 1969.

. . .