FOOT-AND-MOUTH DISEASE OIL ADJUVANTED VACCINE FOR PIGS. II. INTRAPERITONEAL VACCINATION OF YOUNG PIGS WITH DOUBLE EMULSION VACCINE[1]

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SUMMARY

A trivalent double emulsion FMD vaccine was compared at 30 and 110 days post-vaccination (DPV) in pigs with 2 similar vaccines containing antigens concentrated by polyethylene glycol (PEG) precipitation. The antigens in those two vaccines had 1X and 10X the CF capacity of the original antigens. The vaccines were used to vaccinate recently weaned pigs by the intraperitoneal route in a 3 ml dose. The 10X CF vaccine was also used in a 0.3 ml dose.

All vaccines induced good levels of circulating antibodies for the three strains of the FMD virus and the protection against FMD virus subtype O_1 strain Campos was excellent, particularly in the 10X CF vaccine used in the 3 ml dose. No undesirable side effects were observed.

In this experiment a good correlation was observed between antibody levels at 30 DPV and protection at challenge both for the mouse protection test and for the microneutralization test. At 110 DPV there was a poor correlation of the mouse protection test and protection at challenge caused, in particular, by pigs with low mouse protection indices.

During the challenge at 30 days a comparison between foot-inoculation and contact methods of exposure revealed no differences between the two.

INTRODUCTION

An earlier paper (2) reported that a double emulsion (7) foot-and-mouth disease (FMD) oil adjuvanted vaccine, when applied intraperitoneally in recently weaned pigs produced no undesirable local tissue reactions nor macroscopic changes in the regional lymph nodes. Thus, this type of formulation and route of application appeared more suitable for use in the field than primary emulsion vaccine (12). Also the immunogenicity of these vaccines when used intraperitoneally was satisfactory, and the results were not significantly different from subcutaneous or intramuscular application.

The present paper reports some further studies on the intraperitoneal vaccination of young pigs with oil adjuvanted double emulsion FMD vaccines containing different quantities of antigen and adjuvant. At 30 DPV comparisons were also made between the intraplantar needle exposure and contact exposure. Finally, the correlation between circulating antibodies and FMD virus challenge is discussed.

MATERIALS AND METHODS

Vaccines

The antigens used in the formulation of the vaccines were FMD virus O_1 strain Campos, A_{24} strain Cruzeiro and C_3 strain Resende. The virus suspensions were inactivated with binary ethylenimine (3). From each monovalent inactivated antigen suspension a portion was precipitated with 10% (w/v) of polyethylene glycol (PEG) 6000, followed by centrifugation at 800 g for 60 min. The sediment was resuspended in Eagle's medium (pH 7.4) to the extent that the complement fixation (CF) titer (4) was respectively 1X or 10X the CF titer of

[1]This work was supported by grants from the FRIGOBRAS - Cia. Brasileira de Frigoríficos and the Instituto Vallée S.A.

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the original antigen suspension. Table 1 shows the characteristics of each of the antigens.

TABLE 1. *Characteristics of the FMD antigens*[a]

Antigens	Titers		
	$CCID_{50}$	CF	10X conc.[b]
O_1 Campos	7.0	1/12	1/130
A_{24} Cruzeiro	7.0	1/26	1/250
C_3 Resende	7.3	1/16	1/140

[a] Produced in BHK 21 C 13 cells cloned in roller bottles.

[b] Concentration by PEG 6000.

$CCID_{50}$ = Cell culture infectious doses.

CF = Complement fixation.

Equal parts of the monovalent antigen suspensions, with or without PEG treatment, were mixed to form 3 trivalent suspensions: 1) reference - antigens without PEG treatment; 2) 1X CF - PEG - treated antigens with CF titers similar to those of the original antigens; and 3) 10X CF - PEG - treated antigens with a 10X higher CF titer than the original antigens.

With each of the trivalent suspensions a double emulsion oil adjuvanted vaccine was formulated as described (2).

Potency test

Groups of guinea pigs (GP) were inoculated with 0.5 ml of 3-fold dilutions of the reference vaccine (Antigens No. 1) in buffered saline phosphate with 2% polyoxyethylene 20 sorbitan monooleate[4] (pH 7.4). Thirty days later these guinea pigs were inoculated in one footpad with 10^3 GP generalizing doses of FMD virus strain O_1 Campos (8).

The results showed that 0.5 ml of the reference vaccine contained 11 GP PD_{50}.

[4] Tween 80 - ICI America Inc. Atlas Chemical Division.

Pigs

Two-month old recently weaned hybrid Humus-Seghers[5] pigs of approximately 20 kg were used in this experiment. Four groups of 25 pigs each were vaccinated intraperitoneally as follows: pigs of Group 1 received 3 ml of the reference vaccine (Antigens No. 1); Group 2 was vaccinated with 1X CF vaccine (Antigens No. 2) at a 3 ml dose; Groups 3 and 4 were vaccinated with 10X CF vaccine (Antigens No. 3) with 3 ml and 0.3 ml, respectively. Eight pigs were left unvaccinated to serve as future controls and virus donors.

Challenge of immunity

At 30 DPV 16 of the 25 vaccinated pigs of each group were exposed to FMD virus O_1, strain Campos. Eight pigs were inoculated in the heel of the right hind foot with $10^{4.6}$ mouse LD_{50} and 8 were kept as controls. Two of the 4 unvaccinated pigs were similarly exposed and 2 remained as control. The animals were maintained in intimate contact and were all housed in separate isolation units for each type of challenge.

At 110 DPV the remaining pigs of each group were exposed by contact in a similar manner by infecting 2 donor pigs and adding 2 unvaccinated control pigs.

The pigs were examined for FMD on the 12th day after the start of virus exposure.

Antibodies

Serum was collected from all pigs at 0 days and at 30 DPV. Of those pigs not challenged at 30 days, serum was also collected at 60 and 90 DPV.

Circulating neutralizing antibody titers were determined by the microneutralization test (MNT) (6) against the 3 virus strains used for the preparation of the vaccines. The 30 and 90 DPV sera were also used in a mouse protection test (MPT) (5) against FMD virus O_1 strain Campos.

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RESULTS

Table 2 shows the levels of neutralizing antibodies at 30, 60 and 90 DPV against the 3 types of FMD virus. It can be observed that all vaccines induced adequate levels of circulating antibody. The reference vaccine and the 1X CF vaccine which contained the same amount of antigen gave similar results while the 10X CF vaccine induced a higher response when used as a 3 ml dose. However, this same vaccine produced poorer results when used as a 0.3 ml dose, particularly against type O_1 Campos, at 60 and 90 DPV.

The results of the challenge test at 30 and 110 DPV against type O_1 Campos are listed in Table 3.

At 30 and 110 DPV the 10X CF vaccine in the 3 ml dose showed the best protection with none of the exposed animals showing any sign of clinical FMD. The same vaccine in the 0.3 ml dose gave lower protection both at 30 and 110 DPV.

The reference vaccine as well as the 1X CF were equally satisfactory at 30 DPV. At 110 DPV the reference vaccine still induced a good level of protection but the interpretation of the 1X CF vaccine was difficult because 5 animals in this group died between 60 and 90 DPV from causes not related to FMD. The 3 remaining pigs were protected and the antibody responses at 30, 60 and 90 DPV were similar to those of the reference vaccine.

TABLE 2. *Mean and standard deviation of the neutralization titers of sera obtained from pigs vaccinated with double emulsion oil adjuvanted FMD vaccine*

Vaccine	Doses (ml)	Type of virus	Days post-vaccination 0	30	60	90
Reference	3	O	<1.0	2.51 ± 0.56[a]	2.17 ± 0.34[b]	2.55 ± 0.33[b]
		A	<1.0	2.80 ± 0.65	2.85 ± 0.46	2.73 ± 0.51
		C	<1.0	2.72 ± 0.57	2.40 ± 0.39	2.52 ± 0.33
1X FC	3	O	<1.0	2.65 ± 0.50	2.48 ± 0.70	2.75 ± 0.23
		A	<1.0	3.23 ± 0.49	3.3 ± 0.32	3.2 ± 0.09
		C	<1.0	2.78 ± 0.53	2.66 ± 0.35	2.9 ± 0.09
10X FC	3	O	<1.0	2.63 ± 0.34	2.58 ± 0.53	2.58 ± 0.53
		A	<1.0	3.23 ± 0.27	3.27 ± 0.56	3.20 ± 0.57
		C	<1.0	3.0 ± 0.45	2.65 ± 0.57	2.85 ± 0.74
	0.3	O	<1.0	2.18 ± 0.60	1.82 ± 0.23	1.88 ± 0.35
		A	<1.0	2.50 ± 0.73	2.65 ± 0.42	2.37 ± 0.56
		C	<1.0	2.32 ± 0.66	2.17 ± 0.57	1.93 ± 0.43

[a] 25 animals per group.

[b] Means from 9 animals per group with the exception of 1X CF group in which one animal died at 60 DPV and 4 died before completing 90 DPV.

TABLE 3. *Protection of pigs against FMDV O_1 Campos after vaccination with a double emulsion oil adjuvanted FMD vaccine*

Vaccine	Doses (ml)	Challenge			
		30 DPV		110 DPV	
		IDP	Contact	IDP	Contact
Reference	3	7/8[a]	7/8	...	6/9
1X CF	3	7/8	8/8	...	3/3[b]
10X CF	3	8/8	8/8	...	8/9
	0.3	4/8	7/8	...	5/9
Controls		1/2[c]	0/2	0/2	0/2

[a] Number of animals protected over animals challenged.
[b] Five pigs died between 60 and 90 DPV.
[c] One pig died after inoculation.
DPV = Days post-vaccination.
IDP = Intradermoplantar inoculation.

The correlation between protection at challenge and the levels of circulating antibodies (MNT and MPT) at 30 DPV are presented in Table 4. If the value of $\geqslant 2$ is taken to indicate protection for the MNT or the MPT (5), then there exists an excellent correlation between protection and antibody levels, r = 0.83 and 0.79, respectively. Eight out of 64 pigs challenged developed vesicular lesions. Seven of those did not have detectable antibodies in each of the tests (0.0 and 0.9) and developed generalized lesions. One animal of the 8 which had a neutralization titer between 1.5 and 1.9 and an MPI in the 1.0 and 1.9 range, only developed lesions on 2 feet. There were 4 and 3 protected pigs with a value below 2.0 for the MPT and the MNT, respectively.

Using a similar value of 2 the results at 110 DPV, as listed in Table 5, indicated a poor correlation between protection at challenge and the neutralization test and the mouse protection (r = 0.02 and 0.03, respectively).

TABLE 4. *Antibodies of pigs at 30 DPV with a double emulsion oil adjuvanted FMD vaccine in challenge by intradermoplantar inoculation or by contact against FMDV O_1 Campos*

Type of test	Range of antibodies	Number of animals	Number of affected feet				
			0	1	2	3	4
Serumprotection	0.0 – 0.9	8	1	–	–	–	7
	1.0 – 1.9	4	3	–	1	–	–
	2.0 – 2.9	0	–	–	–	–	–
	$\geqslant$ 3.0	52	52	–	–	–	–
	Total	64	56	0	1	0	7
Microneutralization	0.0 – 0.9	7	–	–	–	–	7
	1.0 – 1.4	0	–	–	–	–	–
	1.5 – 1.9	4	3	–	1	–	–
	2.0 – 2.4	17	17	–	–	–	–
	$\geqslant$ 2.5	36	36	–	–	–	–
	Total	64	56	0	1	0	7

TABLE 5. *Antibodies of pigs 90 and 110 DPV with a double emulsion oil adjuvanted FMD vaccine in challenge by intradermoplantar inoculation or by contact against FMDV O_1 Campos*

Type of test	Range of antibodies	Number of animals	Number of affected feet				
			0	1	2	3	4
Serumprotection	0.0 – 0.9	9	6	2	–	–	1
	1.0 – 1.9	3	3	–	–	–	–
	2.0 – 2.9	8	6	2	–	–	–
	⩾ 3.0	10	8	1	1	–	–
	Total	30	23	5	1	0	1
Microneutralization	0.0 – 0.9	0	–	–	–	–	–
	1.0 – 1.4	0	–	–	–	–	–
	1.5 – 1.9	8	6	1	–	–	1
	2.0 – 2.4	12	7	4	1	–	–
	⩾ 2.5	10	10	–	–	–	–
	Total	30	23	5	1	0	1

DISCUSSION

Double emulsion oil adjuvanted FMD vaccine used intraperitoneally in recently weaned pigs induced adequate levels of circulating antibodies, which confirmed earlier observations (2). There were also no undesirable side effects nor abnormalities in the regional lymph nodes.

The protection produced by these vaccines against FMD O_1 virus was excellent, particularly with antigens which were concentrated to a 10-fold CF titer and applied in a 3 ml dose. However, when this same vaccine was used in a 0.3 ml dose the results were inferior to those obtained with the reference vaccine or the 1X CF vaccine containing similar amounts of antigen per dose.

Several investigators (1, 9, 10, 11) have studied vaccines applied in small volumes with purified concentrated antigens for the immunization of pigs. Such smaller volumes are used to reduce or to prevent undesirable tissue reaction to the oil adjuvant. However, results obtained in the present experiment seem to indicate that reducing the quantity of oil adjuvant decreases the immunogenicity of the vaccine.

A good correlation was observed between the circulating antibodies at 30 DPV, between antibody titers or indices and protection of the pigs at challenge (Table 4). If animals with MNT or MPI values < 2 are assumed not to be protected, it can be seen that for the MPT, 4 out of 12 pigs with values lower than 2 did not develop FMD. A fifth pig with an MPI < 2 was partially protected (2 feet). The results of the MNT are quite similar; 3 of 11 pigs did not develop lesions and one pig had lesions on 2 feet. With a value of > 3.0 for the MPI and > 2.0 with MNT all pigs were protected. The correlation between levels of antibodies in both tests and the protection of animals at challenge at 110 DPV was less satisfactory than at 30 DPV. In the MPT this difference is due to the larger number of protected animals with indices < 2 rather than to non-protected pigs with high levels of antibodies. In the MNT there were several pigs (7/20) with titers in the 1.5 - 2.4 range which developed lesions in 1 or 2 feet.

Earlier work (2) indicated that challenge at 110 DPV of vaccinated pigs caused some problems in the interpretation of lesions, such as in the case of pigs with high antibody levels that developed vesicular lesions, and that frequent examination of animals after virus exposure may produce abrasions of skin permitting the entry and local replication of virus, thus causing local lesions in animals with significant levels of antibody. Factors such as the difference in weight of the animals at 30 or 110 DPV may relate to the difference in the challenge results.

Leeuw *et al.* (8) studied the reaction of vaccinated pigs after different methods of virus exposure. In those experiments the pigs were exposed by swabbing, contact or inoculation and kept separately or in groups. Among other conclusions these investigators indicate that the results of such exposure are strongly influenced by diseased roommates and that contact exposure was the most severe of the methods used. In our results there were no differences between inoculation or contact exposure at 30 DPV, probably because of the intimate contact between vaccinated and diseased non-vaccinated pigs. Of 32 pigs challenged in each group 26 were protected after inoculation and 30 by contact. Since no previous stratification on the basis of antibody levels was made, 4 pigs vaccinated with a 0.3 ml dose of the 10X CF vaccine with low antibody levels were in the inoculated group against only one similar animal in the contact group.

At 110 DPV a relatively large number of pigs had antibody levels < 2 (9/12) in the mouse protection test and still were protected. This is particularly noteworthy because of the high level of infectivity in the environment caused by the inoculated and contact control pigs which all became severely ill with generalized lesions. Protection of animals without detectable circulating antibodies has also been reported (8).

No difference was observed between needle or contact challenge. Because of its practicability, the contact challenge may be the method of choice for routine vaccine positive control tests in pigs.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Vicente Astudillo and Dr. Juan Antonio Obiaga for the statistical analysis and Dr. Daniel Abaracón and Eduardo Centeno for the supply of the inactivated FMD antigens and concentration respectively.

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