FOOT-AND-MOUTH DISEASE VACCINES. I. COMPARISON OF VACCINES PREPARED FROM VIRUS INACTIVATED WITH FORMALIN AND ADSORBED ON ALUMINUM HYDROXIDE GEL WITH SAPONIN AND VIRUS INACTIVATED WITH ACETYLETHYLENEIMINE AND EMULSIFIED WITH INCOMPLETE FREUND'S ADJUVANT

by

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INTRODUCTION

Vaccines for the control of foot-and-mouth disease (FMD) in South America are prepared by inactivating the virus suspensions with formaldehyde or acetylethyleneimine (AEI), absorbing the antigen to aluminum hydroxide gel and adding saponin. The duration of immunity obtained with this vaccine is relatively short, and repeated vaccinations at 4-month intervals are an accepted practice. Pigs are particularly difficult to protect with this type of vaccine (1, 2).

Scientists at the Plum Island Animal Disease Center (PIADC) reported favorable results with FMD vaccines prepared from virus inactivated with AEI and emulsified with incomplete Freund's adjuvant (3, 4, 5). A series of cooperative studies by PIADC and the Pan American Foot-and-Mouth Disease Center (PAFMDC) was undertaken in the isolation units at PAFMDC in Rio de Janeiro in an effort to determine the value of such vaccines for future field experiments in South America. Brazilian cattle, pigs and sheep were vaccinated with conventional formaldehyde-inactivated aluminum hydroxide gel-saponin adjuvanted FMD vaccines or with vaccines inactivated with AEI and emulsified with incomplete Freund's adjuvant. Antibody development and the degree of immunity of the animals at various times after vaccination and revaccination with two vaccines were compared.

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MATERIALS AND METHODS

1. Virus

The following South American FMD virus (FMDV) strains were selected for the preparation of the vaccine: subtype O_1, strain Caseros; subtype A_{24}, strain Cruzeiro; and subtype C_3, strain Resende.

2. Vaccines

a) A trivalent aluminum hydroxide-saponin vaccine was prepared by the PAFMDC with the virus suspension produced in Frenkel cultures of bovine tongue epithelium and inactivated with 0.025% formaldehyde at 37° C for 40 hours (6). The characteristics of the antigens before inactivation are given in Table 1.

b) A trivalent vaccine in which the same viruses were used and which was emulsified with an oil adjuvant was prepared at PIADC. The viruses were grown in baby hamster kidney (BHK-21, clone 13) cell cultures, inactivated with 0.05% AEI at 37° C for 48 hours and emulsified with equal parts of oil adjuvant (1 part Arlacel A and 9 parts Bayol F) (5). The characteristics of the antigens are shown in Table 1.

In this paper, the two vaccines will be referred to as aluminum-gel and oil-adjuvanted vaccines, respectively.

3. Innocuity tests

At the PIADC, equal portions of each inactivated antigen used for the oil-adjuvanted vaccines were mixed, and 2 ml of the trivalent suspension was inoculated intradermalingually (IDL) in 20 sites in each of 6 steers. All remained negative for signs of FMD for the 14-day observation period.

At the PAFMDC the aluminum-gel vaccine was inoculated in 100 suckling mice as a 1/5 dilution, 0.05 ml per mouse. None of the mice died during the 10-day observation period.

TABLE 1 - *Infectivity and complement-fixation (CF) titers of FMD virus strains used for production of experimental vaccines*

Subtype	Plum Island Animal Disease Center (BHK-21 culture)		Pan American Foot-and-Mouth Disease Center (Frenkel culture)	
	PFU/ml[a]	CF[b]/vaccine dose	Mouse LD_{50}/vaccine dose	CF[c]
O_1	7.57	1/18.9	7.30	1/6.5
A_{24}	6.87	1/14.7	7.70	1/3
C_3	7.43	1/18.0	7.57	1/16

a) PFU = $\log_{10}$ plaque-forming units/ml.

b) Cowan, K.M.; Trautman, R. Immunochemical studies of foot-and-mouth disease. I. Complement fixation reactions of isolated antigenic components. *J. Immunol. 99:* 729-736, 1967.

c) Alonso Fernández, A.; Federer, K.E.; Gomes, I.; Vieira, A. Comparación serológica e inmunológica de dos subtipos del virus aftoso tipo C Waldmann. *Bltn Centro Panamericano de Fiebre Aftosa 4:* 9-20, 1971.

4. Vaccine safety test

Two ml of the final bottled oil-adjuvanted vaccine was inoculated into 20 sites into the tongue epithelium of each of 6 steers. All remained negative for signs of FMD during the 14-day observation period.

5. Potency tests

At the PIADC, 6 cattle, 6 sheep and 6 pigs were each vaccinated with a 2-ml dose of the vaccine. Cattle and sheep were inoculated subcutaneously on the side of the neck, and the pigs subcutaneously on the back of the ear. Blood samples were collected 7, 14, 21 and 28 days after vaccination for the assay of neutralizing antibodies.

At the PAFMDC, the aluminum-gel vaccine was potency tested in 23 cattle, which were exposed to virus by IDL inoculation of 10^4 mouse LD_{50} 21 days after vaccination.

6. Animals

Cross-bred Zebu cattle, approximately 2 years of age and weighing approximately 200 kg; Merino/Corriedale sheep, 8 to 9 months of age and weighing 20 to 25 kg;and Landrace pigs, 3 to 4 months old and weighing 30 to 40 kg, were used in the experiment. All animals were previously tested for the presence of antibodies against the three subtypes used and were negative.

7. Vaccination

A total of 432 animals were used in these experiments:

a) 32 cattle were each inoculated subcutaneously with 5 ml of aluminum-gel vaccine.

b) 32 cattle were each inoculated subcutaneously with 6 ml of oil-adjuvanted vaccine.

c) 32 cattle were retained as noninoculated control animals to be used when the immunity of the various groups was challenged.

d) 64 sheep were each inoculated subcutaneously with 5 ml of aluminum-gel vaccine.

e) 48 sheep were each inoculated subcutaneously with 6 ml of oil-adjuvanted vaccine.

f) 56 sheep were retained as noninoculated controls to be used when the immunity of the various groups was challenged.

g) 64 pigs were each inoculated with 5 ml of aluminum-gel vaccine.

h) 48 pigs were each inoculated with 6 ml of oil-adjuvanted vaccine.

i) 56 pigs were retained as noninoculated control animals to be used when the immunity of the various groups was challenged.

8. Antibody assay

Blood samples were collected from all animals at monthly intervals. Cattle sera were tested against FMDV, subtype C_3 and the sheep and pig sera against FMDV, subtype O_1; the mouse protection test was used according to the method described (7). The PD_{50} values of sera from the animals used at the PIADC were determined in suckling mice as described (8); the three types of virus were used.

9. Virus exposure

The immunity of the cattle was challenged by IDL inoculation of 10^4 mouse LD_{50} of FMDV, subtype C_3 of cattle origin. Sheep were similarly inoculated with 10^5 mouse LD_{50} of FMDV, subtype O_1 that had been previously passed 6 times in sheep as described (9). Pigs were inoculated into the intraplantar region of one foot with $10^{4.6}$ mouse LD_{50} of FMDV, subtype O_1 of cattle origin. Animals were considered protected if secondary lesions such as foot lesions did not develop. In swine, this would include other than the inoculated foot.

RESULTS

The results of the serum antibody tests (PD_{50} mean endpoints) of the animals used for the potency tests at the PIADC are given in Table 2. These results, which indicate that the vaccine was of borderline quality,were confirmed when these cattle were exposed and 4 out of 12 developed FMD (Table 3).

At the PAFMDC, the potency test of the aluminum-gel vaccine conducted in cattle, when their immunity was challenged at 21 days after vaccination, showed 7 of 7 cattle protected against FMDV type O, 7 of 8 against type A, and 8 of 8 against type C.

The results of the antibody studies and of virus exposure by inoculation for the three species are given in Table 4 and Fig. 1.

Cattle

Results in Fig. 1 show that the primary antibody response of the cattle against subtype C_3 of both the aluminum-gel type vaccine and the oil-adjuvanted vaccine was quite poor and of short duration, with little protection 3 months after vaccination. However, revaccination with both types of vaccine considerably increased the antibody response; this increased response was reflected in the degree of resistance to subtype C_3 exposure 3 and 7 months after revaccination (Table 4).

Sheep

The primary response of sheep was different for the aluminum-gel and the oil vaccines (Fig. 1). The aluminum-gel adjuvanted vaccine antibody levels against subtype O_1 reached a peak at 1 month, but the oil-adjuvanted vaccine antibody levels continued to rise for as long at 3 months after vaccination, followed by a prolonged high plateau. The degree of protection was excellent with the oil vaccine for as long as 9 months after vaccination against FMDV, subtype O_1.

TABLE 2 - *PD_{50}[a] mean endpoint of six animals per species in potency test*

Virus type	DPV[b]	Cattle	Sheep	Pigs
O_1	28	1.57	1.71	1.77
A_{24}	28	1.89	1.50	1.88
C_3	28	1.20	2.24	0.94

a) PD_{50} = $\log_{10}$ of the reciprocal of the serum dilution protecting. 50% of mice against 100 LD_{50} of virus.
b) DPV = days postvaccination.

TABLE 3 - *Response of cattle to potency test challenge with FMDV subtype O_1 in relation to PD_{50} range*

PD_{50} range	Number of cattle	Lesions		
		None	At site of inoculation	Generalized FMD
0 (Control)	3	0	3	3
0.0 - 0.49	1	0	1	1
0.50 - 0.99	1	0	1	1
1.00 - 1.49	1	0	1	1
1.50 - 1.99	2	0	2	1
2.00 - 2.49	4	2	2	0
2.50 - 2.99	2	1	1	0
3.00 - 3.49	1	1	0	0

TABLE 4 - *Number of animals protected on challenge of immunity*

Species	Months	Vaccine	
		Aluminum-Gel	Oil-Adjuvanted
Cattle	3	4/15	5/15
	3[a)]	8/8	8/8
	7[a)]	4/8	6/8
Sheep	1	16/16	-
	3	16/16	14/16
	6	15/16	13/16
	9	13/13	13/14
Pigs	3	0/16	0/16
	1[a)]	0/12	4/12
	3[a)]	-	2/9

a) Months after revaccination.

Pigs

Both vaccines produced only a minimal antibody response after vaccination, and none of the pigs exposed to subtype O_1 at 3 months were protected (Table 4; Fig. 1). Revaccination with the aluminum-gel vaccine resulted in a slight increase in the level of antibodies; this increase afforded no protection when these pigs were exposed to virus 1 month later. The response to the revaccination with oil-adjuvanted vaccine was slightly better than that obtained with the aluminum gel vaccine in regard to antibody development and protection to virus exposure.

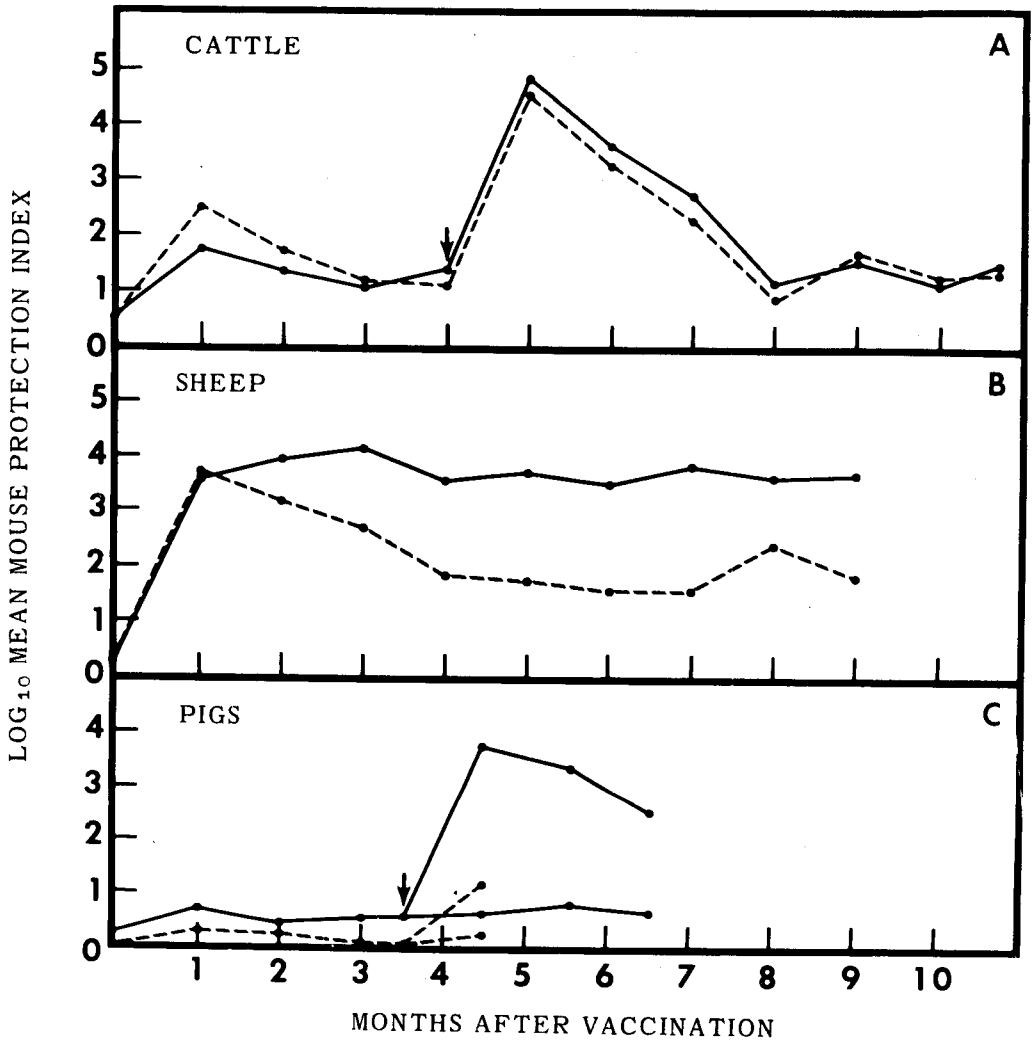

FIGURE 1 - Mean mouse protective index of species vaccinated with formalin-inactivated aluminum hydroxide gel-saponin adjuvanted (aluminum gel) FMD vaccine (------) and acetyl-ethyleneimine inactivated (oil-adjuvanted) vaccine emulsified with incomplete Freund's adjuvant (——): A, response of cattle to FMDV C_3, revaccinated at 120 days; B, response of sheep to FMDV O_1; C, response of pigs to FMDV O_1, revaccinated at 105 days.

↓ = Revaccination.

DISCUSSION

The responses to oil-adjuvanted vaccine were less favorable than those reported earlier (10, 11, 12), in both the actual use at the PAFMDC (Table 4) and the potency tests (Table 2). One may speculate that the amount of antigen contained in the vaccine was not sufficient or that these subtypes are poor antigens. Graves (13) indicated that some disease response is to be expected at challenge with a mean PD_{50} below 2. Little difference was seen in the response of the aluminum-gel and the oil-adjuvanted vaccines in cattle with FMDV C_3 after vaccination or revaccination. Results were more promising with the oil-adjuvanted vaccine in sheep. The aluminum-gel vaccine adequately protected sheep against exposure with subtype O_1, but antibody levels against this subtype were considerably lower than those obtained with the oil-adjuvanted vaccine. Results with these subtypes were poor in pigs with both aluminum-gel vaccine and oil-adjuvanted vaccine, but the response after revaccination was slightly better with the oil-adjuvanted vaccine than with the aluminum-gel vaccine. The clinical signs in pigs vaccinated with the oil-adjuvanted vaccine were less severe than those of the control pigs, and the oil-adjuvanted vaccines offered some degree of protection.

The stability of the vaccine was quite satisfactory. After the PIADC potency test and the revaccination trial at PAFMDC, the vaccine was returned to PIADC and tested by complement fixation and animal inoculation. Results were not different from those originally obtained in the initial testing at PIADC. However, improvement of the vaccine formulation would be required before field application could be considered. Such improvement could include an increase in the amount and stability of the antigens and an increased shelf life beyond 6 months for the consistency of the emulsion.

ABSTRACT

Antibody response of cattle against foot-and-mouth disease virus C_3 of both aluminum-gel type vaccine and oil-adjuvanted vaccine was quite poor and of short duration. Revaccination with both types of vaccine considerably increased the antibody response.

In sheep, antibody levels against O_1 after inoculation with the aluminum-gel adjuvanted vaccine reached a peak at 1 month. The oil vaccine induced excellent protection for as long as 9 months after vaccination against O_1.

Both vaccines produced only a minimal antibody response after vaccination in pigs, but with the oil vaccine, clinical signs were less severe than in control pigs.

The results with the oil vaccines were less favorable than expected. The amount of antigen contained in the vaccine may not have been sufficient or the subtypes used possibly were poor antigens.

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