FOOT-AND-MOUTH DISEASE VACCINES. II. STUDIES ON THE DURATION OF IMMUNITY IN CATTLE AND PIGS

by

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and

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INTRODUCTION

In a companion paper (1), a cooperative study was reported between the Plum Island Animal Disease Center (PIADC) and the Pan American Foot-and-Mouth Disease Center (PAFMDC). In that study, the immune response after vaccination and revaccination of cattle, sheep and pigs to a foot-and-mouth disease (FMD) vaccine emulsified with incomplete Freund's adjuvant was compared with the response to a commonly used formaldehyde-inactivated aluminum-hydroxide-saponin vaccine.

In the present study, only oil-adjuvanted vaccines were tested. The objective was to compare the response of pigs to vaccines prepared by each of the Centers from an identical source of antigen. A vaccine emulsified with incomplete Freund's adjuvant, prepared at the PIADC, was also used to study the duration of immunity of vaccinated and revaccinated cattle.

MATERIALS AND METHODS

1. Virus

The virus strains used were subtypes O_1, A_{24} and C_3 of South American origin and were the same as those described in the companion paper (1).

2. Vaccines

The vaccines were prepared at each Center from the same seed viruses according to the production protocol described (1). A vaccine prepared by the PAFMDC for use in pigs contained more concentrated antigens. For this vaccine, a portion of the inactivated antigens was concentrated by precipitation with ammonium sulfate, dialyzed against 100 volumes of 0.2 M NaCl, 0.05 M phosphate buffer, pH 7.4, and then adjuvanted with oil. The vaccine prepared at the PIADC was tested for innocuity and safety as indicated (2). The infectivity and

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complement-fixation titers of the antigens are given in Table 1.

3. Vaccination

Cross-bred Zebu cattle, 1 to 3 years of age and weighing approximately 200 kg, were vaccinated subcutaneously in the neck with a 6-ml dose of PIADC vaccine. Landrace pigs, 3 to 5 months old and weighing 30 to 40 kg, were vaccinated subcutaneously with a 6-ml dose at the base of the ear. The PAFMDC concentrated vaccine was used in a 2-ml dose and inoculated in the same area.

4. Antibody assay

Blood samples were collected from the pigs and cattle at monthly intervals, and the antibody content of the sera was assayed by the mouse protection test as described (3). The PD_{50} values of the sera from the animals at PIADC were obtained in suckling mice as described (4) with the 3 virus types.

5. Virus exposure

Pigs were exposed to FMD virus by intraplantar inoculation of one foot with $10^{4.6}$ mouse LD_{50} units of one of the 3 subtypes. Cattle were inoculated intradermalingually with 10^4 mouse LD_{50} units of subtype O_1.

RESULTS

The vaccines proved to be innocuous when tested in suckling mice or cattle. The results of the potency tests at the PIADC with the vaccine prepared at that location showed that 5, 4 and 5 out of 6 steers were protected for types O, A and C, respectively. In a potency test in pigs at 28 days after vaccination at the PIADC, 6 out of 6 were protected for type O; pigs vaccinated with a 6-ml dose of trivalent vaccine were not exposed to types A or C. These results and the mean PD_{50} values of these animals as summarized in Table 2 indicate that the potency of the vaccines was acceptable.

Pigs

Local reactions were severe with oil vaccines in several of the pigs at the site of inoculation, starting approximately the 6th day after vaccination. These lesions ranged from 5 to 15 cm in diameter and developed into sterile abscesses or extensive ulceration. The concentrated or regular vaccines were not different in this respect.

The PIADC vaccine induced good immunity against subtype A_{24}; this good immunity was reflected in the high degree of resistance to virus exposure at 1 and 3 months after vaccination (Table 3 and Fig. 1). At 6 months, the mean antibody level against this virus strain remained at the same high level but only 4 of the 14 pigs resisted virus exposure. However, the lesions of the vaccinated pigs were much less severe than those of the unvaccinated control pigs exposed at the same time. The results with subtypes O_1 and C_3 were less favorable than those of subtype A_{24}; but, again, the difference in clinical response of the vaccinated and unvaccinated control pigs was appreciable.

The study of immune response to the PAFMDC vaccine and the PAFMDC concentrated vaccine was ended 3 months after vaccination because of the rather disappointing antibody levels and protection against virus exposures (Table 3 and Fig. 1). Again, the response of subtype A_{24} was better than that of subtypes O_1 and C_3.

Cattle

Local tissue reaction to the vaccine was only minimal at the site of inoculation. The results of the vaccination and revaccination of cattle with the PIADC vaccine are shown in Table 3 and Fig. 2. One month after vaccination, levels of antibodies were in the protective range (MPI ≥ 2), and 13 out of 16 cattle were protected against clinical disease after exposure to subtype O_1. Antibody

levels remained stable, and protection was similar 4 and 6 months after vaccination. Revaccination increased antibody levels, and high plateaus were established. Approximately 6 months after revaccination, the level of antibodies was declining slowly; but at that time, over 90% of the cattle were protected (5). At the end of the observation period (18 months), the number of cattle protected against clinical disease after virus exposure was lower than the average mouse protection test results indicated. However, on an individual basis, the mouse protection test results correlated well with the protection of the animal.

TABLE 1 - *Infectivity and complement-fixation (CF) tests of FMD virus used to produce experimental vaccines emulsified with incomplete Freund's adjuvant*

Subtype	Plum Island Animal Disease Center		Pan American FMD Center		
			Regular vaccine		Concentrated vaccine
	PFU[a]	CF	PFU[a]	CF	CF
O_1	N.A.[b]	1/54	7.4	1/32	1/80
A_{24}	N.A.	1/50	7.0	1/11	1/100
C_3	N.A.	1/56	6.9	1/13	1/130

a) PFU = $\log_{10}$ plaque-forming units/ml.
b) N.A. = data not available.

TABLE 2 - *PD_{50}[a] value mean endpoints of six animals per species in the potency test at the Plum Island Animal Disease Center*

Virus type	DPV[b]	Cattle	Pigs
O_1	28	1.87	1.33
A_{24}	28	2.87	2.87
C_3	28	2.34	2.13

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 - *Animals protected on challenge of immunity after vaccination with oil-adjuvanted vaccine*

Species	Months		Challenge virus	Plum Island Animal Disease Center vaccine[a]	Pan American FMD Center[a]	
	1st vaccination	After revaccination			Regular vaccine	Concentrated vaccine
Pigs	1		A_{24}	10/12	7/12	3/8
	1		O_1	4/12	3/12	0/8
	3		O_1	1/12	0/11	-
	3		C_3	2/12	0/11	0/7
	3.5		A_{24}	7/8	-	0/8
	6		A_{24}	4/14	-	-
Cattle	1		O_1	13/16	-	-
	4		O_1	12/16	-	-
	6		O_1	13/16	-	-
	12	6	O_1	15/16	-	-
	18	12	O_1	9/14	-	-

a) number of animals protected/number of animals tested.
- not carried out.

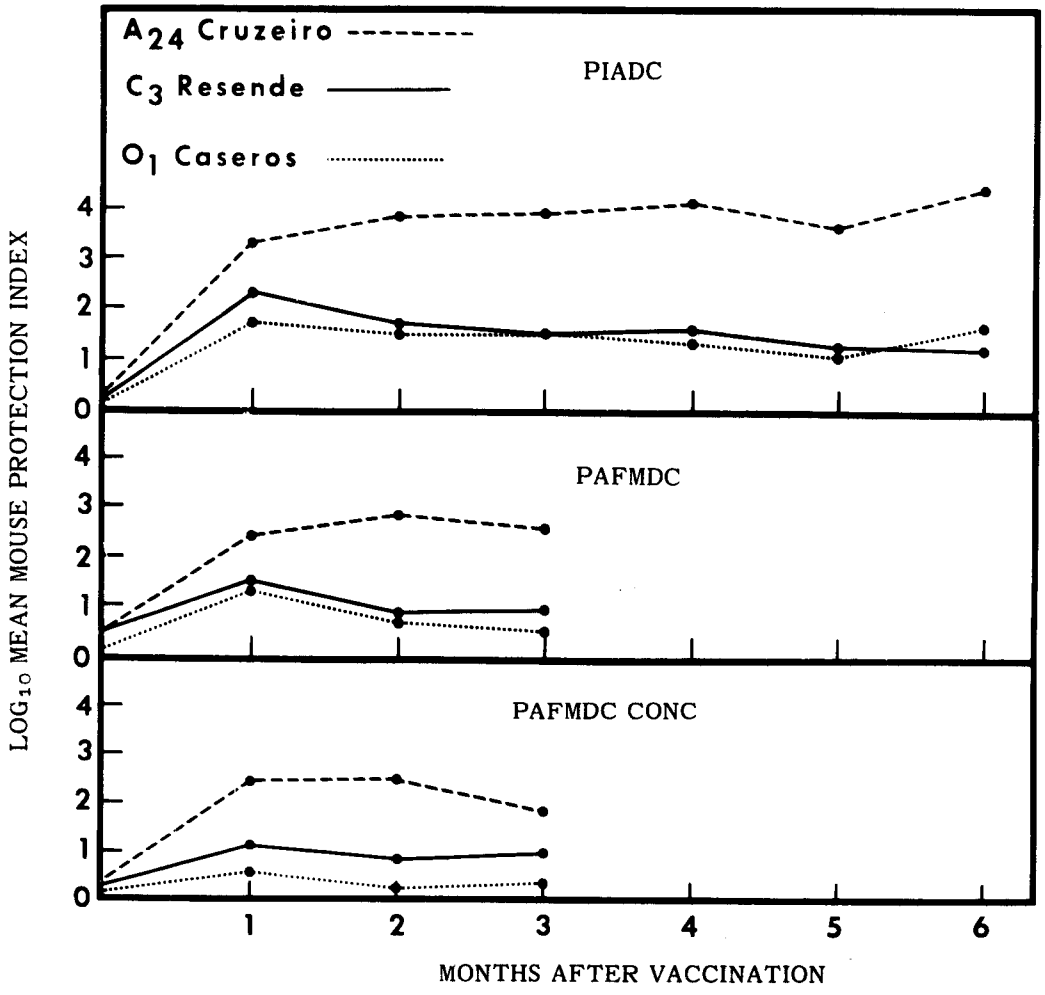

FIGURE 1 - Mean protection indices of pigs vaccinated with trivalent experimental FMD oil-adjuvanted vaccines prepared at PIADC and at the PAFMDC.

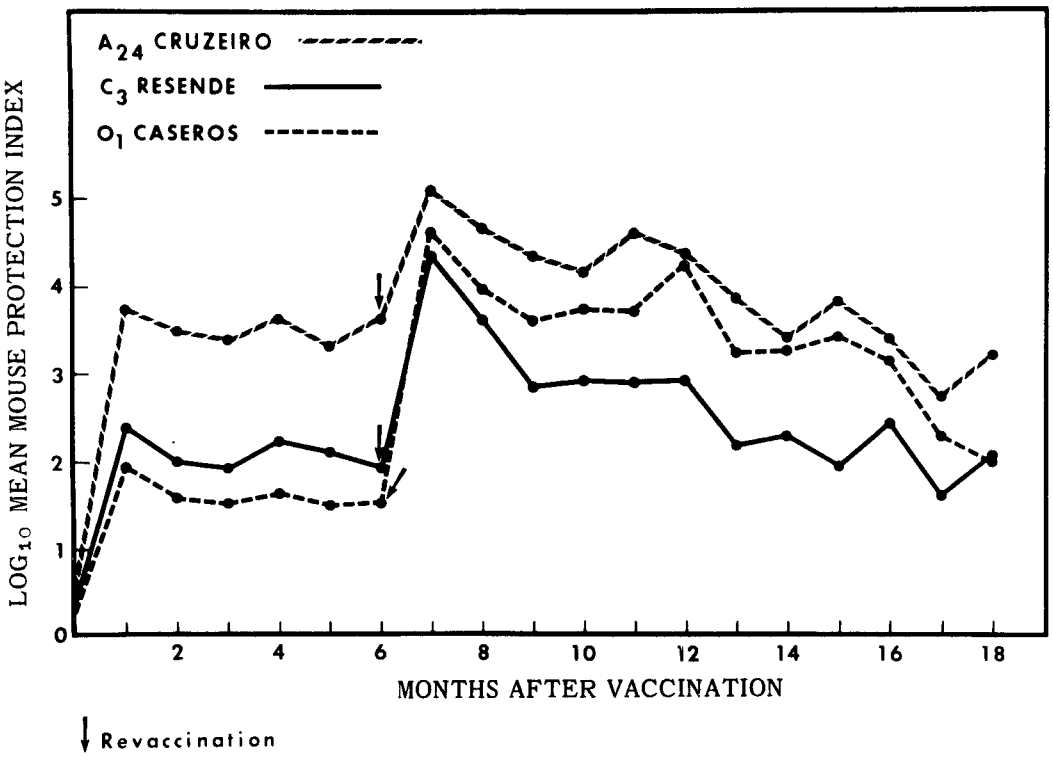

FIGURE 2 - Mean protection indices of cattle vaccinated with a trivalent experimental oil-adjuvanted vaccine and revaccinated with a similar vaccine 6 months after the initial vaccination.

DISCUSSION

Infectivity titers of the virus used for the preparation of the present vaccines were similar to those used for the earlier study (1), but the higher complement-fixation titers indicated a larger antigenic mass. The results of the potency tests also indicated that the present vaccines should afford better protection. The dose response was studied at PAFMDC with the PIADC oil-adjuvanted vaccine in pigs, and sera were tested at the PAFMDC for antibodies. Analysis of the serum protection indices showed a marked difference in antibody among the three types. The antibody response to type A_{24} antigen was significantly higher than the response to the type O_1 antigen. This response is in agreement with the results obtained in the potency tests and with those presented in Table 3 and Figs. 1 and 2.

The local tissue reaction at the site of vaccine inoculation in pigs was more of a problem than anticipated (6). Possibly, the

relatively thin white skin of the Landrace pigs and the large dose of vaccines used in this experiment favored the development of the inflammatory reactions. Other inoculation sites and a reduction of the dose in future experiments should be considered in efforts to resolve the problem of these local reactions before field application of this type of vaccine in pigs can be recommended. However, a smaller dose apparently could have been used because in the dose response studies, the response increased significantly when the dose of vaccine was increased from 0.5 ml to 2.0 ml; but the response did not increase significantly above 2.0 ml when the dose was increased to 8.0 ml (PIADC). Also, present experimental methods in which a purified antigen in a total dose volume of 0.25 ml is used as discussed elsewhere (7) would perhaps help to resolve the inflammatory reactions at sites of inoculation. No correlation could be established between the presence, absence or extent of the local reactions and the immune response, but workers at the PAFMDC have observed that the immune response of guinea pigs that developed abscesses at the site of inoculation with inactivated-saponin vaccines was poorer than the response of others without such reactions but inoculated with the same vaccine (A.A. Fernández, personal communication). Cattle had no complications after vaccination.

The immune response and the protection of pigs with the PIADC oil vaccine were considerably better than those reported from earlier cooperative studies between both Centers (1). The differences observed between the PIADC vaccines and those prepared at the PAFMDC possibly relate to differences in emulsification techniques or the amount of antigenic mass, or both. However, concentration of the antigen in the PAFMDC vaccine did not increase its potency in pigs. This finding appears to indicate other factors in the formulation process such as degradation of the antigen. Further, we might note that tests in guinea pigs at the PIADC showed that the PIADC vaccine was uniformly stable during the 8 months of the test period.

The results of the vaccination of cattle indicate that with AEI-inactivated oil-adjuvanted vaccines, adequate protection can be expected for a period of at least 6 months. A solid population immunity apparently can be induced, particularly when vaccine is applied systematically twice a year. An extension of the vaccination interval from 4 to 6 months would possibly reduce by about 1/3 the expense of the vaccination campaigns now in progress in South America; thus, a larger scale field experiment with the oil-adjuvanted vaccines appears to be justified.

ABSTRACT

In pigs, the vaccine induced good immunity against foot-and-mouth disease virus A_{24}, as reflected in the high degree of resistence to virus exposure at 1 and 3 months after vaccination. At 6 months, the mean antibody level against this virus strain remained high but only 4 of the 14 pigs resisted virus exposure. However, lesions of the vaccinated pigs were much less severe than those of unvaccinated control pigs exposed at the same time. The response of A_{24} was better than that of O_1 and C_3.

The results of cattle vaccination indicate that with AEI-inactivated oil-adjuvanted vaccines adequate protection can be expected for a period of at least 6 months. One month after vaccination, 13 out of 16 cattle were protected against clinical disease after exposure to O_1. Antibody levels remained stable, and protection was similar 4

and 6 months after vaccination. Revaccination boosted the antibody levels, with 90% of the cattle protected 6 months later. Local tissue reaction to the vaccine was only minimal at the site of inoculation.

However, the oil vaccines caused rather severe local reactions in several of the pigs at the site of inoculation, starting approximately the 6th day after vaccination.

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