OIL-ADJUVANT VACCINE AGAINST FOOT-AND-MOUTH DISEASE[1]

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SUMMARY

The preparation of an oil-adjuvant vaccine against foot-and-mouth disease (FMD) is described and some pertinent details are given. Results of immunogenicity studies in cattle in laboratory and field experiments are presented and the incidence of FMD in cattle populations vaccinated with oil-adjuvant vaccine in large scale field experiments in Argentina and Brazil is discussed. The systematic application of oil-adjuvant FMD vaccine gives the vaccinated cattle population a much greater protection than vaccination with the conventional aluminum hydroxide vaccines. It therefore should become an important tool in the programs for the control of FMD in South America.

INTRODUCTION

For almost 50 years now the standard vaccine against FMD has been prepared with inactivated antigen adsorbed to aluminum hydroxide. Although the methods of antigen production and inactivation have changed over the years, most of the FMD vaccines today are still prepared with aluminum hydroxide adjuvant, which was already used in the first effective vaccine in 1937.

The first study of the use of an oil adjuvant with inactivated FMD virus antigen was published in 1963 (*8*). This adjuvant was subsequently studied more in relation to preparation of an effective FMD vaccine for pigs. In 1968 the Pan American Foot-and-Mouth Disease Center (PAFMDC) began laboratory and field studies of an oil-adjuvant vaccine for application in cattle. The field studies began in Brazil and were later extended to Argentina, Bolivia, Colombia, Ecuador, Paraguay, Peru and Uruguay (*6*).

VACCINE PRODUCTION

Antigen production

The majority of FMD vaccine production laboratories today use cell cultures, mainly of BHK-21 cells, for the replication of FMD virus. The PAFMDC also uses these cells in monolayer or suspension cultures for this purpose.

The cell cultures are infected with a seed virus preparation which has a low passage level in cell cultures, a high infectivity titer and is controlled serologically for virus strain specificity. The virus suspensions are purified after harvest by intensive treatment with chloroform and clarification by filtration or centrifugation. Inactivation is done in most laboratories now with an alkylating agent which gives an inactivation reaction of the first order or a linear reaction. The inactivant preferred by many vaccine production laboratories is binary ethylenimine (BEI), which was developed at the PAFMDC (*4*). The Center uses BEI at 3 mM for 24 hours at 26°C. The inocuity test is done in monolayer cell cultures in three serial passages.

The amount of viral antigen in each virus suspension is determined by infectivity titration, at the Center by a test for plaque forming units in monolayer cell cultures. The purity and subtype specificity of each virus suspension is controlled serologically by the complement fixation test. A more precise determination of the amount of viral antigen can be done by centrifugation of the virus suspension in a density gradient of sucrose or cesium chloride. The Center uses cesium chloride density gradient centrifugation, as it also allows the sample to be used for testing the integrity of the antigenic polypeptide by poly-

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acrylamide gel electrophoresis (PAGE). In the regular FMD virus antigen production in monolayer and suspension cell cultures at the PAFMDC the values for antigenic mass for the four virus strains used (O_1 Campos, A_{24} Cruzeiro, A Venceslau and C_3 Indaial) are usually between 1.5 and 3 microgram per ml.

Antigen preparation

The preparation of plurivalent FMD vaccines with aluminum hydroxide adjuvant is usually done by mixing inactivated monovalent vaccines. In the case of oil-adjuvant vaccines and with the use of first order rate inactivants it is possible to mix monovalent antigen suspensions before addition of the adjuvant. The different antigens are mixed in such a manner that a single vaccine dose contains sufficient antigen of each virus type to comply with the potency requirements of the government control authority.

The amount of antigen to be used in a vaccine is determined in many laboratories on the basis of the infectivity and complement fixation titers of the virus suspensions. The more precise determination of antigenic mass by density gradient centrifugation allows however a better standardization of the antigen content of FMD vaccines.

After the potency controls of a large number of vaccines, which were prepared on the basis of antigens with antigenic mass determinations, the PAFMDC now uses the following parameters for the antigen content per dose of each virus type: virus O_1 Campos $\geqslant$ 2.2 microgram, virus A $\geqslant$ 1.4 microgram (about equal amounts of A_{24} Cruzeiro and A Venceslau) and virus C_3 Indaial $\geqslant$ 1.0 microgram. The standard dose of vaccine is 5 ml and the total amount of antigen is contained in 2.5 ml of antigen suspension without any concentration.

Emulsification

The final step in the production of an oil-adjuvant FMD vaccine is the mixture of the viral antigen with an equal amount of light mineral oil (Marcol 52), which contains 10% of an emulsifier (Montanide 888), and the preparation of a primary water-in-oil emulsion.

For this purpose the oil-emulsifier mixture is sterilized by filtration through Millipore cartridge filters and then added to the antigen preparation. This antigen-oil-emulsifier mixture is passed through an industrial in-line Silverson-type emulsifier developed at the Center and the degree of dispersion of the emulsion is controlled by resistance measurements with a locally made conductivity meter. In order to achieve the desired resistance of more than 100 mega Ohm the vaccine is usually passed three times through the emulsifier.

The stability of the emulsion of the final vaccine preparation is controlled by a centrifugation test (3000 rpm for 3 hours) and by holding vaccine samples at 37°C for 15 days.

Potency testing

The recommendations of the PAFMDC for FMD vaccine potency values of oil-adjuvant vaccines are an expected percentage of protection (EPP) of 85 in cattle of nine to twelve months old. In direct challenge tests in cattle at least 12 to 16 vaccinated animals should be protected (*7*).

Each batch of vaccine prepared at the Center is tested after bottling for potency in guinea pigs and cattle. In guinea pigs the 50% guinea pig protective dose ($GPPD_{50}$) is determined by injection of 0.25 ml of vaccine diluted with active diluent and challenge with the homologous vaccine virus strains. The $GPPD_{50}$ value is calculated for the injected vaccine volume, i.e. 0.25 ml. However a direct relation between $GPPD_{50}$ value and protection in cattle has not been established and the $GPPD_{50}$ test is therefore considered more to be a screening test and not a definite test for vaccine potency.

Potency in cattle is tested by vaccination of animals which had no prior contact with FMD and through antibody assays with their sera taken at 21 to 28 days after vaccination. Neutralizing antibodies are determined by virus neutralization tests in cell culture or in newborn mice, also called mouse protection test (MPT). The MPT titers are used to calculate the mean EPP

values (*11, 13, 14*). For vaccines prepared with the before mentioned antigen quantities the mean EPP is a minimum of 85 but usually falls between 90 and 97. All valencias in the vaccines are tested individually in each batch of vaccine.

Official government control laboratories in Argentina, Brazil and Uruguay have tested the oil-adjuvant vaccine of the Center in direct challenge tests in cattle for potency, stability and duration of immunity.

IMMUNOGENICITY OF OIL-ADJUVANT FMD VACCINE

Experimental studies

The immunogenicity of oil-adjuvant FMD vaccines as well as the duration of immunity induced by these vaccines in young and adult cattle at primovaccination and at revaccination was studied in extensive laboratory experiments at the PAFMDC and field trials in Brazil (*1, 2, 3, 6, 12*). Some of the results obtained are given in Fig. 1 for primovaccination and in Fig. 2 for revaccination.

The serum antibody titers are higher for the oil-adjuvant vaccine in comparison to the aluminum hydroxide vaccine for a longer period of time already in primovaccinated animals. This effect is much more pronounced upon revaccination. The recommendation for application of the oil-adjuvant vaccine is therefore that young animals under two years of age should be revaccinated at six month intervals while for animals more than two years old and previously immunized one annual revaccination is sufficient.

Field challenge

All laboratory and field studies of the immunogenicity of oil-adjuvant FMD vaccine indicated that this type of vaccine induced high levels of antibody to FMD virus and for longer periods of time than the conventional aluminum hydroxide FMD vaccine.

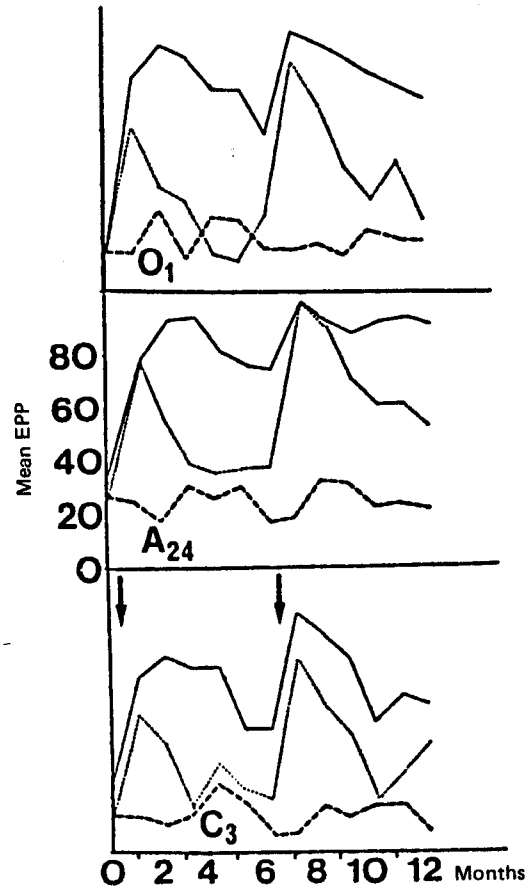
Two countries in South America, Argentina and Brazil, tested the oil-adjuvant vaccine in large scale field application programs in comparison to the standard aluminum hydroxide (saponin) vaccine. In Argentina about 68,000 cattle were vaccinated from 1977 to 1981 in Hipólito Yrigoyen, Province of Buenos Aires, with oil-adjuvant FMD vaccine and the incidence of FMD in these animals compared with that in a cattle population in the same area vaccinated with commercial aluminum hydroxide vaccine. Caggiano and collaborators reported on this experiment in 1982 (*5*) and the results are summarized in Table 1.

Recently Dora and collaborators in Brazil published a report (*9*) comparing the incidence of FMD during an epidemic in Bagé, Rio Grande do Sul, in 1980 in two cattle populations vaccinated with aluminum hydroxide and oil-adjuvant

TABLE 1. *Field challenge of oil-adjuvant foot-and-mouth disease vaccine. Hipólito Yrigoyen, Buenos Aires province, Argentina, 1977-1981*

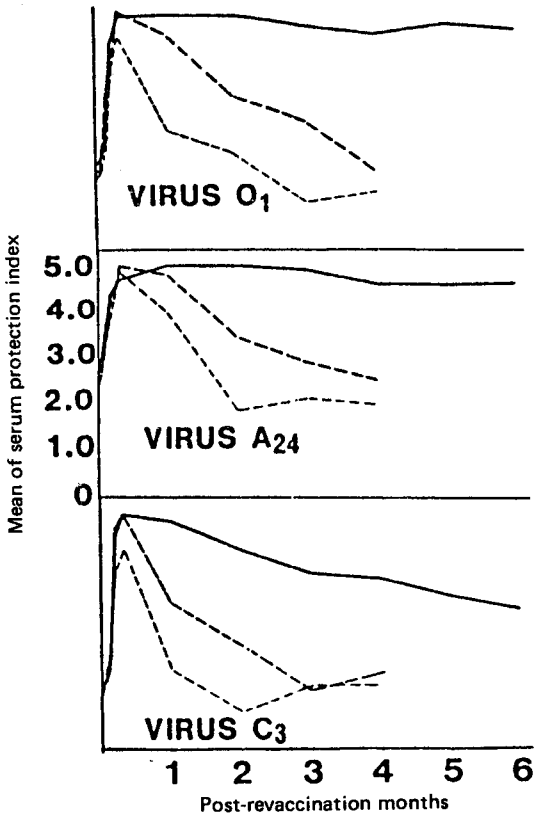
	Vaccines	
Cattle	Aluminum hydroxide	Oil adjuv.
Herds vaccinated	1,719	494
Animals vaccinated	620,462	67,715
Herds affected	365	23
Animals exposed	245,516	2,874
Animals diseased	34,373	441
Morbidity/1000	55	7

Source: Data from Caggiano *et al.* (*5*).



Oil-adjuvant vaccine
Aluminum hydroxide vaccine
Controls

FIGURE 1. *Expected percentage of protection (EPP) of cattle for FMD virus subtypes O_1, A_{24} and C_3.*



Oil-adjuvant vaccine
Aluminum hydroxide-saponin vaccine
Antigen without adjuvant

FIGURE 2. *Mean of serum protection index of cattle revaccinated with oil-adjuvant, aluminum hydroxide-saponin or antigen without adjuvant vaccines.*

vaccine respectively. The results of their publication are summarized in Table 2.

Although in the Argentinian experiment the number of animals vaccinated with oil-adjuvant vaccine is considerably smaller than in the control group, it can clearly be seen that the disease incidence is greatly reduced in the oil-adjuvant group. The morbidity rate in the aluminum hydroxide vaccine group is 55 per 1000 animals while it is only 7 for the oil-adjuvant group.

The Brazilian experiment has comparable cattle populations of over 200,000 animals for each vaccine type. The morbidity rates for the aluminum hydroxide vaccine and for the oil-adjuvant vaccine were 45 and 6 respectively, which is surprisingly similar to those in the Argentinian experiment (Table 3). Also very interesting is a comparison of the animals vaccinated once or two and more times with the oil-adjuvant vaccine. The primovaccinated animals

TABLE 2. *Field challenge of oil adjuvant foot-and-mouth disease vaccine. Bagé, Rio Grande do Sul, Brazil, 1980*

Cattle	Alum. hydr. vaccine	Oil adjuvant vaccine		
		Total	Primovaccinated	Revaccinated
Herds vaccinated	1,813	620	316	304
Animals vaccinated	220,532	240,616	87,831	152,785
Herds affected	73	20	9	11
Animals exposed	30,780	17,164	5,647	11,517
Animals diseased	9,958	1,546	1,218	328
Morbidity/1000	45	6	14	2

Source: Data from Dora *et al. (9)*.

had a morbidity rate that was lower by two thirds in comparison to the aluminum hydroxide vaccine. But the animals vaccinated two or more times with this type of vaccine had a morbidity rate more than twenty times lower than the animals vaccinated with aluminum hydroxide vaccine.

These figures show that the incidence of FMD is greatly reduced in animal populations vaccinated with an oil-adjuvant vaccine. One can only agree with the conclusion of Dora and his collaborators, that if all the animals in the particular area had been vaccinated with an oil-adjuvant vaccine the incidence of the disease would have been insignificant. This opinion is supported by the observation that the cattle population in Bagé, which continues to be vaccinated with oil-adjuvant vaccine, has not had a single case of FMD in the five years following the described epidemic in 1980, although there are still outbreaks of FMD in Rio Grande do Sul (*10*). However, the incidence of FMD in Rio Grande do Sul has been reduced sharply as almost half the bovine population in this State, 5.5 million animals out of a total of about 12 million cattle, is now systematically vaccinated with oil-adjuvant vaccine.

TABLE 3. *Morbidity rate (per 1000) of foot-and-mouth disease in two large scale field challenge experiments with aluminum hydroxide and oil-adjuvant vaccines*

Country	Vaccines	
	Aluminum hydroxide	Oil adjuvant
Argentina 1977-81	55	7
Brazil 1980	45	6

PRESENT AND FUTURE FMD OIL-ADJUVANT VACCINE PRODUCTION

The PAFMDC prepares at present inactivated oil-adjuvant FMD vaccines for Government field projects in Argentina, Bolivia, Brazil, Ecuador, Paraguay, Peru, Uruguay and Venezuela and keeps a stock of 50,000 vaccine doses for emergency application at outbreaks in disease-free areas.

The excellent experience with this vaccine in FMD epidemics in southern Brazil and the great interest of farmers in the application of this vaccine lead government authorities in Brazil to establish oil-adjuvant vaccine production

laboratories in Campinas (Federal Government) and Porto Alegre (State Government, Rio Grande do Sul), which are in operation since 1984.

Private vaccine production laboratories in Brazil are interested in this vaccine and have already produced experimental batches. Through the experience of government field projects, supported by the PAFMDC, several countries in South America are also interested in the production of this type of FMD vaccine in the near future. It is therefore very likely that the production volume of oil-adjuvant FMD vaccine in South America will soon increase considerably. A systematic and massive application of this type of vaccine mainly in primary endemic areas should have a profound effect on the epidemiological situation of FMD on this continent.

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