INFLUENCE OF THE DEGREE OF DISPERSION IN THE AQUEOUS PHASE ON THE IMMUNOGENICITY OF OIL-ADJUVANTED FOOT-AND-MOUTH DISEASE VACCINE

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SUMMARY

Both primary emulsion (water-in-oil) and double emulsion (water-in-oil-in-water) foot-and-mouth disease vaccines with different viscosities produced excellent persistent levels of neutralizing antibodies. The degree of dispersion of the aqueous antigenic phase in the oily phase, which determined the viscosity of the vaccine, did not have a demonstrable effect on the long-term immunogenicity of these vaccines.

INTRODUCTION

The immune response following vaccination with a water-in-oil type emulsion vaccine may depend, among other factors, on the degree of dispersion of the aqueous phase in the continuous oily phase. For a given ratio of aqueous and oily phase a higher degree of dispersion of the aqueous phase results in a higher viscosity of the emulsion. Such a high degree of dispersion usually means a higher stability of the emulsion during storage although such vaccines are more difficult to handle in the field than those of low viscosity.

Berlin (*5*) concluded in his studies of oil-adjuvanted influenza vaccine that the adjuvant effect may be adversely influenced by extremely high viscosity regardless of whether differences between emulsified vaccines were produced by altering the viscosity of the external phase, the proportion of the dispersed aqueous phase or the amount of agitation used in their preparation.

The same investigator (*5*) also found that emulsified vaccines exhibiting intermediate stability had a greater adjuvant effect than vaccines with a brief or prolonged stability.

From a practical point of view for the production of foot-and-mouth disease (FMD) vaccines it would therefore be important to know if the degree of dispersion of the aqueous phase would significantly influence the immune response and, if so, what would be the ideal compromise between immunogenicity, dispersion (viscosity) and stability of the emulsion.

For several years the Pan American Foot-and-Mouth Disease Center has used a water-in-oil type emulsion for the formulation of oil adjuvanted inactivated FMD vaccines for use in cattle (*1, 2, 6, 7*), pigs (*3, 4, 7*) and sheep (*6, 7*).

These vaccines consist of aqueous antigen emulsified with an equal volume of the oily phase (9 parts of mineral oil[3] and one part of mannide monooleate[4]). The emulsion is considered satisfactory when a drop of the emulsion in cold water remains a perfect sphere (*5*). Moreover the emulsion when stored at 4°C should remain stable for at least 12 months and at 37°C for at least two weeks. In this paper we refer to this type of emulsion as the "standard" vaccine.

In order to study the influence of the degree of dispersion of the aqueous phase on the immunogenicity of the emulsion, three vaccines were tested in cattle: one low viscosity emulsion a poor dispersion of the aqueous phase, the standard vaccine and an emulsion with the highest possible viscosity for the given ratio of the oily and aqueous phase.

In addition, 3 double emulsion (water-in-oil-in-water) vaccines prepared from these primary emulsions were tested in cattle (*9*).

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MATERIALS AND METHODS

1. Vaccine

The antigens for the vaccines were prepared by standard methods used in the vaccine plant of the Pan American Foot-and-Mouth Disease Center. These particular antigens were produced in BHK cells grown in suspension in a 200-liter vessel. The characteristics of the antigens are listed in Table 1.

TABLE 1. *Characteristics of the FMD antigens used for the preparation of the experimental vaccine*

Antigen	Strain	CFT[a]	CCID[b]
O_1	Campos	1/20	7.0
A	25% Cruzeiro	1/22	7.3
	25% Venceslau	1/16	7.6
	50% Bage	1/12	7.4
C_3	Resende	1/22	7.5

[a]CFT = 50% complement fixation titer ($4HCU_{50}$–90').
[b]Cell culture infectious doses 50%/ml.

Vaccines 1 (lowest viscosity) and 2 (standard vaccine) were made in a 50-liter emulsifier vessel. Vaccine 1 was collected as soon as the conductivity of the emulsion was near zero, and emulsification in the 50-liter vessel was terminated when the characteristics of the "standard" (Vaccine 2) were reached. A 1000 ml portion of the emulsion was further emulsified with a benchtop emulsifier[5] until the maximum viscosity was obtained which could still be applied by special syringe (highest viscosity).

The three vaccines had the following characteristics: Vaccine 1 (lowest viscosity) did not form a droplet in cold water and was not stable at 37°C for more than 24 hours; Vaccine 2 (standard viscosity) was in all aspects like the standard vaccine of the Center (*1, 2*); and Vaccine 3 (highest viscosity) was very stable even at 37°C but was too viscous to be practical under field conditions.

The emulsion of all 3 vaccines were stable at storage for more than 12 months at 4°C. Double emulsions were prepared from Vaccines 1, 2 and 3 by emulsification in a benchtop emulsifier with equal parts of phosphate buffer solution (PBS) with 2% of polyoxyethylene 20 sorbitan monooleate[6]. These double emulsion vaccines (identified as 4, 5 and 6, respectively) had equal low viscosity.

2. Potency tests

For potency testing in guinea pigs and cattle each vaccine was serially diluted in an emulsion having the same characteristics as the vaccine itself.

Guinea pigs: 3-4 months old guinea pigs weighing 550 ± 50 g were used in these experiments. They were inoculated intramuscularly with 0.25 ml of primary emulsion vaccine or with 0.5 ml of double emulsion vaccine. At 30 days postvaccination (DPV) the guinea pigs were inoculated intradermally in the foot pad with 0.1 ml of virus suspension containing 10^3 guinea pig ID_{50} of FMD virus type O_1 Campos. Guinea pigs with generalized lesions at the non-inoculated feet were scored as "not protected".

Cattle: Hereford steers, 2-3 years old, weighing approximately 200 kg raised in isolation on islands in the lake of the Rio Negro river in Uruguay were used in these experiments. They were inoculated intramuscularly with 5 ml of Vaccine 2 in the following dilutions: 1:1, 1:10, 1:40 and 1:160. For each dilution 8 cattle were used. At 30 DPV the cattle were exposed to FMD virus O_1 Campos by inoculation in the tongue epithelium of 10^4 bovine ID_{50}. Any animal developing one or more foot lesions was scored as "not protected". Serum was collected before vaccination and at the day of challenge (30 DPV) for antibody studies.

3. Main experiment

Cattle used in the main experiment were similar to those described above. During the experiment they were maintained in strict isolation on the same islands as the cattle used for the potency tests.

[5]Silverson - Machine (Sales) Ltd. London.

[6]Tween 80, ICI American Inc. Atlas Chemicals Division

Six groups of 12 cattle each were used for Vaccines 1-6. The cattle were vaccinated intramuscularly at the side of the neck. A 5 ml dose of the primary emulsion vaccine was used. The double emulsions were given in 10 ml doses since these vaccines contained half the amount of antigen and oil adjuvant per ml.

Serum was collected for antibody assay before vaccination and at monthly intervals up to 180 days after vaccination.

4. Antibody assay

Microneutralization tests with the sera were made as described by Ferreira (*8*).

RESULTS

Potency tests

Table 2 lists the results of the potency tests in guinea pigs for subtype O_1 virus of the standard vaccine and of the double emulsion Vaccines 4, 5 and 6.

TABLE 2. *Potency test of oil-adjuvanted vaccines in guinea pigs for subtype O_1 FMD virus*

Type of emulsion	Dilution of vaccine	Vaccine No.		
		1	2	3
Primary	1:10		6/6[a]	
	1:40	NT[b]	1/6	NT
	1:160		0/6	
	$GPPD_{50}$[c]		25	
		4	5	6
Double	1:4	1/6	4/6	6/6
	1:16	0/6	0/6	0/6
	1:64	0/6	0/6	0/6
	$GPPD_{50}$[d]	<4	5	8

[a]Number of guinea pigs protected/number of guinea pigs inoculated.
[b]Not tested.
[c]$GPPD_{50}$ = Guinea pig protective dose 50% in 0.25 ml of vaccine, intramuscularly.
[d]$GPPD_{50}$ = Guinea pig protective dose 50% in 0.50 ml of vaccine, intramuscularly.

The standard vaccine when diluted in an emulsion without antigen (active diluent) contained 25 guinea pig PD_{50} per 0.25 ml. The double emulsion Vaccines 4, 5 and 6, diluted in a double emulsion without antigen, contained <4, 5, 8 guinea pig PD_{50} per 0.5 ml, respectively.

Results of the serum neutralization and the challenge tests in cattle following inoculation of the standard vaccine (No. 2) are shown in Table 3 and shows this standard emulsion vaccine contained 40 bovine PD_{50} per 5 ml dose for subtype O_1 virus.

TABLE 3. *Mean serum neutralization titer[a] and 30-day challenge results of cattle inoculated with primary oil emulsion vaccine in various dilutions*

Vaccine dilution[b]	Neutralizing antibodies: Virus		Challenge
	O_1 Campos	A_{24} Cruzeiro	O_1 Campos
1:1	3.24 ± 0.44	2.98 ± 0.39	8/8[c]
1:10	2.81 ± 0.70	2.66 ± 0.54	6/8
1:40	2.87 ± 0.61	2.48 ± 0.42	4/8
1:160	2.19 ± 0.57	2.04 ± 0.47	2/8
Controls	<1.0	<1.0	0/4[d]

[a]Microneutralization test. Reciprocal of $\log_{10}$ of serum dilution protecting 50% of cell cultures against approximately 100 ID_{50}.
[b]Dilution in primary emulsion without FMD virus antigen.
[c]Protected/Total.
[d]One animal died as consequence of FMD.

Long-term immune response

The mean serum antibody titers for subtype A_{24} virus are shown in Fig. 1. It can be observed that no real differences existed among the three viscosities with regard to long-term response. However, the double emulsion vaccines consistently induced a somewhat lower antibody response than the primary emulsion. The results of antibody assays for virus type O_1 are shown in Fig. 2. The overall response for type O was somewhat better than for virus A. At 30 DPV the emulsions with the highest dispersion of the aqueous phase (Vaccines 1 and 4) induced the highest levels of antibody. However, in the long-term response no differences between the vaccines were observed probably because peak antibody titers were obtained with all of them which obliterated any differences.

Both types O and A produced excellent levels of antibodies which persisted throughout the observation period. From these results we conclude that in this experiment the degree of dispersion of the aqueous phase in the primary emulsion had little influence on the immunogenicity of the vaccine in cattle.

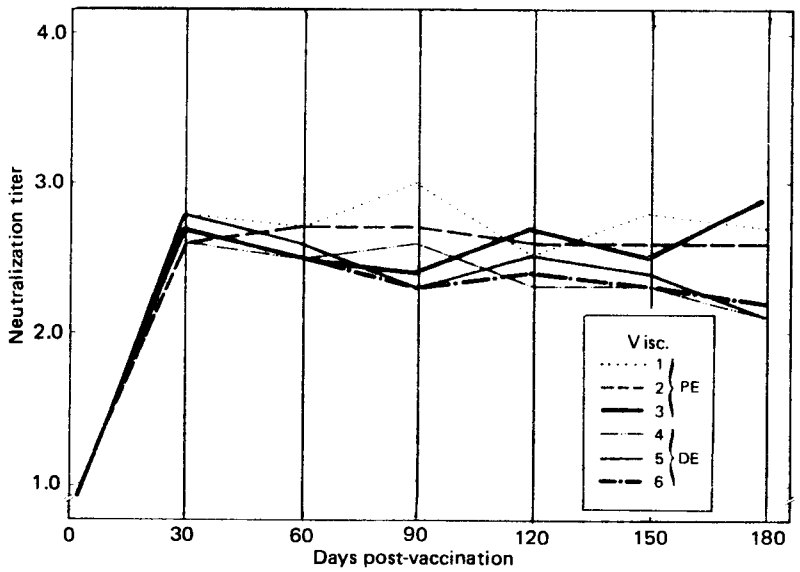

FIGURE 1. *Neutralization titers of subtype A_{24} (Cruzeiro) of primary emulsion inactivated oil adjuvanted vaccines (PE) with different viscosities and of double emulsion vaccines (DE) prepared from the PE vaccines.*

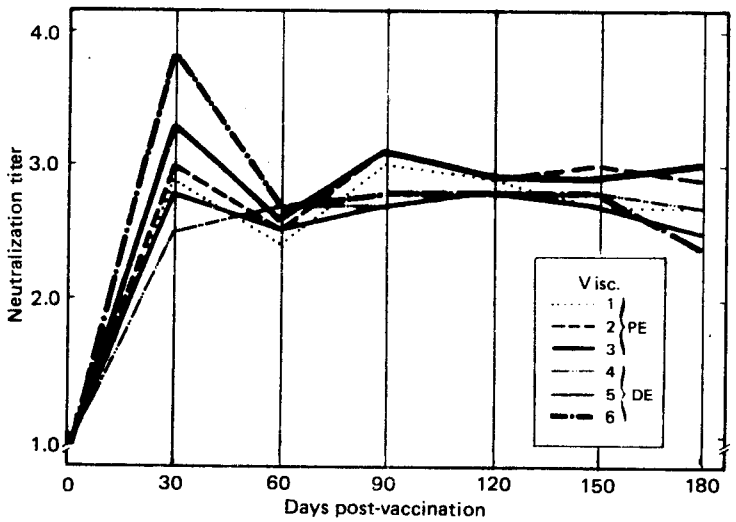

FIGURE 2. *Neutralization titers of subtype O_1 (Campos) of primary emulsion inactivated oil adjuvanted vaccines (PE) with different viscosities and of double emulsion vaccines (DE) prepared from the PE vaccines.*

DISCUSSION

Excellent levels of neutralizing antibody were induced both by the primary and the double emulsion vaccines, even though the primary emulsions performed slightly better. Subtype O_1 antigen induced higher antibody levels than the A antigen. This is contrary to the results in earlier experiments (*1, 2, 6, 7*) in which the A antigen performed better than the O_1 Campos antigen and may be a result of the mixture of A strains used for the vaccines in this experiment. Because of the epidemiological situation in Brazil at the time of preparation of the main batch (Vaccine 2) and its intended use in the field, three strains of FMD A virus were incorporated in the vaccine in the following ratio: 25% A Cruzeiro, 25% A Venceslau and 50% A Bage by volume. The incorporation of these three strains produced a broad coverage against the field strains but resulted in lower titers for the individual vaccine strains.

The results of the GP PD_{50} tests of the double emulsion vaccines agree with the response of cattle at 30 DPV. However, after 60 DPV all vaccines gave a similar response in cattle.

It appears from this experiment that the degree of dispersion of the aqueous antigen phase in the oily phase is not extremely critical and that with even relatively poor dispersion, adequate vaccines may be prepared. It should be kept in mind however that a low degree of dispersion might mean a low stability of the emulsion and that an additional stabilizer for the vaccine might be needed.

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