FORMULATION OF OIL-ADJUVANTED FOOT-AND-MOUTH DISEASE VACCINES CONTAINING ANTIGEN ADSORBED TO ALUMINUM HYDROXIDE

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SHORT COMMUNICATION

Several papers have been published by workers of the Pan American Foot-and-Mouth Disease Center (PAFMDC) on oil-adjuvanted foot-and-mouth disease (FMD) vaccines. These vaccines consist of primary emulsions type water-in-oil with the FMD antigens replicated on BHK_{21} cells in roller bottles or suspension and inactivated with binary ethylenimine (BEI) (*2, 5*) in the aqueous phase and the oily phase of 90% Marcol 52[3] and 10% of mannide monooleate (*1, 3, 4, 6*). This vaccine is used in cattle intramuscularly in a 5 ml dose.

Since, therefore the volume of antigen suspension is fixed at 2.5 ml per dose it is necessary to use some sort of antigen concentration technique in case a higher antigenic mass is to be incorporated in the vaccine. Also the concentration of antigens offers certain advantages which must be considered. For instance, the reduction of the volume of antigen by concentration requires less storage capacity of the 4°C cold rooms which is an important consideration in industrial production. Also concentration of antigen permits increasing the antigenic mass in the vaccine which may be important if the vaccines must be used against a new emerging strain which deviates from the vaccine strains. Finally, the concentrated antigens can be used in formulations with a lower water-in-oil ratio, which result in more fluid emulsions.

This communication reports on the formulation of vaccines with antigens adsorbed to aluminum hydroxide gel. This technique is simple and well known in the FMD vaccine production laboratories and is very efficient since 99.9% of the FMD antigen is adsorbed while the major part of the non viral proteins are discarded with the supernatant.

Results of various cattle experiments with vaccines which contained antigens concentrated in this form are described.

The basic formulation of the experimental vaccines consisted of a water-in-oil type emulsion consisting of an aqueous phase, containing the antigen adsorbed to aluminum hydroxide gel and a double volume of the oily phase (90% Marcol 52 and 10% of mannide monooleate). The oil-aqueous phase ratio and dose volume varied somewhat in the various experiments.

In all experiments a reference control vaccine was used which was formulated according to described standard techniques of the PAFMDC and which contained the same antigenic mass as the experimental vaccines. For the experimental vaccines the inactivated virus suspensions were mixed with $AL(OH)_3$ (at 2.0% of AL_2O_3) in a proportion of 92 parts of virus suspension and 8 parts hydroxide. After adsorption the supernatant was discarded to arrive at vaccine which at 2 or 3 ml of the final dose contained an equal amount of antigens than the 5 ml dose of the reference vaccine.

All experiments were done on farms with cattle free of FMD for several years and which were kept under continuous surveillance for continuous FMD viral activity. The experimental cattle which were free of FMD antibody were of rather homogeneous in age within the experiments but varied between experiments from 6 to 18 months at the moment of first vaccination. Antibody assays were made by the mouse protection test (*7*) and the results evaluated as expected percentages of protection (EPP) (*8*).

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[3] Marcol 52 - Exxon Corporation, U.S.A.

In 7 experiments (Tables 1, 2 and 3) in which 208 cattle were vaccinated with oil vaccines it was shown that antigens which performed well with regular oil-adjuvanted vaccines performed at least as well when adsorbed to aluminum hydroxide. A reduction of the vaccine dose, which was possible because of the concentration of the antigens may be a further advantage in addition to those already mentioned. It results in substantial savings of oil and emulsifier both of which are relatively expensive imported products. However, if the dose is too small a large portion of the vaccine may be lost because of back flow at the time of vaccination under field conditions. The aluminum hydroxide incorporated in the experimental vaccines in the concentration used did not produce unusual undesirable side effects.

In experiment No. 8 (Table 4) vaccines containing either antigens adsorbed to aluminum hydroxide or free antigens (control vaccine) stored for 1 year at 4°C did not have any detectable loss of potency.

TABLE 1. *Expected percentages of protection (EPP) of cattle vaccinated with oil-adjuvanted vaccine, with or without concentration to aluminum hydroxide*

Experiment No.	Vaccine	Cattle dose (ml)	Trivalent antigen per cattle dose (ml)	Cattle per group	Virus	EPP MPV 0	1	3	6
1	Exper.[a]	2	2.5	12	O	< 30	90	87	
					A	< 30	98	91	
					C	< 30	98	87	
	Control[b]	5	2.5	12	O	< 30	99	83	
					A	< 30	91	90	
					C	< 30	99	83	
2	Exper.	3	2.5	12	O	< 30	79	68	
					A	< 30	88	88	
					C	< 30	97	81	
	Control	5	2.5	12	O	< 30	90	83	
					A	< 30	91	88	
					C	< 30	98	89	
3	Exper.	3	2.5	10	O	< 30	99	85	
					A	< 30	95	95	
					C	< 30	98	91	
	Control	5	2.5	10	O	< 30	99	77	
					A	< 30	96	94	
					C	< 30	96	88	
4	Exper.	3	2.5	12	O	< 30	99	70	60
					A	< 30	98	89	79
					C	< 30	97	74	75
	Control	5	2.5	12	O	< 30	89	52	46
					A	< 30	89	85	75
					C	< 30	95	63	62

[a] Experimental oil vaccine adsorbed to $AL(OH)_3$, aqueous + oil phases relationship = 1 + 2.

[b] Standard oil vaccine, aqueous + oil phases relationship = 1 + 2.

MPV = Months post-vaccination.

TABLE 2. *Expected percentages of protection (EPP) of cattle vaccinated and revaccinated at 6 months with oil-adjuvanted vaccine, with or without concentration to aluminum hydroxide*

Experiment No.	Vaccine	Cattle dose (ml)	Trivalent antigen per cattle dose (ml)	Cattle per group	Virus	EPP MPV / MPR	0	1	2	4	6	0	6	12
5	Exper.[a]	2	2,5	12	O		22	95	88	77	47	97	98	
					A		22	99	98	98	96	96	98	
					C		29	98	89	60	64	85	90	
	Control[b]	5	2,5	12	O		24	84	85	58	55	85	91	
					A		21	98	99	98	97	97	95	
					C		30	95	82	65	69	85	81	

[a] Experimental vaccine adsorbed to $AL(OH)_3$, aqueous + oil phases relationship = 1 + 2.
[b] Standard oil vaccine, aqueous + oil phases relationship = 1 + 1.
MPV Months post-vaccination.
MPR Months post-revaccination.

TABLE 3. *Expected percentages of protection (EPP) of cattle vaccinated and revaccinated at 3 months with oil-adjuvanted vaccine, with or without concentration to aluminum hydroxide*

Experiment No.	Vaccine	Cattle dose (ml)	Trivalent antigen per cattle dose (ml)	Cattle per group	Virus	EPP MPV / MPR	0	1	3	0	3	6	12
6	Exper.[a]	2	2.5	10	O		< 30	97	91	96	82	76	
					A		< 30	99	99	87	95	84	
					C		< 30	92	95	96	92	91	
	Control[b]	5	2.5	10	O		< 30	98	93	95	79	84	
					A		< 30	99	99	95	89	94	
					C		< 30	99	99	95	89	94	
7	Exper.	3	2.5	12	O		< 30	92	81	91	86	73	
					A		< 30	88	87	91	84	62	
					C		< 30	92	81	95	89	78	
	Control	5	2.5	12	O		< 30	82	69	91	88	78	
					A		< 30	82	80	93	87	62	
					C		< 30	87	76	94	82	81	

[a] Experimental vaccine adsorbed to $AL(OH)_3$, aqueous + oil phases relationship = 1 + 2.
[b] Standard oil vaccine, aqueous + oil phases relationship = 1 + 1.
MPV = Months post-vaccination.
MPR = Months post-revaccination.

To evaluate the local reactions which the oil vaccines containing aluminum hydroxide might cause, several hundred of cattle were injected with aluminum hydroxide vaccine on one side of the neck and with the control vaccine in the other side either intramuscularly or subcutaneously. No local

reactions were observed with the intramuscular route. Slightly more reaction was observed with the vaccines containing aluminum hydroxide after subcutaneous injection. However, these experiments with cattle vaccinated with both types of vaccines showed that the degree of local reaction depends much on the individual animal. Cattle which got a local reaction from the vaccine containing aluminum hydroxide usually also reacted with the control vaccine to some degree.

TABLE 4. *Expected percentages of protection (EPP) at 30 days post-vaccination with experimental and standard vaccines recently formulated and after storage for 1 year at 4°C.*

Experiment No.	Vaccine	Cattle dose (ml)	Antigen per cattle dose (ml)	Months of storage at 4°C	EPP[a] Virus O	A	C
8	Exper.[b]	2.0	2.5	1	95	99	98
				12	99	96	98
	Control[c]	5.0	2.5	1	84	98	95
				12	93	96	87

[a] Groups of 12 cattle.
[b] Experimental vaccine adsorbed to $Al(OH)_3$, aqueous + oil phases relationship = 0.7 + 1.3.
[c] Standard oil vaccine, aqueous + oil phases relationship = 1+ 1.

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