SHELF LIFE OF INACTIVATED OIL-ADJUVANTED FOOT-AND-MOUTH DISEASE VACCINE

D. Abaracon[1]; *N. Magallanes*[2]; *E.G. Charles*[3]; *L.A. Durini*[3]; *E. Frick*[3]; *G. Fernández de Albarracín*[3]; *E. Degiorgi de Burghi*[3]; *T. Radisich*[3]

SUMMARY

The shelf life of an inactivated oil-adjuvanted foot-and-mouth disease vaccine at 4°C was tested for a storage period of 15 months. No appreciable vaccine potency loss could be detected during that period by the direct challenge test in cattle and antibody assay. An aluminum hydroxide vaccine tested simultaneously up to 13 months also retained its immunogenicity.

INTRODUCTION

An earlier study (*6*) noted that oil-adjuvanted foot-and-mouth disease (FMD) vaccines could maintain their immunogenicity after long storage periods but no systematic observations were made of the same vaccine after different storage time.

In the present study the shelf life of an oil-adjuvanted vaccine up to 15-month storage at 4°C was tested. For reference an inactivated FMD vaccine was included in the test which contained the same antigens absorbed to aluminum hydroxide and to which saponin was added. Both vaccines were tested in cattle after various storage periods.

MATERIALS AND METHODS

1. Virus

Foot-and-mouth disease virus (FMDV) strains O_1 Campos, A_{24} Cruzeiro and C_3 Resende were used. These viruses were produced at the Pan American Foot-and-Mouth Disease Center (PAFMDC) in cultures of $BHK_{21}C13$ cells. The infectious virus suspensions were inactivated with binary ethylenimine (BEI) (*4*). The characteristics of these antigens are presented in Table 1.

TABLE 1. *Characteristics of the foot-and-mouth disease virus antigens used for vaccine preparation*

Antigens	Infectivity titers	CFT[b]
O_1 Campos	7.5[a]	1/20
A_{24} Cruzeiro	8.2	1/18
C_3 Resende	8.2	1/22

[a] Log_{10} BHK cell culture ID_{50}%/ml.

[b] CFT = 50% complement fixation titer ($4HCU_{50}$–90').

2. Trivalent oil-andjuvanted vaccine

The trivalent oil-adjuvanted vaccine consisted of a mixture of inactivated FMD antigens in aqueous suspension emulsified with equal parts of the oil phase (Marcol 52, 90% and Arlacel A, 10%) (*2*). A batch of 30 liters of vaccine containing a mixture of the three antigens was prepared as described (*3*) in a 50-liter emulsification vessel. Each dose of 5 ml trivalent oil vaccine was applied intramuscularly and contained 0.83 ml of each of the antigen suspensions.

3. Trivalent saponin-hydroxide vaccine

A batch of 72 liters of vaccine was prepared according to the method described earlier (*1*), using the same antigens as the oil vaccines. A 5 ml dose applied subcutaneously, contained the equivalent

[1] Pan American Foot-and-Mouth Disease Center, PAHO/WHO, Caixa Postal 589, 20000 Rio de Janeiro-RJ, Brazil.

[2] PAHO/WHO Consultant in Argentina. Present address: Director General de Servicios Veterinarios, Ministerio de Agricultura y Pesca, Constituyente 1476, Montevideo, Uruguay.

[3] Servicio de Laboratorios (SELAB), SENASA, Chorroarin 134, Buenos Aires, Argentina.

of the 3 ml type O, 2 ml type A and 2 ml type C antigen suspensions.

4. Vaccine controls

The oil vaccine was tested for viscosity, type of emulsion, stability (by centrifugation at 1000 g during one hour and storage at 37° and 55° C) and a complement fixation (CF) titer determination after rupture of the emulsion by the addition of vegetable oil (*2*). The results of these controls indicated that the vaccine was a water-in-oil type emulsion with adequate viscosity and stability. No appreciable loss of CF titer had occurred during formulation of the vaccine.

The oil and aluminum hydroxide-saponin vaccines were also tested for sterility in Sabouraud agar, thioglycollate and tryptose phosphate broth with negative results.

5. Potency test in guinea pigs after vaccine formulation

Three-to-four month old guinea pigs weighing 550 ± 50 g were used. Groups of 6 guinea pigs were inoculated intramuscularly with 0.25 ml. The C Index was determined at 30 days according to standard techniques (*5*) by challenge of the guinea pigs in one foot pad with strains O_1 Campos, A_{24} Cruzeiro or C_3 Resende. All C indexes against the three virus strains were >4.0 for both vaccines.

6. Cattle tests

Two-years old Hereford steers weighing 250-280 kg, originating from the FMD-free area in Argentina were vaccinated after various vaccine storage periods as indicated in Table 2 and were challenged at 30 days post-vaccination (DPV). Shortly before challenge the animals were transported to the National Service of Animal Health (SENASA) isolation unit in Buenos Aires where they were inoculated intradermolingually (IDL) with approximately 10,000 mouse/ID_{50}. The types of virus used are shown in Table 2. The cattle were examined 48 hours after inoculation to check for tongue lesions and at 7 days to examine the feet.

TABLE 2. *Protection of cattle vaccinated with FMD vaccines stored for different periods after formulation*

Vaccine	Challenge FMD strains	Months of storage of vaccine at 4°C			
		1	8	13	15
Oil adjuvanted	O_1	9/9[a]	–	–	12/12
	A_{24}	8/8	12/12	12/12	–
	C_3	8/8	–	–	12/12
Aluminum-hydroxide-saponin adjuvanted	O_1	8/8	–	–	–
	A_{24}	8/8	12/12	11/12	–
	C_3	8/8	–	–	–

[a] Number of cattle protected/Total number.

7. Antibody tests

Blood samples were collected from the cattle before vaccination and before exposure to virus. The serum antibodies were assayed by the mouse protection tests as described (*7*) and the results expressed by the expected percentage of protection (*8*).

RESULTS

Cattle vaccinated with the oil-adjuvanted vaccine stored for 1, 8, 13 and 15 months were fully protected against challenge. Cattle vaccinated with aluminum hydroxide-saponin vaccine stored for 1 and 8 months were also totally protected, as were eleven of twelve cattle vaccinated with the same vaccine, after a storage period of 13 months.

Results of the mouse protection tests made with the bovine sera are shown in Table 3. With both vaccines the antibody response after prolonged storage periods remained similar.

DISCUSSION

The present study provides further information on the shelf life of an oil vaccine stored at 4°C for 15 months. No appreciable loss of protection in cattle could be detected during storage. A reference vaccine with aluminum hydroxide adjuvant retained its immunogenicity when tested

TABLE 3. *Mean expected percentage of protection indices of cattle after vaccination with FMD vaccines stored for different periods after formulation*

Vaccine	FMD virus	Months of storage at 4°C			
		1	8	13	15
Oil adjuvant	O_1	97 ± 5	98 ± 3	94 ± 10	98 ± 1
	A_{24}	96 ± 7	98 ± 2	88 ± 17	. . .
	C_3	99	97 ± 5	80 ± 20	96 ± 5
Aluminum-hydroxide-saponin adjuvanted	O_1	93 ± 12	96 ± 3	78 ± 29	
	A_{24}	92 ± 14	98 ± 3	93 ± 8	
	C_3	98 ± 3	91 ± 4	81 ± 20	

. . . Not done.

after 13 months of storage. Results of the mouse protection test confirmed these observations.

The direct challenge method used in the present study does not differentiate the potency of two vaccines, both protecting all vaccinated cattle. In future experiments the use of the 50% protection dose method is indicated. Further studies are needed on the duration of immunity of vaccines before and after storage for prolonged periods.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Ivo Gomes for carrying out the mouse protection test.

REFERENCES

1. ABARACON, D.; GIACOMETTI, H.; MESQUITA, J.A. El uso de la etilenimina binaria (BEI) como inactivante de virus de la fiebre aftosa producido por diferentes técnicas semi-industriales. (The use of binary ethylenimine (BEI) for the inactivation of foot-and-mouth disease virus produced by different semi-industrial techniques). *Bltn Centro Panamericano Fiebre Aftosa 33-34:* 1-5, 7-11, 1979.
2. AUGÉ DE MELLO, P.; ASTUDILLO, V.; GOMES, I.; CAMPOS GARCIA, J.T. Aplicación en el campo de vacuna antiaftosa oleosa e inactivada: vacunación y revacunación de bovinos jóvenes. (Field application of inactivated oil adjuvanted foot-and-mouth disease vaccine: vaccination and revaccination of young cattle). *Bltn Centro Panamericano Fiebre Aftosa 19-20:* 31-38, 39-47, 1975.
3. AUGÉ DE MELLO, P.; MESQUITA, J.A. Preparation of water-in-oil emulsion foot-and-mouth disease vaccine in a semi-industrial scale at the Pan American Foot-and-Mouth Disease Center. (In preparation).
4. BAHNEMANN, H.G. Binary ethylenimine as an inactivant for foot-and-mouth disease virus and its application for vaccine production. *Arch. Virol. 47:* 47-56, 1975.
5. CENTRO PANAMERICANO DE FIEBRE AFTOSA. Manual de procedimientos para el control de vacuna antiaftosa. *Ser. Man. Técn. 2*, 1980.
6. CENTRO PANAMERICANO DE FIEBRE AFTOSA; DIRECCION DE LUCHA CONTRA LA FIEBRE AFTOSA. Prueba de potencia para vacunas contra la fiebre aftosa de adyuvante oleoso: ensayos de DP_{50} en cobayos y en bovinos de una vacuna preparada en forma semi-industrial con una emulsión del tipo agua

en aceite. (Potency testing of oil adjuvanted foot-and-mouth disease vaccine: PD_{50} assays of a semi-industrial water-in-oil type emulsion vaccine in guinea pigs and cattle). *Bltn Centro Panamericano Fiebre Aftosa 29-30:* 55-59, 61-65, 1978.

7. CUNHA, R.G.; BAPTISTA Jr., J.A.; SERRÃO, U.M.; TORTURELLA, I. El uso de los ratones lactantes en la evaluación de los anticuerpos contra el virus de la fiebre aftosa y su significación inmunológica. *Gac. Vet.*, B. Aires, *19* (11): 243-267, 1957.
8. GOMES, I.; ASTUDILLO, V. Foot-and-mouth disease: evaluation of mouse protection test results in relation to cattle immunity. *Bltn Centro Panamericano Fiebre Aftosa 17-18:* 9-16, 1975.