

## FIELD APPLICATION OF INACTIVATED OIL ADJUVANTED FOOT-AND-MOUTH DISEASE VIRUS VACCINE: VACCINATION AND REVACCINATION OF YOUNG CATTLE

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### INTRODUCTION

Studies at the Plum Island Animal Disease Center (PIADC) and the Pan-American Foot-and-Mouth Disease Center (PAFMDC) demonstrated that under laboratory conditions cattle could be adequately protected against foot-and-mouth disease (FMD) with vaccines prepared from FMD virus inactivated with N-acetylenimine (AEI) and adjuvanted with mineral oil (12, 13). In view of those favorable results a long-term program was implemented to study the use of such vaccines under controlled field conditions.

This paper describes the results of antibody studies after the vaccination and revaccination of young cattle with oil adjuvanted AEI inactivated FMD virus vaccines. The immune response of a group of these calves was compared with those of another vaccinated with an aluminum-hydroxide vaccine prepared from the same AEI inactivated antigens. The overall immune response of the calf population vaccinated with the experimental oil-vaccine was also assessed.

### MATERIALS AND METHODS

#### *Experimental station*

The site selected for these studies was

the Ministry of Agriculture's "Cinco Cruzes" experimental station in Bagé, in the state of Rio Grande do Sul. The farm comprises nearly 2,800 hectares. The cattle population consists of a closed herd of high quality crossbred beef cattle of 5/8 of Aberdeen-Angus and 3/8 Nelore and of a dairy herd of approximately 250 Jersey and Red Dane cows. The total number of beef cattle fluctuates by season between 1,600 and 2,500 head. Calves are born in September and October, and they are weaned 6-7 months later. The animals are weighed at monthly intervals until they are 2-2.5 years old, at which time the steers are sent to the slaughterhouse.

Besides FMD vaccinations, an active livestock health control program is carried out which includes regular examination and treatment for ecto and endoparasites, and brucellosis, anthrax and blackleg vaccinations.

#### *Vaccination scheme*

In accordance with the herd management practices and weather conditions, it was decided to vaccinate the cattle with the experimental vaccines during the period April/May and September/October of each year. A 6-month vaccination interval was chosen because earlier laboratory experiments (13) had indicated that these vaccines

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could adequately protect cattle during that period.

All cattle at the station not included in the experiment continued to be vaccinated with commercial AEI inactivated aluminum hydroxide vaccine at 3-month intervals.

#### *Experimental groups*

The 353 calves born in 1971 were 5-7 months old at the start of the experiment. They formed a homogeneous group and were maintained under identical conditions throughout the study.

#### *1. Comparison of adjuvants*

For the comparison of immune response induced by oil vs. aluminum-hydroxide adjuvant vaccines, 30 male and 30 female calves were randomly selected and divided into 3 groups. The first group was vaccinated with the oil vaccine and the second with the aluminum hydroxide. The third was not vaccinated and served as a control. After 6 months the first and second groups were revaccinated with the same type of vaccine (Table 1). All cattle were bled for assay of circulating antibodies before vaccination and at monthly intervals.

#### *2. Population immunity*

In order to study the immune response to the oil vaccine of the population as a whole, the remaining 293 calves were vaccinated at the beginning of the experiment and 277 revaccinated six months later. For the bimonthly serum surveys, a new random selection of 40-50 calves for bleeding was made, taking into account the sex distribution of the calf population.

#### *3. Duration of immunity after one vaccination*

Sixteen calves from the preceding group were randomly selected and identified after the first vaccination and were not revaccinated later. They were bled at monthly

intervals from 6 to 12 months after vaccination.

#### *Antigens*

The following strains of FMD virus of South American origin were used: O<sub>1</sub> Campos, A<sub>24</sub> Cruzeiro and C<sub>3</sub> Resende. These viruses were first adapted to growth in BHK-21 cells (5). Stationary BHK cell cultures grown in Roux flasks were inoculated with virus in 50 ml of Eagle modified medium without serum. After 20 hours incubation at 37° C the virus suspensions were harvested, shaken with 10% (v/v) of trifluor-trichloroethane for 10 minutes, clarified by centrifugation at 800 x g for 30 minutes, and stored at 4° C until inactivation.

Infectivity titers were determined by plaque assay and expressed as PFU/vaccine dose.

Complement fixation was carried out by the regular PAFMDC method (10) with 4 units of complement, 90 minutes of incubation at 37° C and reading 50% hemolysis in a spectrophotometer\*, and assayed at the beginning and at the end of the inactivation process (Table 1).

#### *Inactivation process*

Each virus suspension was inactivated with 0.05% of N-acetylenimine (AEI) for 24 hours under continuous stirring at 37° C in a waterbath. The action of AEI was neutralized by adding sodium thiosulfate in the final concentration of 2% (w/v) after inactivation. Rates of inactivation were determined by the method described by Graves (3).

#### *Inocuity test*

After inactivation, each of the antigen suspensions was inoculated intraperitoneally in 120 unweaned 7-day old mice, 0.1 ml/mouse. They were observed for 10 days, and the suspension was considered to be

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\* Coleman Jr. Mod. 6A.

inocuous if no mice died of FMD virus infection.

#### *Vaccine formulation*

Two batches of vaccine were prepared for vaccination and revaccination, respectively. Equal portions of each of the three antigens were mixed, and the resulting trivalent product was divided in two portions to which different adjuvants were added:

##### *1. Oil*

A mixture of mannide monooleate\* and mineral oil\*\* in proportion of 1:10 was emulsified with an equal quantity of inactivated trivalent virus suspension by means of a homogenizer\*\*\* to form a water-in-oil emulsion.

##### *2. Aluminum hydroxide*

Equal parts of a colloidal suspension of  $\text{Al}(\text{OH})_3$  (concentration of 2%  $\text{Al}_2\text{O}_3$ ) and of the inactivated trivalent antigen suspensions were slowly added and mixed over a 10-minute period.

#### *Potency test*

The potency of the vaccines 21 days after vaccination was assayed in guinea pigs using the C Index method and in cattle using the modified K Index method (10). The vaccines passed these tests with indices of  $>2.0$  (C) and  $>1.5$  (K).

#### *Antigen control*

After vaccine formulation and prior to use of the vaccine, the emulsion was "broken" by adding soybean oil to the vaccine (4 parts oil + 1 part vaccine), mixing and

centrifugation for 30 min (Dr. J.H. Graves, personal communication). After centrifugation the aqueous phase was assayed by complement fixation (CF) test.

#### *Antibody assay*

Antibody levels were assayed by the mouse protection test (1). Of all groups a mean expected percentage of protection (EPP) was calculated according to the method proposed by Gomes and Astudillo (2). According to their definition, protection in cattle is defined as absence of foot lesions after virus exposure by tongue inoculation.

## RESULTS

#### *Comparison of vaccines adjuvanted with oil or with aluminum hydroxide gel*

The expected percentages of protection (EPP) for the 3 virus types in cattle vaccinated with oil or aluminum-gel vaccine and in the unvaccinated control cattle are presented in Fig. 1.

The aluminum-gel vaccine induced an EPP of approximately 60-70% at 30 days, although these values dropped to less than 50% at 60 days and by 90 days had nearly reached the EPP level of the unvaccinated cattle.

With the oil vaccine the EPP was more than 70% at 30 days, and the protection level continued to rise up to 60 days after vaccination, when an EPP of about 80-90% was reached. These peak levels were maintained for nearly 2 months, after which a slow decline occurred. At 6 months there still was considerable protection ( $O_1$  and  $C_3$  56-54%, respectively, and A 73%).

Revaccination produced a booster effect with both vaccines, although the aluminum-gel vaccine protection levels decreased quite rapidly. With the  $O_1$  and  $C_3$  vaccine the levels of protection were similar to the unvaccinated control 4 months after revac-

\* Arlacel A - ICI America Inc., Atlas Chemicals Division.

\*\* Marcol 52 - Exxon Corporation U.S.A.

\*\*\* Silversen - Machines (Sales) Ltd. London.

cination. The oil vaccine induced high levels of protection at revaccination which lasted much longer. The persistence of protection against subtype C<sub>3</sub> was the least satisfactory of the 3 viruses in both types of vaccine.

#### *Population immunity*

Table 2 lists the 95% confidence limits of the expected percentage of protection from a sample of calves vaccinated and revaccinated with the oil adjuvanted vaccine.

It can be observed that an excellent population immunity was obtained with vac-

inations every six months. These results also confirm that the A<sub>24</sub> virus is the best antigen, followed by type O<sub>1</sub>, and that type C<sub>3</sub> is the least efficient antigen.

#### *Duration of immunity after one vaccination*

Table 3 lists the duration of immunity in the 16 cattle vaccinated only once with oil adjuvanted vaccine. Protection against subtypes O<sub>1</sub> and C<sub>3</sub> persisted around 70% of the cattle for as long as 9 months after vaccination. The response to subtype A<sub>24</sub> was particularly good with more than 70% protection at 12 months post-vaccination.

TABLE 1 - *Characteristics of two batches of foot-and-mouth disease virus antigens used for the preparation of vaccine for vaccination and revaccination, respectively*

FMD virus	Batch					
	I			II		
	Titers		Rate of inactivation PFU/min	Titers		Rate of inactivation PFU/min
	PFU	CF		PFU	CF	
O <sub>1</sub>	7.57	1/25	-0.038*	7.29	1/30	-0.038
A <sub>24</sub>	7.54	1/20	-0.017	7.69	1/15	-0.038
C <sub>3</sub>	7.44	1/25	-0.017	7.92	1/25	-0.027

PFU = log<sub>10</sub> plaque forming units/vaccine dose.

CF = serum dilution giving 50% hemolysis before and after inactivation (see text).

\* = decrease of viral infectivity in log<sub>10</sub> PFU per minute.

TABLE 2 - *Population immunity after vaccination and revaccination of cattle with oil adjuvanted FMD vaccine*

Average age of cattle (months)	Months after vaccination		Expected percentage of protection against FMD virus		
	1st	2nd	O <sub>1</sub>	A <sub>24</sub>	C <sub>24</sub>
6	0		22 ± 12	21 ± 12	31 ± 13
8	2		94 ± 7	97 ± 5	85 ± 10
10	4		86 ± 10	90 ± 8	82 ± 11
12	6	0	84 ± 10	94 ± 7	74 ± 12
14	8	2	96 ± 10	100 ± 0	95 ± 6
16	10	4	87 ± 10	97 ± 5	83 ± 11
18	12	6	78 ± 12	91 ± 8	70 ± 13

TABLE 3 - *Duration of immunity of cattle vaccinated once with oil adjuvanted foot-and-mouth disease vaccine*

Average age of cattle (months)	Months after vaccination	Expected percentage of protection against FMD virus		
		O <sub>1</sub>	A <sub>24</sub>	C <sub>24</sub>
12	6	84	85	72
13	7	68	79	64
14	8	76	89	53
15	9	68	84	70
16	10	58	79	64
17	11	33	77	49
18	12	49	72	41

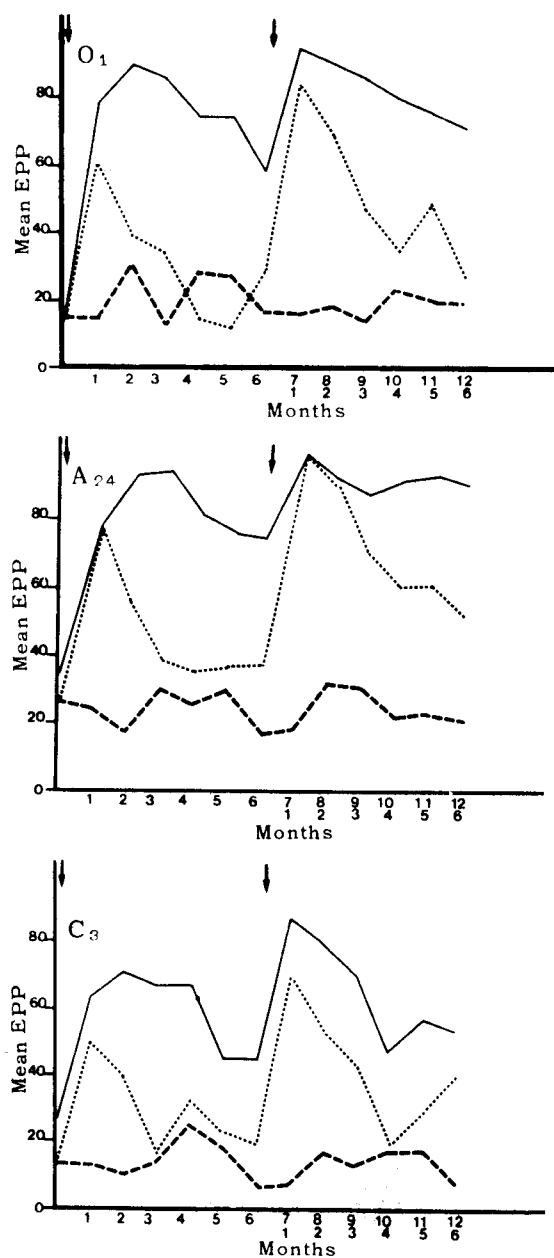


FIGURE 1 - Mean of expected percentages of protection (EPP) of cattle against FMD virus of subtypes O<sub>1</sub>, A<sub>24</sub> and C<sub>3</sub> after vaccination with aluminum-gel vaccine or oil adjuvanted vaccine and of unvaccinated control cattle.

———— oil adjuvanted vaccine.

..... aluminum-gel vaccine.

----- unvaccinated controls.

↓ ↓ vaccination/revaccination.

### DISCUSSION

It has long been recognized that young cattle are difficult to protect adequately with the formalin inactivated aluminum-gel vaccine, even though repeated vaccination may lead to a longer lasting immunity (4, 6, 7, 8, 9). Present results support this observation. The results obtained with oil adjuvanted vaccines show that under field conditions these vaccines produce higher, longer lasting immunity in young cattle than aluminum-gel vaccines prepared from identical inactivated antigens.

These experiments show the immunogenic differences among the virus strains used for the preparation of the vaccines. The ability of the antigens to induce protection in the cattle varied greatly even though the viral infectivity and complement fixation (CF) titers were similar. In other studies of oil adjuvanted FMD vaccines, the O<sub>1</sub> strain Caseros was the poorest of the immunogens (12, 13). Thus, selection of the most immunogenic vaccine strain is critical for the preparation of a satisfactory vaccine.

Other studies have shown that oil adjuvanted vaccines can induce a high degree of protection in adult cattle (11). The present population immunity study demonstrated that vaccination at 6-month intervals will also protect a high percentage of young cattle. It thus appears feasible to vaccinate only twice a year with oil adjuvanted vaccines instead of 3 times per year with conventional vaccines. The booster effect at 6 months was satisfactory, although further

studies are needed to establish the optimum vaccination interval.

The vaccinations were carried out under controlled field conditions; and in the next experimental phase the vaccine should be tested in a larger cattle population at risk.

### ABSTRACT

The serum protection test results of a group of 5-7 months old cattle vaccinated and revaccinated under controlled field conditions with oil adjuvanted foot-and-mouth disease vaccine were compared with those of cattle vaccinated with aluminum-hydroxide vaccine. The same acetylenimine (AEI) inactivated antigens were used in both vaccines. The mean expected percentage of protection showed that the oil-adjuvanted vaccine produced a higher and longer lasting protection than the aluminum-gel vaccines. Revaccination with the oil vaccine 6 months later produced a good anamnestic response with high levels of protection which lasted for a long time.

A bimonthly serum survey of the calf population of 293 animals vaccinated with the oil vaccine at 6-month intervals showed that under field conditions this type of vaccine produced high level of long lasting protection. Six months after application of oil vaccines, around 70% of the young cattle were expected to be protected. These levels of protection persisted for up to 9 months for types O and C, and 12 months for type A.

The choice of virus strains to be used for the preparation of the vaccine proved to be important.

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