

ANAMNESTIC RESPONSE IN CATTLE AFTER REVACCINATION WITH OIL ADJUVANTED FOOT-AND-MOUTH DISEASE VACCINES

*P. Augé de Mello**; *Ivo Gomes**

SUMMARY

The immune response to three inactivated foot-and-mouth disease (FMD) vaccines was compared: the first vaccine was prepared with oil adjuvant, the second with aluminum hydroxide-saponin and the third did not contain adjuvant. With all three vaccines an anamnestic response was observed on the 4th day after revaccination, and from the 6th day on the expected percentage of protection was 99%.

The anamnestic response of the oil adjuvanted vaccine was characterized by high antibody levels which persisted for a long period. Moreover, the A₂₄ Cruzeiro strain in the oil adjuvanted vaccine gave a wide coverage for least 180 days against three immunologically different A strains. After an initial favorable anamnestic response the aluminum hydroxide-saponin vaccine at 60 days post-revaccination (DPR) gave low mouse protection indices against those different samples.

The results showed that oil adjuvanted FMD vaccines can be very useful for strategic ring vaccinations in view of the excellent anamnestic response of long duration and wide immunological coverage.

INTRODUCTION

Experiments both in the laboratory (13) and in the field under controlled conditions have shown that oil adjuvanted FMD vaccines can efficiently protect young and adult cattle. The duration of this protection allows for vaccination at 6-month intervals of animals less than 2 years (3) and a yearly vaccination (4) of older animals.

The speed at which the anamnestic response occurs after FMD vaccination is of great importance

for ring vaccination, which is one of the options in the strategy of FMD control during an outbreak (6). It is thus important to know what the action of oil adjuvanted vaccines would be under such conditions, particularly since one of the characteristics of this adjuvant appears to be its slow release of the antigen (12), attaining a maximum induction of antibodies as late as 60 days after the first vaccination (3).

The present work compares the anamnestic response, as well as the duration of immunity, of three inactivated FMD vaccines: one with oil adjuvant; the another with aluminum hydroxide-saponin, and a third with a virus suspension containing no adjuvant. The immunological coverage of the A₂₄ strain is also compared to three serologically different A strains: A Bagé (1976), A Venceslau (1976) (9) and A Macabú (1977) (14).

MATERIALS AND METHODS

Antigens

The following FMD virus subtypes were used: O₁ strain Campos, A₂₄ strain Cruzeiro and C₃ strain Resende. All three strains were produced in BHK₂₁ C₁₃ cells in roller bottle cultures. The virus suspensions were inactivated by 2-bromoethylamine in alkaline solution (BEI) at a concentration of 0.001 M during 24 h at 37° C (5). The infective titers were determined in cell culture and expressed as 50% infective cell culture (CCID₅₀/vaccine dose). Complement fixation titers were determined before and after the inactivation process. After inactivation the antigen suspensions were assayed for the absence of infectivity in cell culture with the minimum of three passages. All tests were made in accordance with the standards

* Pan American Foot-and-Mouth Disease Center, Caixa Postal 589, ZC-00, Rio de Janeiro, RJ, Brazil.

for FMD vaccine control (7). Table 1 specifies the characteristics of the antigens.

TABLE 1. *Characteristics of the antigens*

| Foot-and-mouth disease antigens | Titers | |
|---------------------------------|---------------------------|------|
| | CCID ₅₀ /doses | CF |
| O ₁ Campos | 7.5 | 1/14 |
| A ₂₄ Cruzeiro | 7.5 | 1/14 |
| C ₃ Resende | 7.3 | 1/15 |

CCID₅₀ = 50% Infective cell culture doses.

CF = Complement fixation with 4 units of complement at 90 minutes of incubation at 37° C.

Preparation of the vaccines

A trivalent mixture of the antigens was prepared by mixing equal parts of each of the inactivated virus suspensions. The trivalent suspension was divided in three parts so that each vaccine contained the same amount of antigen.

Oil adjuvanted vaccine

A mixture of manide mono-oleate* and mineral oil** in the proportion of 1:10 was emulsified with an equal amount of trivalent suspension (3).

Aluminum hydroxide-saponin vaccine

A colloidal suspension of Al(OH)₃ (minimum concentration of 2% Al₂O₃) was mixed in equal parts with the inactivated suspension. After complete homogenization of the suspension saponin was added so that each vaccine dose would contain 5 mg.

Virus suspension without antigen

The trivalent suspensions were mixed with

equal parts of buffer phosphate 0.04 M and 50% glycerin.

Cattle

Thirty zebu cattle 4-6 months old were used; they had no history of FMD vaccination or exposure and were free of FMD circulating antibodies. They were vaccinated with a trivalent aluminum hydroxide-saponin vaccine produced in the production plant of the Pan American Foot-and-Mouth Disease Center, containing the equivalent of 7 ml of trivalent virus suspension (O₁, A₂₄ and C₃). The cattle were inoculated subcutaneously with 5 ml.

Vaccination

Five months after the first vaccination the cattle were bled and divided randomly into three groups. One group was revaccinated with the oil adjuvanted vaccine, the second group with the aluminum hydroxide-saponin vaccine and the third with the virus suspension without antigen. Five ml doses were used for all vaccines, the aluminum hydroxide-saponin vaccine was given subcutaneously and the others by the intramuscular route.

Antibody studies

The cattle were bled at 2, 4, 6 and 10 days post-revaccination (DPR) and at 30 days intervals up to 120 days. The animals revaccinated with oil vaccine were bled up to 180 days. The levels of circulating antibodies were determined by the mouse protection test in suckling mice (8).

RESULTS

The mouse protection indices (MPI) for the three types of FMD virus and for the three experimental groups are listed in Table 2. The anamnestic response was already detected at 4 DPR and at day 6 reached an expected percentage of protection (EPP) of 99% (10) in all cases.

Figures 1, 2 and 3 show the antibody curves for the three types of virus with different treatments. The effect of adjuvant on the persistence of the

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

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

TABLE 2. Anamnestic response of cattle after revaccination with foot-and-mouth disease vaccine

| Vaccine | Foot-and-mouth disease virus | Days post-revaccination | | | | |
|----------------------------|------------------------------|-------------------------|-----------|-----------|-----------|-----------|
| | | 0* | 2 | 4 | 6 | 10 |
| Oil adjuvanted | O ₁ | 1.8 ± 1.2** | 1.9 ± 1.1 | 2.4 ± 1.1 | 4.3 ± 0.5 | 5.0 |
| | A ₂₄ | 2.5 ± 1.2 | 2.8 ± 0.9 | 3.4 ± 0.6 | 4.4 ± 0.6 | 5.0 |
| | C ₃ | 1.3 ± 0.2 | 1.4 ± 0.6 | 1.8 ± 0.8 | 4.5 ± 0.5 | 5.0 |
| Aluminum hydroxide-saponin | O ₁ | 1.5 ± 0.5 | 1.7 ± 0.6 | 3.0 ± 0.8 | 4.0 ± 0.5 | 5.0 |
| | A ₂₄ | 2.2 ± 0.8 | 2.6 ± 1.0 | 3.3 ± 0.6 | 4.3 ± 0.8 | 5.0 |
| | C ₃ | 1.1 ± 0.6 | 1.4 ± 0.4 | 2.0 ± 0.6 | 4.6 ± 0.5 | 5.0 |
| Without adjuvant | O ₁ | 1.8 ± 0.9 | 1.7 ± 0.7 | 2.5 ± 1.1 | 4.4 ± 0.5 | 4.7 ± 0.7 |
| | A ₂₄ | 2.7 ± 1.0 | 3.0 ± 0.8 | 3.5 ± 0.8 | 4.5 ± 0.9 | 4.9 ± 0.2 |
| | C ₃ | 1.7 ± 0.7 | 1.6 ± 0.5 | 2.0 ± 0.9 | 3.8 ± 1.1 | 4.2 ± 1.2 |

* After the first vaccination with inactivated aluminum hydroxide-saponin vaccine.

** Mean of the mouse protection indices and standard deviation. Values of ≥ 3,6 represent an expected percentage of protection (EPP) of 99% (10).

circulating antibodies was quite clear. Oil adjuvanted vaccines induced circulating antibodies which persisted for more than 6 months for the three types of virus. This prolonged duration of immunity was not observed with the aluminum hydroxide-saponin vaccines, which induced maximum antibodies levels at 30 DPR followed by a rather steep decrease.

Antibody levels of the cattle group which had received the virus suspension without adjuvant decreased rapidly even though each of the groups showed an anamnestic response similar to the other two groups.

In all treatments strains O₁ Campos and C₃ Resende were immunogenically inferior to strain A₂₄ Cruzeiro. Table 3 lists the immunological response of the A₂₄ Cruzeiro aluminum hydroxide-saponin vaccine and the oil adjuvanted vaccine against the virus strains A Bagé, A Venceslau and A Macabú. Immunological coverage of the induced antibodies for the vaccine prepared with the aluminum hydroxide-saponin was unsatisfactory from 60 days onward. The oil vaccine showed high levels of coverage for the strains under study for a period of more than 180 days.

DISCUSSION

Even though the highest level of antibodies occurs after 60 days in animals vaccinated for the first time with oil adjuvanted FMD vaccine (3), Graves *et al.* observed high levels of protection between 3 and 14 days after the first vaccination (11) of cattle and swine vaccinated with an oil adjuvanted vaccine. We found that the immediate antibody response to FMD virus antigens does not depend on of the presence of the adjuvant. The anamnestic response of cattle reached EPP values of 99% (10) from the 6th day on independent of whether or not adjuvant was incorporated in the vaccine. However the persistence of antibodies depended on the type of adjuvant used. In this regard the oil adjuvant induced the highest level of antibodies and the longest duration of protection, confirming earlier results (3, 4, 13).

The differences in the immunological coverage induced by the different adjuvants was of great importance. Thus the A₂₄ strain Cruzeiro with oil adjuvanted vaccine gave a wide coverage up to 180 DPR against three immunologically different A strains while the vaccine prepared with the

FIGURE 1. Mean mouse protection index type O_1 of cattle revaccinated with oil adjuvanted vaccine, aluminum hydroxide-saponin vaccine or antigen without adjuvant.

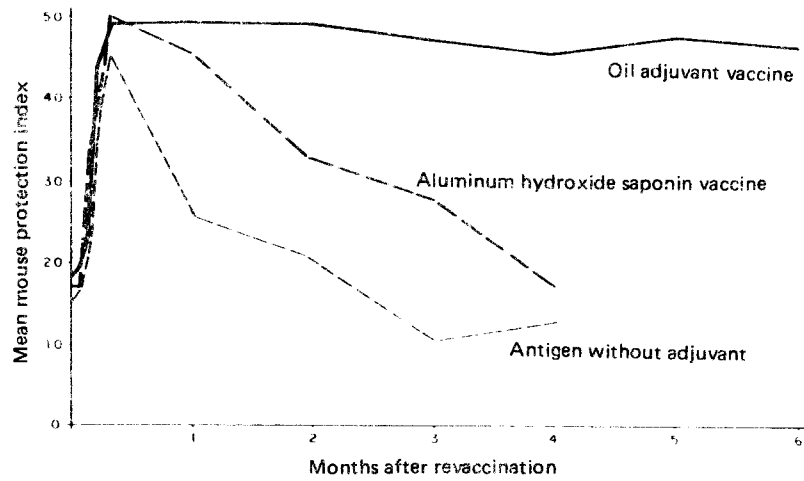


FIGURE 2. Mean mouse protection index type A_{24} of cattle revaccinated with oil adjuvanted vaccine, aluminum hydroxide-saponin vaccine or antigen without adjuvant.

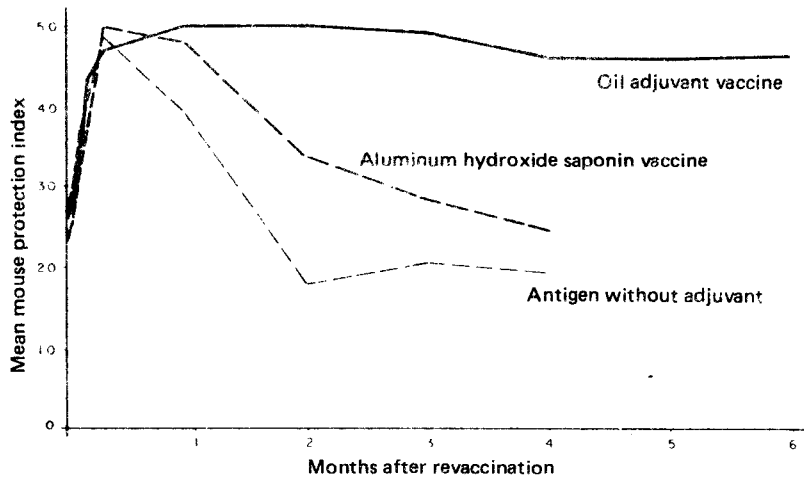


FIGURE 3. Mean mouse protection index type C_3 of cattle revaccinated with oil adjuvanted vaccine, aluminum hydroxide-saponin vaccine or antigen without adjuvant.

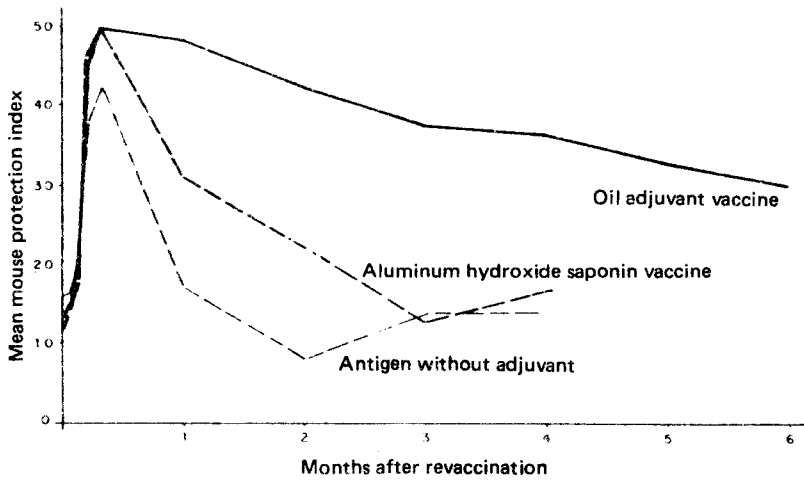


TABLE 3. Immunological response of A₂₄ Cruzeiro strain against 3 virus A strains immunologically different

| Vaccine | Days post-revaccination | A virus strains | | | |
|----------------------------|-------------------------|-----------------|-----------|-----------|-----------|
| | | Cruzeiro | Bagé | Venceslau | Macabú |
| Oil adjuvanted | 60 | 5.0* | 3.4 ± 0.9 | 2.9 ± 1.2 | 3.6 ± 0.9 |
| | 120 | 4.6 ± 0.4 | 4.3 ± 1.2 | 3.4 ± 1.0 | 3.3 ± 0.8 |
| | 180 | 4.7 ± 0.5 | 3.8 ± 0.9 | 2.8 ± 1.2 | 3.1 ± 1.1 |
| Aluminum hydroxide-saponin | 60 | 3.4 ± 1.1 | 1.8 ± 0.9 | 0.4 ± 0.5 | 1.5 ± 0.4 |
| | 120 | 2.4 ± 0.6 | 1.2 ± 0.9 | 1.1 ± 0.9 | 1.4 ± 0.3 |

* Mean of mouse protection indices and standard deviation.

aluminum hydroxide-saponin gave low MPIs against the strains studied already at 60 DPR. This study confirms earlier work (1, 2, 9) that oil adjuvanted vaccines notably increase the immunological coverage of the FMD virus antigens.

The results obtained by Graves *et al.* (11) in

animals vaccinated for the first time and the excellent anamnestic response with a wide immunological coverage up to 180 DPR indicates that oil adjuvant FMD vaccine could be used in strategic ring vaccinations.

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