OIL ADJUVANTED FOOT-AND-MOUTH DISEASE VACCINE: A COOPERATIVE STUDY

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and the
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ABSTRACT

A summation of cooperative studies on foot-and-mouth disease virus vaccines between the Argentine Instituto Nacional de Tecnología Agropecuaria (INTA), the Pan American Foot-and-Mouth Disease Center, Brazil, and the United States Plum Island Animal Disease Center, is presented. These studies involve the immune response obtained in steers from (1) Frenkel virus vaccines prepared in oil and aluminum hydroxide adjuvants. When the immunity of the steers was challenged at 30 days, the Frenkel vaccine adjuvanted with aluminum hydroxide and saponin gave the best protection. In the major study (11), the response of steers from 3 BHK produced virus vaccines emulsified with oil adjuvant was studied. The three vaccines were prepared: one in which the virus was used in the concentration in which it was harvested; the other two from virus which was concentrated 100-fold by the polyethylene-glycol technique and then diluted to a 20X and 1X concentration. The antibody response and protection afforded by the 3 vaccines were similar. It was concluded that animals in the three groups were afforded similar protection when their immunity was challenged, indicating that these differing concentrations of antigen provided no significant difference in protection.

INTRODUCTION

A summation of results obtained in cooperative studies on foot-and-mouth disease virus (FMDV) vaccines conducted in Argentina by the Instituto Nacional de Tecnología Agropecuaria (INTA), the Pan American Foot-and-Mouth Disease Center (PAFMDC), Brazil, and the United States Plum Island Animal Disease Center (PIADC) over a period of several years (1968-1975) is reported (9, 11, 12). In this report, the immune response of oil adjuvant and aluminum hydroxide vaccines in cattle (part I), and the immune response after vaccination and revaccination with an FMD vaccine emulsified with oil adjuvant (part II) are evaluated. In the preliminary studies workers compared results of vaccines in which the viruses used for antigen were grown in baby hamster kidney (BHK) cells, inactivated with acetyleneimine (AEI) and emulsified with oil adjuvant (PIADC and INTA) and vaccines in which the virus used as antigen was grown in Frenkel cultures, inactivated with formaldehyde and combined with aluminum hydroxide and saponin adjuvant. The vaccinated cattle were kept at a holding unit in the Valdés Peninsula, Chubut Province, Argentina.

MATERIALS AND METHODS

Selection of strains of FMDV

On the advice of representatives of the Pan American Foot-and-Mouth Disease Center, the strains of FMDV subtype O1 Caseros, subtype A24 strain Cruzeiro, and subtype C3 Resende selected for the vaccine preparation were the same as those previously reported (9, 11).

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Vaccines

Four trivalent vaccines were prepared for the
initial part (I) of these studies: 1) FMDV grown
in Frenkel cultures, inactivated with formalde-
hyde and combined with aluminum hydroxide
saponin adjuvant; 2) FMDV grown as in 1) but
combined with INTA oil formulation; 3) FMDV
grown in BHK cell cultures, inactivated with AEI
and emulsified with PIADC oil adjuvant; and 4) 
similar to 3) but emulsified with the INTA oil
adjuvant (1, 2, 10).

Three trivalent vaccines were used in II, the
second major part of the study as reported (9)
and consisted of: 1) Vaccine 1 - virus grown in
BHK cells, inactivated with AEI and emulsified
with oil adjuvant (INTA formulation) (2); 2) Vac-
cine 2 - virus grown as above, concentrated 100-
fold by the polyethylene glycol technique (13)
and then reconstituted to contain approximately
the same antigen mass as Vaccine 1; and 3) Vac-
cine 3 - virus processed as in Vaccine 2, but ad-
justed to contain 20-fold the antigenic mass as
Vaccines 1 and 2.

Safety testing

The infectivity of each antigen prepared at
PIADC was tested by inoculating the tongues of
each of 6 steers at 20 sites with 0.1 ml of the inac-
tivated virus (8). All antigens and final products
were further tested in a similar manner in Argent-
a on before use in part I and at PIADC before use
in part II.

Potency tests

The BHK antigens prepared at PIADC for part I
were tested as monovalent and trivalent vaccines
before their transportation to Argentina. The
monovalent vaccines were each inoculated into 6
steers whose immunity was challenged at 30 days
postvaccination (DPV) with their homologous
viruses. The same antigens were formulated into
a trivalent vaccine, and a 5-ml dose of this vaccine
was inoculated into each of 6 steers whose immu-
nity was also challenged at 30 DPV.

In Argentina, a 30-day potency test was con-
ducted with each vaccine--1, 2, 3 and 4. Twenty-
seven steers were inoculated with each vaccine,
and these animals, totalling 108, with controls
were kept in the isolation station at Puerto La-
rarde in Peninsula Valdés.

In the potency test for II, the second part of
the study, 12 steers were inoculated subcutaneous-
ly, each with a 5-ml dose of vaccine, 4 steers were
used with each vaccine--1, 2 and 3. A 50% bovine
protective dose for O virus was determined for
Vaccines 1 and 2 (9).

Vaccination

A total of 371 steers between 1 and 2 years
old were selected for the study (II). They were
from Chubut, the southern province of Argentina,
situated in the FMD-free area of Patagonia, and
were transferred to the cattle holding station in
the Valdés Peninsula. Serums from the selected
steers were examined for FMD antibodies at INTA
by the mouse protection test (5). Only those with
a serum-neutralizing index of less than 1 were se-
lected for use in the study. They were divided
into groups of 52—a group of 52 animals for each
of the 3 vaccines and a group of 52 as nonvacci-
nated controls. Each group of 52 animals was
selected from the 371 animals by pseudo random
number selection on a computer (9).

Serology

Blood samples were collected from these vac-
cinated animals and appropriate control animals
at 0, 30, 60, 120, 180, 240, 300, 365, 455 and
545 DPV. Serums were assayed for FMDV O, A
and C antibodies at PIADC by a variety of sero-
logical techniques, including radial immunodiffu-
sion (4), microneutralization (14), plaque reduc-
tion (3), mouse PD₅₀ (6) and serum protection test in
suckling mice (5).

The bioassay values against FMDV A for the 5
assay techniques (9), in conjunction with the re-
results of immunity, were analyzed by stepwise lin-
ear discriminant analysis. With analysis, the assay
techniques that most effectively discriminated
between protected and unprotected animals were
the mouse protection and the PD₅₀ test in suck-
lings mice.
Challenge of immunity

The steers in the potency study conducted in Argentina (initial, part I) were transported by truck from the Valdés Peninsula to Buenos Aires, divided into 3 groups and kept in isolation barns designated for the appropriate virus type (O, A or C) to be used in the challenge of immunity. Each group, including the control group, consisted of 9 randomly selected animals from the different vaccine groups. The challenge virus consisted of bovine tongue epithelium passaged in the bovine within 60 days, and recently titrated in mice. The dose of challenge virus consisted of 10,000 mouse LD_{50} contained in a 1-ml volume and inoculated at 4 sites in the tongue intradermally, 0.25 ml/site. At 48 hours postchallenge, the control animals from each group were examined to assure that the challenge dose of virus was effective, and tissue was collected for complement-fixation (CF) typing to ascertain that the infection was caused by the correct challenge virus. All animals were examined at 5 and 10 days postchallenge, and final results were summarized.

In the major study (II), each vaccinated group of steers was divided into 3 groups of 16 animals each by random number selection on a computer. Four spare animals in each group of 52 constituted replacements for animals which died or were injured. The immunity of all groups was challenged by exposure to FMDV A as follows: one group at 6 months, one group at 12 months, and one group revaccinated at 6 months and challenged 12 months later.

RESULTS

Safety test

All antigens and vaccine samples from the initial study (I) and the major study (II) proved innocuous to the inoculated steers.

Potency tests

The results of the challenge of immunity obtained in study I are presented in Table 1.

In study II, 1 of 4 steers inoculated with Vaccine 1 developed one secondary lesion on the gum; the steers inoculated with Vaccines 2 and 3 were resistant to infection.

Serology

The antibody assays of the steer serums (part I) were measured by a seroneutralization test in tissue culture and recorded as neutralization indices (Table 2).

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**TABLE 1. Initial study results, portion of animals protected from challenge, infected with O, A and C FMDV at 30 days postvaccination**

<table>
<thead>
<tr>
<th>Vaccines</th>
<th>Challenge FMDV type</th>
<th>Frenkel, Al(OH)_{3} &amp; asponin</th>
<th>Frenkel, INTA oil</th>
<th>BHK, PIADC oil</th>
<th>BHK, INTA oil</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O</td>
<td>9/9*</td>
<td>1/9</td>
<td>6/9</td>
<td>3/8</td>
<td>0/9</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>9/9</td>
<td>3/9</td>
<td>5/9</td>
<td>5/9</td>
<td>0/9</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>9/9</td>
<td>7/9</td>
<td>8/9</td>
<td>9/9</td>
<td>1/9</td>
</tr>
</tbody>
</table>

*Code: Numerator, number of animals protected; denominator, number of animals challenged.
TABLE 2. Neutralizing indices of bovine serums from study I against A₂₄, A₂₄-₂₅ and A₂₅ FMDV

<table>
<thead>
<tr>
<th>Vaccines</th>
<th>Index with FMDV*</th>
<th>A₂₄</th>
<th>A₂₄-₂₅</th>
<th>A₂₅</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frenkel, Al (OH)₃ and saponin</td>
<td>3.32</td>
<td>2.02</td>
<td>1.73</td>
<td></td>
</tr>
<tr>
<td>Frenkel, INTA oil</td>
<td>1.01</td>
<td>1.78</td>
<td>1.27</td>
<td></td>
</tr>
<tr>
<td>BHK, PIADC oil</td>
<td>3.86</td>
<td>0.62</td>
<td>0.73</td>
<td></td>
</tr>
<tr>
<td>BHK, INTA oil</td>
<td>4.04</td>
<td>1.42</td>
<td>0.45</td>
<td></td>
</tr>
</tbody>
</table>

*ₐ₋₄ = virus grown in BHK cells; A₂₄-₂₅ = viruses grown in Frenkel cultures.

The titer of the serums against A by the 5 techniques (part II), in conjunction with the results of the challenge of immunity, were analyzed by discriminant analysis.

The results of the analysis of the data for the 3 challenge-of-immunity groups at 180, 365 and 545 DPV indicate that the mouse protection test was the most effective discriminator between the protected and unprotected animals.

With the same data, which were tested by the 5 serological techniques but including an additional 34 animals that were not assayed by radioimmunodiffusion, the PD₅₀ test in suckling mice appeared to be the most effective discriminator.

Immunity

The results of the challenge of immunity of the animals (part II) are shown in Table 3.

In general, although all groups had considerable protection when their immunity was challenged with FMDV A, there was little difference between vaccines, time of exposure and results of revaccination.

The results of the challenge data were examined by Chi-square techniques in comparing vaccines, duration of immunity and possible differential behavior of the vaccines over time or the holding periods under different vaccines. No Chi-square value approached significance. Immunity was the same at 12 months as at 6 months and was the same after revaccination at 6 months and holding for an additional 12 months as at 12 months. The response to the vaccines over time did not change nor did the immunity at different times differ among vaccines. The vaccines protected against FMDV in about three-fourths of vaccinated steers for at least 1 year.

DISCUSSION

In study I, the best results were obtained with vaccine produced from Frenkel cultures and combined with aluminum hydroxide and saponin adjuvant (Tables 1 and 2). All the steers vaccinated with this vaccine were protected when their immunity was challenged at 30 DPV. Under the conditions of the study, these animals were completely protected against FMDV, types O, A and C.

The vaccines prepared from antigens grown in BHK cultures, inactivated with AEI and emulsified with oil adjuvants (PIADC and INTA formulations) protected 67% and 63% respectively, of the test animals.

The vaccine prepared from the antigens grown in Frenkel cultures, inactivated with formaldehyde and emulsified with oil (INTA) protected only 42% of the steers and produced the poorest response of the 4 vaccines.

TABLE 3. Results of challenge of immunity with FMDV A

<table>
<thead>
<tr>
<th>Vaccine No.</th>
<th>Days postvaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>180</td>
</tr>
<tr>
<td>1</td>
<td>13/16</td>
</tr>
<tr>
<td>2 (1X)</td>
<td>12/16</td>
</tr>
<tr>
<td>3 (20X)</td>
<td>15/16</td>
</tr>
<tr>
<td>Total</td>
<td>40/48</td>
</tr>
</tbody>
</table>

a Vaccine No. 1, virus grown in BHK cells (not concentrated); vaccine No. 2, virus grown in BHK cells, concentrated 100-fold and reconstituted to contain the same antigen mass as No. 1; vaccine No. 3, virus as in No. 2, but adjusted to contain 20-fold the antigenic mass of No. 1 and No. 2.

b Revaccinated at 180 days after initial vaccination.

c Code: Numerator, number of animals protected; denominator, number of animals challenged.
Before the challenge of immunity of study I, CF tests at PAFMDC and at INTA indicated that the FMDV used for the A antigen in the Frenkel vaccines included 2 strains of A virus—A24 and A25. The information from PAFMDC indicated that the A antigen in both BHK vaccines was A24 and did not include A25 as in the vaccines made from the Frenkel antigens. The average neutralization indices for the 4 vaccines obtained with each of the 3 virus types are given in Table 2. With type A24 virus, the average protection index of the 2 BHK vaccines (which were uniformly high and very similar) was significantly higher than that of either of the 2 Frenkel vaccines.

The actual effect of the challenge with FMDV A24 that contained A25 is rather difficult to determine. However, Graves et al. (7) reported that immunity is highly dependent on the relatedness of the subtype of the virus used for the exposure to that used to prepare the vaccine. Possibly, therefore, the poorer protection afforded by the BHK antigens, which contained only FMDV A24, was related to the challenge virus which consisted of FMDV A24-25.

In study II, reactions obtained at the sites of vaccination, particularly when the animals were revaccinated, were severe although they disappeared within 30 days. The immunity of the steers was challenged with A virus; however, from the antibody measurements as assayed by a number of different methods, one could assume that the test was reasonably successful for 2 of the 3 virus types tested—A and C. The antibody response obtained with the O virus with the exception of the potency test was disappointing (9). One could speculate that this virus is more sensitive and fragile than types A and C and thus did not remain as stable in the emulsified vaccine.

The results of the challenge of immunity at 6 months after vaccination, 12 months after vaccination, and 12 months after revaccination (at 6 months) were only slightly different, indicating that further studies to determine the optimal time of revaccination with such products are needed.

The response obtained with the 3 vaccines (part II) was very similar. The 20-fold concentrated vaccine did not produce a greater antibody response nor any greater degree of protection than either the crude or the 1X vaccine.

ACKNOWLEDGMENTS

The technical assistance of the staff at both INTA and PIADC is acknowledged. We also thank E. Fred Schultz, Jr., Statistician, Program Analysis and Coordination Staff; and John G. Phillips, Consulting Statistician, Federal Research, Science and Education Administration, USDA, for their analysis of the serological and immunological data.

REFERENCES

8. HENDERSON, W.M. Significance of tests for non-
infectivity of foot-and-mouth disease vaccines. J.
9. INSTITUTO NACIONAL DE TECNOLOGIA
AGROPECUARIA; PLUM ISLAND ANIMAL
DISEASE CENTER. Foot-and-mouth disease: A
vaccine study. Int. Symp. on Foot-and-Mouth Dis-
133, 1977.
10. McKERCHER, P.D.; GIORDANO, A.R. Immune
response of steers inoculated with chemically-treated
foot-and-mouth disease virus preparations previously
studied in swine. Arch. ges. Virusforsch. 20: 190-
197, 1967.
11. PLUM ISLAND ANIMAL DISEASE CENTER; PAN
AMERICAN FOOT-AND-MOUTH DISEASE
CENTER. Foot-and-mouth disease vaccines. II.
Studies on the duration of immunity in cattle and
pigs. Blnn Centro Panamericano Fiebre Aftosa 19-20:
24-30, 1975.
12. RIVENSON, S.; IBARRA, O.; GAGGINO, O.P.;
LAPORTE, O.; GARCIA OLANO, H.; PIZZI, J.C.;
MARANGUNICH, L. Oily vaccines in immunity to
foot-and-mouth disease. Presented at the World
Meat Congress held in Buenos Aires, Argentina,
August 3-6, 1976.
13. WAGNER, G.G.; CARD, J.L.; COWAN, K.M.
Imunochemical studies of foot-and-mouth dis-
ease. VII. Characterization of foot-and-mouth dis-
ease virus concentrated by polyethylene glycol
precipitation. Arch. ges. Virusforsch. 30: 343-352,
1970.
14. WAGNER, G.G.; McVICAR, J.W. Foot-and-mouth
disease virus antibodies: Comparison of a tissue cul-
ture microneutralization test with the assay in suck-