

IMMUNE RESPONSE INDUCED BY OIL-ADJUVANTED FOOT-AND-MOUTH DISEASE VACCINE IN CATTLE IN TROPICAL AREAS OF COLOMBIA¹

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SUMMARY

The immune response induced by oil-adjuvanted foot-and-mouth disease vaccine was evaluated by assaying the titers of neutralizing antibodies at 2, 4, 6, 7, 9, 12, 15 and 18 months after a first and second dose of vaccine was administered to groups of cows, heifers and calves under varying management conditions and having a history of periodic vaccination with saponin-hydroxide vaccine.

6000 doses of oil-adjuvanted vaccine prepared by the Pan American Foot-and-Mouth Disease Center (Rio de Janeiro, Brazil) with virus strains A₂₇-Cundinamarca/75-8046 and O₁-Campos/Brazil, were applied. Four cattle ranches in different tropical regions were selected for the application of 3000 initial doses followed by a second inoculation 6 and 12 months later. 430 blood samples were taken at each stage of blood sample collection.

The cows and heifers showed high antibody titers at 2 months after the first vaccination when challenged by O and A virus types. Titers were observed to decline at 4 and 6 months, with a difference not greater than 1 log in relation to the titers initially obtained at two months. Likewise, this difference did not exceed 1.5 log in relation to the final value obtained at 12 months. Higher and more persistent titers were obtained during the 12 months following application of the second dose of oil-adjuvanted vaccine.

The results obtained suggest a minimum of three vaccinations at 6-month intervals, followed by annual vaccination of cattle younger than 18 months. The second dose for cattle aged 18 to 36 months should be given at 6 months, followed by yearly vaccination thereafter. The adult cattle population having a history of periodic immunization every 4 months with saponin-hydroxide vaccine could be given one dose yearly.

INTRODUCTION

Cooperative studies to assess the immunity induced by oil-adjuvanted foot-and-mouth disease (FMD) vaccine have been conducted by the Pan American Foot-and-Mouth Disease Center (PAFMDC) in Brazil, the National Institute of Agricultural Technology (INTA) in Argentina, and the Plum Island Animal Disease Center (PIADC) in the U.S.A. (5, 9). The inoculation of oil-adjuvanted vaccine in cattle and pigs yielded higher neutralizing titers for longer durations, in comparison to the titers induced by conventional aluminum-hydroxide vaccines (10).

The PAFMDC has been engaged in both laboratory and field research of various aspects related to the production and immunological effect of oil-adjuvanted FMD vaccine. The research has considered, among others, cattle and pig populations, type of cattle-raising and management techniques, breeds, ages, routes of application, virus strains, potency and efficacy tests, etc. Promising results have been obtained for the protection of swine and cattle populations under conditions in South America (5).

Preliminary studies to evaluate oil-adjuvanted vaccines were conducted at the Zooprophyllactic Institute in Colombia (11). A vaccine prepared at the PAFMDC with virus strains from Colombia was given to a group of 1000 cattle on a ranch located in the area (Urabá-Antioqueño) covered

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by the joint program of the Colombian Agriculture and Livestock Institute (ICA) and the U.S. Department of Agriculture (USDA). The immune response determined by evaluating neutralizing antibody titers at 4, 6, 8 and 12 months post-vaccination (MPV) indicated that a uniform and lasting immunity can be induced with one or two annual vaccinations of oil-adjuvanted vaccine (6).

This study aimed to determine the response at varying MPV's, in terms of neutralizing antibodies titers induced by FMD oil-adjuvanted vaccine in groups of young and adult cattle. The cattle were observed at three different locations in Colombia, and had a history of systematic vaccination every four months with domestically produced saponin-hydroxide vaccine.

MATERIALS AND METHODS

Location

The study was conducted on four ranches located in different cattle-raising regions of Colombia. Their geographic location is as follows: "Bejuquillo" in the municipality of Mutatá and "La Patria" in the department of Antioquia, at an altitude of 20 to 30 meters above sea level; the "La Romelia" ranch situated in the municipality of Chinchiná in the department of Caldas, at 1500 to 2000 meters above sea level, and the "Carimagua" National Research Center in the department of Meta, some 150 to 175 meters above sea level.

Vaccine

The 6000 bovine doses of oil-adjuvanted vaccine (Series OL-135) were prepared by the Pan American Foot-and-Mouth Disease Center from the A₂₇-Cundinamarca/75-8046 (Colombia) and O₁-Campos (Brazil) virus strains. The results of the guinea pig potency tests yielded 64 and 51 guinea pig 50% protective doses (GPPD₅₀) per 0.25 ml of vaccine challenged by the homologous O and A viruses, respectively.

Vaccination and blood samples

On the "Bejuquillo" and "La Patria" ranches 2100 doses were administered in the first vaccination to groups of 840 and 1260 pure and

crossbred zebu cattle, under and over 2 years old, respectively.

The same groups of cattle were given a second vaccination six months later. The initial 6-month period of immunization was evaluated by taking blood samples from 132 cattle under two years and from 90 cattle over 2 years, at 0, 2, 4 and 6 MPV. The evaluation 12 months after the second vaccination was conducted using the same groups of cattle with blood samples taken at 1 and 6 months. Due to the exit of cattle from the region, the full 12-month scheduled evaluation was not conducted on these two ranches.

The first vaccination at the "Carimagua" National Research Center administered 300 doses to crossbred zebu cattle --85 calves, 114 heifers and 101 cows. At six months the second vaccination was given to 50% of the cattle (57 heifers and 50 cows). The rest of the animals received their second vaccination 12 months after the first; the group of 85 calves was revaccinated twice at 6-month intervals.

The blood samples were taken from 41 calves, 61 heifers and 56 cows at 0, 2, 4, and 6 months after the first vaccination. In order to assess an initial 12-month period of immunization of these groups of cattle, blood samples were taken at 9 and 12 months from 29 heifers and 28 cows that were not revaccinated at 6 months. Blood samples were drawn from those same groups of cattle at 1, 3 and 6 months after the second vaccination, which was administered 12 months after the first. Blood samples were taken at 1, 3, 6, 9 and 12 months from the groups of cattle that were revaccinated at 6 months --32 heifers and 28 cows-- for an annual evaluation. Blood samples were collected at 2, 4 and 6 months from the 41 calves during the three 6-month periods following the vaccinations.

The first vaccination of 300 doses on the "La Romelia" ranch was given to groups of 160 cows, 60 heifers and 80 calves of the Holstein and Brown Swiss breeds.

All the groups mentioned were given the second vaccination six months later. Blood samples were collected from 15 cows and 15 heifers at 2, 4 and 6 months after the first vaccination, and at 1, 3, 6 and 12 months after the second vaccination.

Blood samples were taken from a group of 20 calves 2, 4 and 6 months after the first vaccination, 1, 3 and 6 months after the second and 6 months after the third.

Neutralizing antibodies

The neutralizing antibodies titers were determined by the microneutralization test with BHK₂₁ cells (1) challenged by two-fold dilutions of the sera and 10^{1.7} 50% tissue culture infecting doses (TCID₅₀) of the viruses homologous to those used to prepare the vaccine. The Spearman-Kärber method was utilized to obtain the sera neutralizing titer (12).

RESULTS

The results obtained during the initial 6-month evaluation period following the first inoculation on the "Bejuquillo" and "La Patria" ranches where blood samples were collected at 0, 2, 4 and 6 months, indicated high antibody titers against A and O virus for both age groups when the blood samples taken at 2 MPV. However, the titers declined steadily at 4 and 6 MPV, and the final average approached log 2. During the six months following the second vaccination, at the month the immune response averaged higher, with a less pronounced drop in the titers over the six months, in comparison with the results observed after the first vaccination in the two age groups, against the two virus types (Table and Figure 1).

The study conducted at "Carimagua" indicates, for the groups of cows and heifers, that the titers of the neutralizing antibodies during the six-month period after the first vaccination were considerably high. They averaged above log 3 and showed a less evident declining trend against both types of virus. The groups of cows and heifers studied during the 12 months following the first vaccination showed at two months an average response above log 3. The decline was steady and the final average value at 12 months exceeded log 2. Those same groups yielded higher and more stable titers during the 6 and 12 months following the second vaccination. During the first period after the first vaccination, the group of calves yielded

neutralizing antibody titers tending to decline more sharply; the final values were substantially below log 2. However greater response and persistence in the titers were observed after the application of two more vaccinations every six months (Tables and Figures 2 and 3).

The results for the groups of cows, heifers and calves on the "La Romelia" ranch were similar in intensity and persistence to the titers observed in

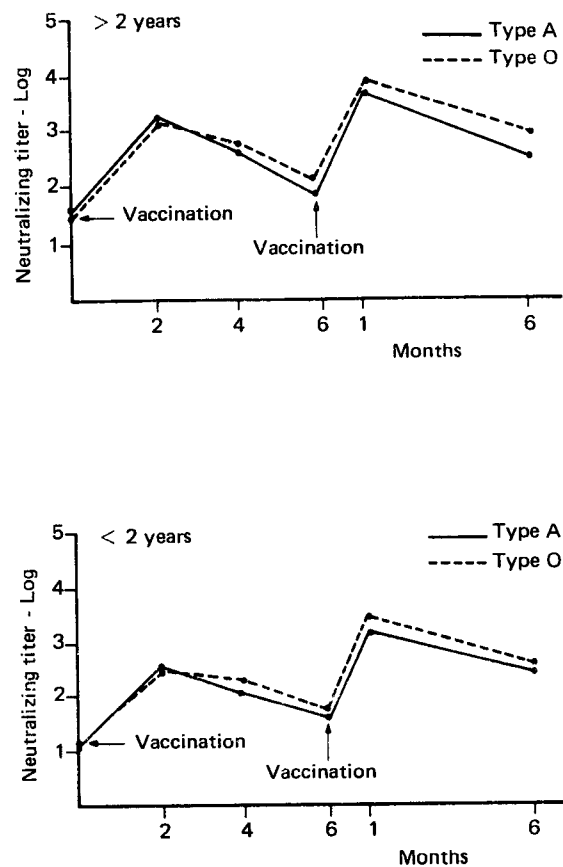


FIGURE 1. Mean titers of neutralizing antibodies against FMD virus types A and O, induced by oil-adjuvanted vaccine in cattle from the "La Patria" and "Bejuquillo" ranches (Antioquia), Colombia, 1982.

TABLE 1. Mean titers of neutralizing antibodies against FMD virus types A and O, induced by oil-adjuvanted vaccine in cattle from the "La Patria" and "Bejuquillo" ranches (Antioquia), Colombia, 1982.

Age (years)	Number of blood samples	Months post vaccination							
		0	2	4	6	1	6		
Virus A ₂₇ -Cundinamarca/75-8046									
>2	90	1.64 ± 0.54	V ± 0.27	3.20 ± 0.20	2.55 ± 0.20	1.85 ± 0.67	V ± 0.28	3.58 ± 0.28	2.45 ± 0.64
<2	132	1.04 ± 0.27	V ± 0.51	2.61 ± 0.37	2.01 ± 0.37	1.68 ± 0.61	V ± 0.35	3.10 ± 0.35	2.45 ± 0.48
Virus O ₁ -Campos/Brazil									
>2	90	1.56 ± 0.56	V ± 0.47	3.15 ± 0.18	2.85 ± 0.18	2.10 ± 0.35	V ± 0.30	3.72 ± 0.30	2.91 ± 0.59
<2	132	1.07 ± 0.28	V ± 0.41	2.59 ± 0.39	2.34 ± 0.39	1.73 ± 0.48	V ± 0.28	3.45 ± 0.28	2.75 ± 0.60

V = Vaccination.

TABLE 2. Mean titers of neutralizing antibodies against FMD virus types A and O, induced by oil-adjuvanted vaccine in cattle from the Carimagua National Research Center (Meta), Colombia, 1982.

Cattle	Number of blood samples	Months post-vaccination											
		0	2	4	6	9	12	1	3	6			
Virus A ₂₇ -Cundinamarca/75-8046													
Cows	28	1.92 ± 0.45	V ± 0.38	3.36 ± 0.57	2.99 ± 0.56	2.56 ± 0.34	2.21 ± 0.36	2.00 ± 0.36	V ± 0.20	3.64 ± 0.23	3.54 ± 0.23	3.20 ± 0.45	
Heifers	29	1.54 ± 0.41	V ± 0.39	3.12 ± 0.39	2.31 ± 0.47	2.31 ± 0.47	1.92 ± 0.30	V ± 0.30	3.32 ± 0.32	3.32 ± 0.32	3.32 ± 0.32	3.28 ± 0.34	
		0	2	4	6	9	12	1	3	6			
Cows	41	1.06 ± 0.23	V ± 0.40	2.67 ± 0.30	2.18 ± 0.30	1.67 ± 0.22	V ± 0.38	3.24 ± 0.37	2.61 ± 0.37	2.21 ± 0.30	V ± 0.30	3.19 ± 0.41	2.75 ± 0.25
		0	2	4	6	9	12	1	3	6			
Virus O ₁ -Campos/Brazil													
Cows	28	1.96 ± 0.31	V ± 0.31	3.55 ± 0.37	3.20 ± 0.37	2.96 ± 0.46	2.28 ± 0.27	2.05 ± 0.21	V ± 0.21	3.98 ± 0.10	3.66 ± 0.12	3.68 ± 0.12	
Heifers	29	1.39 ± 0.51	V ± 0.40	3.15 ± 0.40	2.82 ± 0.58	2.82 ± 0.58	2.11 ± 0.55	V ± 0.55	3.60 ± 0.23	3.60 ± 0.23	3.60 ± 0.23	3.46 ± 0.25	
		0	2	4	6	9	12	1	3	6			
Calves	41	0.77 ± 0.48	V ± 0.49	2.70 ± 0.27	2.35 ± 0.27	2.00 ± 0.32	V ± 0.32	3.28 ± 0.34	3.01 ± 0.22	2.38 ± 0.30	V ± 0.30	3.60 ± 0.22	2.83 ± 0.28

V = Vaccination.

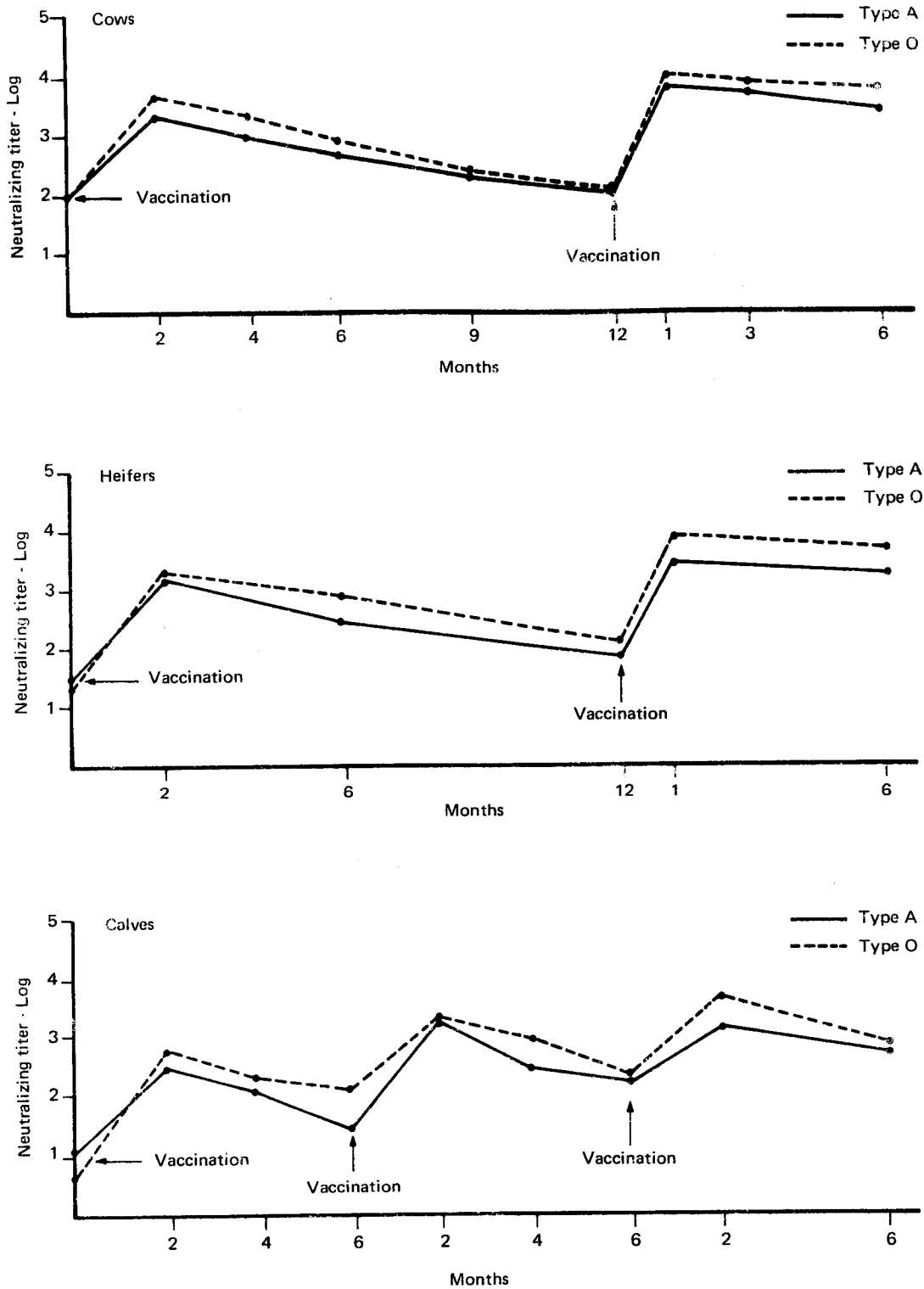


FIGURE 2. Mean titers of neutralizing antibodies against FMD virus types A and O, induced with oil-adjuvanted vaccine in cattle from the "Carimagua" National Research Center (Meta), Colombia, 1982.

TABLE 3. Mean titers of neutralizing antibodies against FMD virus types A and O, induced by oil-adjuvanted vaccine in cattle from the Carimagua National Research Center (Meta), Colombia, 1982.

Cattle	Number of blood samples	Months post-vaccination									
		0	2	4	6	1	3	6	9	12	
Virus A₂₇-Cundinamarca/75-8046											
Cows	28	1.76 ± 0.54	V 3.19 ± 0.49	3.12 ± 0.49	2.12 ± 0.67	V 3.70 ± 0.16	3.53 ± 0.26	3.45 ± 0.33	2.81 ± 0.35	2.70 ± 0.33	
Heifers	32	1.45 ± 0.50	V 3.23 ± 0.34		2.20 ± 0.39	V 3.50 ± 0.25		3.38 ± 0.21		2.78 ± 0.21	
Virus O₁-Campos/Brazil											
Cows	28	1.48 ± 0.48	V 3.29 ± 0.36	3.07 ± 0.43	2.87 ± 0.50	V 3.68 ± 0.20	3.43 ± 0.25	3.49 ± 0.25	3.07 ± 0.31	2.54 ± 0.31	
Heifers	32	1.21 ± 0.58	V 3.09 ± 0.35		2.69 ± 0.51	V 3.64 ± 0.18		3.37 ± 0.34		3.32 ± 0.51	

V = Vaccination.

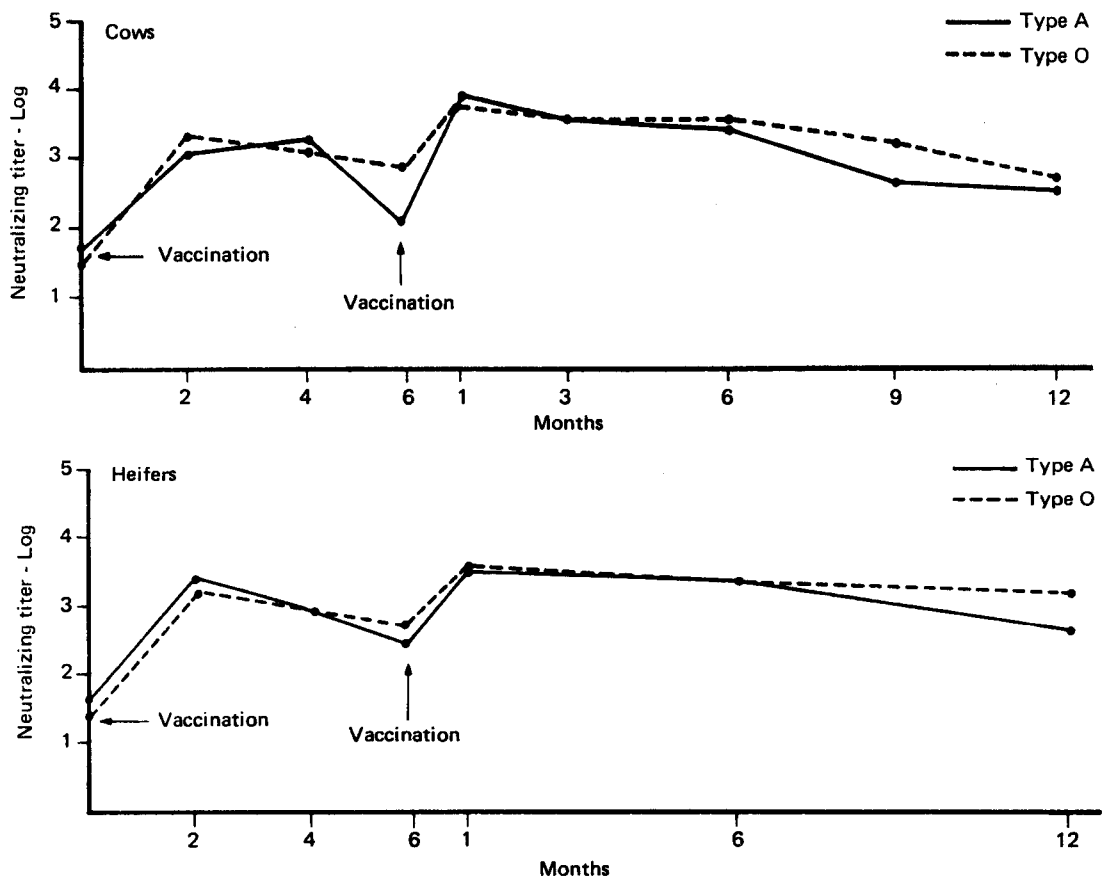


FIGURE 3. Mean titers of neutralizing antibodies against FMD virus types A and O, induced with oil-adjuvanted vaccine in cattle from the "Carimagua" National Research Center (Meta), Colombia, 1982.

the cattle at "Carimagua". The second inoculation of oil-adjuvanted vaccine given to the three groups 6 months after the first vaccination induced higher and more persistent titers when tested against the two virus types. An average higher than log 2 was observed at 12 months. Revaccination of the calves every 6 months induced an increase in the titers which yielded greater and greater persistence (Table and Figure 4).

None of the groups or breeds of cattle evidenced undesirable reactions to the oil-adjuvanted vaccines.

DISCUSSION

The results of neutralizing antibody titers induced by an oil-adjuvanted vaccine administered to groups of cattle of different ages, which had

been vaccinated every four months with saponin-hydroxide vaccine, once again demonstrated the possibility of obtaining prolonged periods of immunity characterized by the presence of rather uniform antibody titers. Estupiñán *et al.* (6) obtained a similar immune response when they determined the neutralizing antibody titers for two groups of cattle under and over two years of age that had been inoculated with oil-adjuvanted vaccine. The response was evaluated at 4, 6, 8 and 12 MPV and yielded antibody levels higher than those obtained in other studies aiming to evaluate aluminum hydroxide vaccines.

The results recorded in groups of heifers and calves offer a very promising condition for obtaining a solid immunity. However, booster vaccinations are required every six months in order to maintain the immune level, which tends to

TABLE 4. Mean titers of neutralizing antibodies against FMD virus types A and O, induced with oil-adjuvanted vaccine in cattle from the "La Romelia" ranch (Caldas), Colombia, 1982.

Cattle	Number of blood samples	Months post-vaccination							
		0	2	4	6	1	3	6	12
Virus A ₂₇ -Cundinamarca/75-8046									
Cows	15	1.94 ±0.55	3.30 ±0.39	2.54 ±0.38	2.24 ±0.25	3.44 ±0.15	2.99 ±0.30	2.96 ±0.47	2.62 ±0.38
Heifers	15	1.57 ±0.16	2.95 ±0.34	2.57 ±0.39	2.33 ±0.40	3.25 ±0.40	3.03 ±0.33	2.79 ±0.33	2.42 ±0.39
Calves	20	0.74 ±0.15	2.58 ±0.41	2.13 ±0.44	1.76 ±0.24	3.43 ±0.28	2.78 ±0.23	2.10 ±0.38	3.21 ±0.32
Virus O ₁ -Campos/Brazil									
Cows	15	1.44 ±0.31	3.29 ±0.32	2.90 ±0.24	2.66 ±0.49	3.65 ±0.17	3.08 ±0.24	3.20 ±0.32	2.83 ±0.42
Heifers	15	1.21 ±0.22	3.21 ±0.15	3.16 ±0.11	2.80 ±0.26	3.69 ±0.15	3.12 ±0.21	3.46 ±0.28	2.89 ±0.37
Calves	20	0.72 ±0.18	2.75 ±0.25	2.24 ±0.32	1.85 ±0.22	3.41 ±0.22	3.21 ±0.31	2.25 ±0.30	3.44 ±0.23

V = Vaccination.

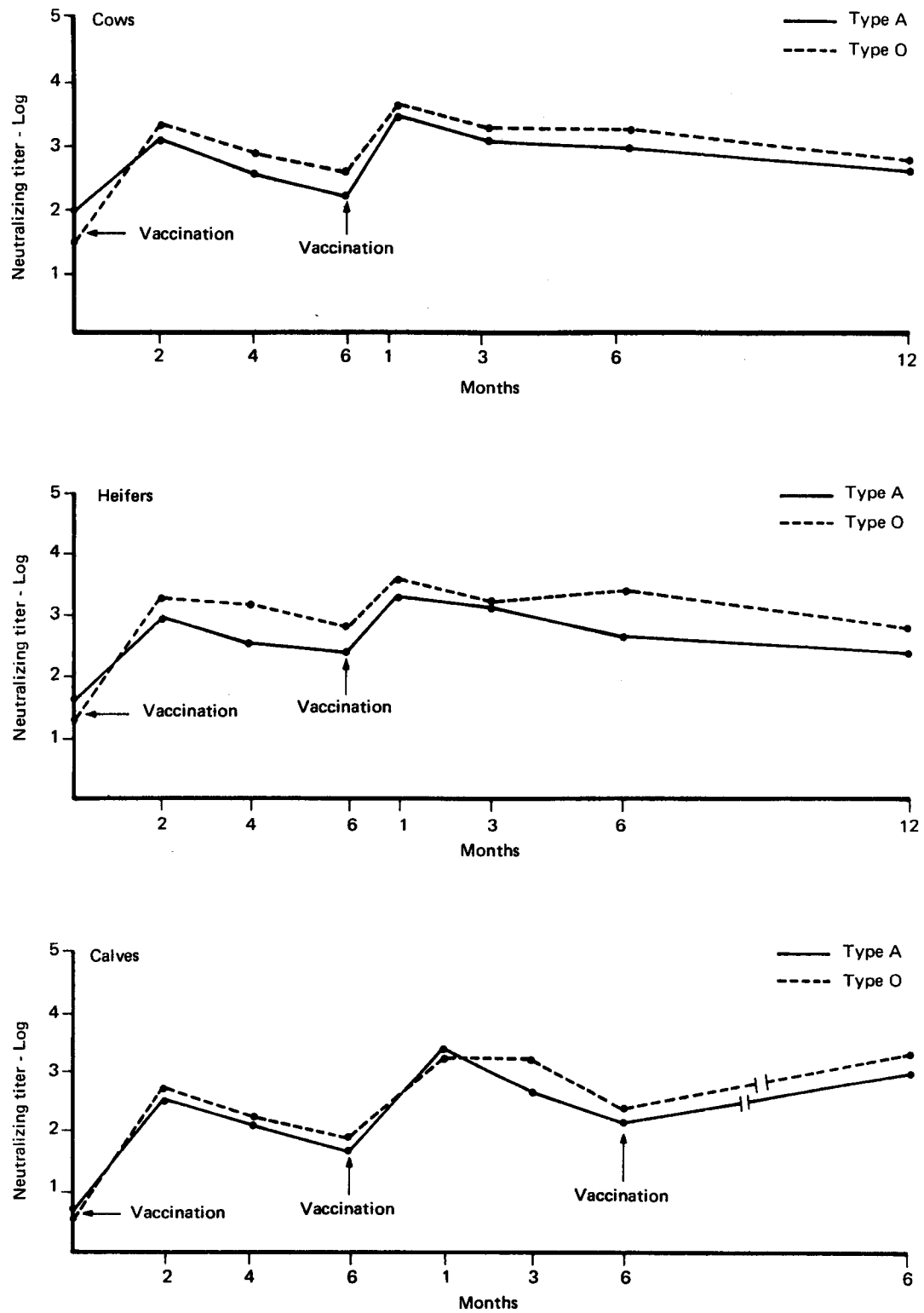


FIGURE 4. Mean titers of neutralizing antibodies against FMD virus types A and O, induced with oil-adjuvanted vaccine in cattle from the "Romelia" ranch (Caldas), Colombia, 1982.

decline considerably in young cattle. A study by Honigman *et al.* (8) of postvaccination immunity against FMD virus in calves, using saponin-hydroxide vaccine, demonstrated that 6 to 9-month-old cattle (at the outset of the experiment) vaccinated 3 times at 4-month intervals, yielded an immune response 50% lower than that of cattle vaccinated 5 times at 2-month intervals. Studies by Augé de Mello *et al.* (3) demonstrated that when groups of young cattle in the field were submitted to vaccination and revaccination with oil-adjuvanted and aluminum-hydroxide inactivated FMD vaccine, the animals inoculated with the aluminum-hydroxide vaccine yielded an expected percentage of protection (EPP) ranging from 60 to 70% at 30 days post-vaccination (DPV). That value decreased to 50% at 60 days post-vaccination and, at 90 DPV, was the same as the control animals. But with the oil-adjuvanted vaccine the EPP at 30 DPV was higher than 70% and continued rising to 60 days after the vaccination, when it reached 80 to 90%. The EPP at 6 months was still substantial, not less than 54%. The expected percentages of protection induced with the oil-adjuvanted vaccine were high and persisted for a long time in comparison to the aluminum-hydroxide vaccine.

Observations drawn from the present study indicate that adult cattle populations having a history of systematic vaccination with saponin-hydroxide vaccine show satisfactory immunity when they receive at least two doses of oil-adjuvanted vaccine every 6 months. The time span between vaccinations can even be lengthened up to a year. The antibody titers observed in the groups of cows, heifers and calves on the four selected facilities were similar in the degree of initial response as well as in the persistence of the titers against O and A virus types. In general, the titers tended to decline during the 12-month period following the first vaccination. The difference between the values at 2 months and at 6 MPV did not exceed 1 log. Likewise, that difference did not exceed 1.5 log in relation to the final value obtained at 12 MPV. Higher and more persistent titers were observed during the 6 and 12 months following the second application of oil-adjuvanted vaccine.

A study involving cattle vaccinated three times with oil-adjuvanted vaccine at 6-month intervals confirmed the presence of a solid immunity 12 months after immunization with FMD virus. The disease generalized in only one of the 30 cattle. The high antibody levels observed in this study are comparable to those observed by Gomes *et al.* (7), who demonstrated protection against exposure to virus by the intra-dermolingual route and by contact with diseased cattle and pigs. A similar study by Augé de Mello *et al.* (2), involving 2-year-old cattle that had been vaccinated three times at 6-months intervals with inactivated oil-adjuvanted vaccine, produced a very satisfactory immune response; 12 months after the fourth vaccination the EPP was over 80%. Similar protection studies were conducted on cattle at 1, 3, 6 and 12 MPV, using oil-adjuvanted vaccine prepared from O, A and C virus types. Satisfactory immunity results were obtained at the different times against O and A virus types (4, 9).

Similar to the findings by Augé de Mello *et al.* (2), the results obtained in this study suggest the following initial immunization scheme with oil-adjuvanted FMD vaccine: a vaccination every 12 months for adult cattle populations where a high number of animals in the region have a history of periodic vaccination every 4 months with saponin-hydroxide vaccine. Otherwise a second vaccination is recommended 6 months later and thereafter every 12 months. In the case of cattle aged from 18 to 36 months, regardless of their immunization history, they should receive the second vaccination at 6 MPV and then every 12 months. Cattle under 18 months should be vaccinated at least three times every 6 months and every 12 months thereafter.

It is important to stress that the quality of the antigens utilized and the high percentage of vaccination coverage are fundamental to the success of a regional foot-and-mouth disease control campaign.

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