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Response to foot-and-mouth disease vaccines in newborn calves. Influence of age, colostral antibodies and adjuvants*

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SUMMARY

Oil-emulsified (OE) and aqueous (Aq) vaccines were prepared with the same batch of inactivated A_{24} 8345 foot and mouth disease virus (FMDV). Calves born to vaccinated dams did not respond to the Aq vaccine 30 or 90 days post partum. When the OE vaccine was used on a similar group of calves, no responses were elicited up to 21 days post partum. However, calves 30 or more days old responded like adult cattle to the OE vaccine. When the OE vaccine was used in colostral antibody-free calves 3–30 days old, all animals showed good antibody responses but, in calves vaccinated 3 or 7 days post partum, antibodies were detectable only after a considerable period of time. Our results show that both passively acquired colostral antibodies and age are important in the response of very young calves to FMDV oil vaccines. From a practical point of view, in endemic areas where adult cattle are periodically vaccinated, vaccination of calves between 30 and 60 days post partum with OE vaccines would lead to high levels of herd protection.

INTRODUCTION

Foot and mouth disease virus (FMDV) is the aetiological agent of an acute febrile disease that causes enormous economic losses in many countries of the world. In endemic areas inactivated aqueous (Aq) vaccines with aluminium hydroxide and saponin adjuvants are often used but these vaccines induce protection for very short periods only, and repeated vaccinations at 4-month intervals are necessary (INTA & PIADC, 1977; Rivenson *et al.* 1982). One of the

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principal problems in mass immunization against FMDV is inducing protection of young calves, since it has been shown that newborn calves with maternal antibodies give very poor or no response to aqueous (Aq) FMDV vaccines (Nicholls *et al.* 1984), and that epidemic waves start in many countries with infection of these unprotected young calves (COSALFA, 1981).

The lack of response of newborn calves may be due to interference by passively acquired colostral antibodies (CAb) (Brun *et al.* 1974; Graves, 1963; Nicholls *et al.* 1984). Several workers have studied the immune response of young calves against FMDV and have shown that, using Aq-FMDV vaccines on the offspring from immunized dams, the latter not only fail to respond but their serum antibody titres drop after vaccination. Hence vaccination with Aq-FMDV has no positive effect on protection up to 5 months of age (Graves, 1963; Nicholls *et al.* 1984). Although Nicholls *et al.* (1985) have shown that, in the absence of CAb 1-week-old calves responded to FMDV vaccination as well as adult cattle, preliminary experiments in our Institute (Sadir, A. M. *et al.* unpublished results) showed very poor responses ($\log_{10}$ neutralizing titres $\leqslant 1{\cdot}67$) to a commercial FMDV trivalent Aq vaccine in calves of less than 30 days of age, even if they were born to non-vaccinated dams. Recently, oil-emulsion (OE) vaccines have been introduced and it has been demonstrated, both in laboratory and in controlled field conditions, that they confer a much longer lasting protection in cattle (INTA & PIADC, 1977; Rivenson *et al.* 1982). Furthermore, it has been shown that, in other species, vaccination of newborn animals with OE-FMDV vaccines induces immune responses even in the presence of colostral antibody (CAb) (Cunliffe & Graves, 1970; Morgan & McKercher, 1979), although some contradictory evidence has recently appeared (Francis & Black, 1986).

The aim of our work was to study the immune response of new-born calves, born to vaccinated and nonvaccinated dams, against FMDV vaccines in order to determine the best immunization programme in endemic areas. We here show that, although both age and the presence of CAb are important in the response to FMDV vaccine, when using OE vaccines on calves 30 or more days old, very good responses are obtained, even in the presence of CAb.

MATERIALS AND METHODS

Animals

Twenty-six calves of the Holando-Argentino breed, aged 1-90 days, born from dams vaccinated and nonvaccinated against FMDV were used. Offspring from nonvaccinated dams were born and kept during the whole experiment in a FMDV-free area (Chubut, Argentina).

Calves born to immunized cows were provided by the INTA-Castelar dairy farm. These dams had been revaccinated approximately 70 days before parturition with the OE-FMDV vaccine and had high anti-FMDV antibody titres (Sadir *et al.* 1984). Virus infection associated antibody (VIA) tests were negative in calves and dams during the entire experimental period (McVicar & Sutmoller, 1870).

Vaccine

Vaccines were prepared from FMDV, strain A_{24} 8345. The virus obtained from infected bovine tongue epithelium stocks was passaged seven times in primary

bovine kidney cultures (BK), once in BHK-21 clone 13 cells, and then grown in BHK-21 monolayers in roller bottles. The final viral titre was $10^{7.2}$ 50% tissue culture infectious doses ($TCID_{50}$) per ml, its complement fixation titre was 16 after inactivation and the 146S concentration was 1·8 μg/ml.

The viral suspension was inactivated with binary 2-bromo-ethyleneimine as described by Bahnemann (1975). Tests for residual live virus in the antigen before emulsification were done in 100 suckling mice. The sterility was checked in anaerobic and Sabouraud culture media.

The OE vaccine was prepared according to the INTA formulation with Marcol 52 (42·5%), Arlacel 83 (6·55%) and Tween 40 (0·95%) using a Silverson type emulsifier (Rivenson *et al.* 1982). The Aq-FMDV control vaccine was produced by adsorption of the same viral suspension on to aluminium hydroxide (40% v/v and 2·5% Al_2O_3) with saponin and merthiolate added to a final concentration of 0·1% and 1/30000 respectively.

Immunization schedules

All calves were vaccinated by subcutaneous inoculation of 3 ml of vaccine per calf. All calves were first bled for serological tests and then vaccinated and revaccinated 30 days post partum (dpp) with 3 ml of OE vaccine. Calves born from nonimmunized dams received the same dose of OE vaccines at days 3, 7, 21 and 39 dpp; some were revaccinated as shown in Table 1.

Serology

Serum samples were obtained 7, 14, 21, 30 and 60 days postvaccination (dpv) and 7, 14, 21, 30, 60, 90, 120, 150 and 180 days post revaccination (dpr).

Neutralizing antibody titres were determined in each sample by the fixed virus–variable serum method, using 100 $TCID_{50}$ per tube. Results are expressed as the $\log_{10}$ dilution of serum protecting half of the monolayers.

Decay rate of colostral antibodies

Three calves, born 60–70 days after the last vaccination of their dams, were studied. Blood samples were periodically collected from 5 to 180 dpp and their neutralizing antibodies measured. Individual slopes were studied by linear regression analysis, and the weighted mean slope was estimated ($b = -0{\cdot}014$) in order to determine the half-life of CAb. The experimental error mean square (EEMS) was 0·46.

Challenge

Four calves born to immunized dams, vaccinated at 1, 30, 60 and 90 days of age with OE vaccines and revaccinated 60 days later, were challenged with 10000 50% suckling mouse doses 180 dpr.

RESULTS

Half-life of colostral antibodies

In order to be able to detect active immune responses in newborn calves, the half-life of CAb was first ascertained (Table 2). The mean slope from three

Table 1. *Experimental animals and immunization schedules*

Calf	Dam†	Adjuvant	Vaccination (days post partum)	Revaccination (days post partum)
1	Vacc.	OE*	6	No
2	Vacc.	OE	13	No
3	Vacc.	OE	13	No
4	Vacc.	OE	18	No
5	Vacc.	OE	4	61
6	Vacc.	OE	5	65
7	Vacc.	OE	5	65
8	Vacc.	OE	5	65
9	Vacc.	OE	10	70
10	Vacc.	OE	16	76
11	Vacc.	OE	21	81
12	Vacc.	OE	30	90
13	Vacc.	OE	45	105
14	Vacc.	OE	60	120
15	Vacc.	OE	90	150
16	Nonvacc.	OE	7	No
17	Nonvacc.	OE	21	No
18	Nonvacc.	OE	30	No
19	Nonvacc.	OE	3	63
20	Nonvacc.	OE	7	63
21	Nonvacc.	OE	21	81
22	Nonvacc.	OE	30	90
Aq_{1-2}	Vacc.	Aq‡	39	90
C_{1-2-3}	Vacc.	—	No	No

* OE, oil-emulsified vaccine.
† Vacc, Vaccinated; Nonvacc., Nonvaccinated.
‡ Aq; aqueous vaccine with hydroxysaponin adjuvant.

Table 2. *Decline of maternally derived antibodies with time*

Calf no.	Calf age (days)	NT*	Slope†
C 1	5	2·30	
	18	1·30	
	25	1·20	−0·028
	55	⩽0·70	
	178	⩽0·70	
C 2	11	3·10	
	48	2·10	−0·020
	90	1·50	
C 3	16	2·90	
	23	2·70	
	30	2·50	−0·012
	60	2·30	
	116	1·80	
	170	0·90	

* NT, neutralizing titres ($\log_{10}$).
† The weighted mean slope was −0·014.

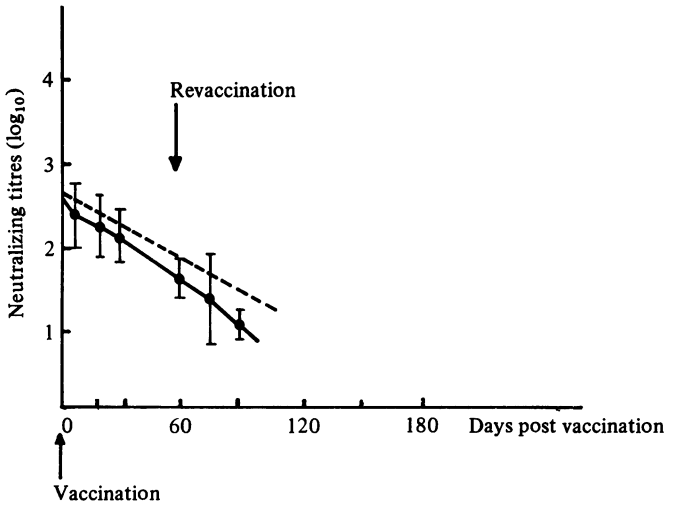

Fig. 1. Response to an aqueous foot-and-mouth disease vaccine in the presence of colostral antibodies. Calves were vaccinated when 30 days old and revaccinated 60 days later and their neutralizing antibodies tested as described in material and methods. (---): theoretical half-life slope of passively acquired antibodies; (—): actual titres in vaccinated calves.

non-immunized calves had a value of b = −0·140, giving a half-life value of 21·5 days, coincident with other data in the literature (Nicholls *et al.* 1984).

Effect of Aq-vaccine in calves born to vaccinated dams

As may be seen in Fig. 1, the antibody titres in calves with CAb were lower than expected from their theoretical half-life slope; furthermore, even when the calves were revaccinated 60 dpv, good responses could not be elicited.

Effect of OE-FMDV vaccines in calves born from vaccinated dams

Calves vaccinated 1 to 21 dpp had mean initial CAb log_{10} titres of 3·18 ± 0·66. At 60 dpv, their titres were similar or lower than expected from the theoretical half-life slope. In 4 calves which were not revaccinated no antibody rise could be detected up to 120 dpv (Fig. 2). In 5 out of 7 calves, revaccination induced a moderate antibody response (Fig. 2); the oldest calf of this group gave a good response, while one animal gave a very low and protracted response to revaccination.

Calves vaccinated 30 or more dpp had a mean prevaccination antibody titre of 1·67 ± 0·7; at 60 dpv their titre had risen to 2·17 ± 0·37 showing that vaccination at this age was effective; revaccination induced a further rise in antibody titres in all animals within this group (Fig. 3).

Effect of OE-FMDV vaccine on calves born from non-vaccinated dams

Calves vaccinated 21 and 30 dpp (Fig. 4, C and D) gave good antibody responses detectable 7 or 14 dpv. Calves vaccinated 3 and 7 dpp (Fig 4A and B) also gave good antibody responses, but with a lag of 30 to 60 dpv. Non-revaccinated calves

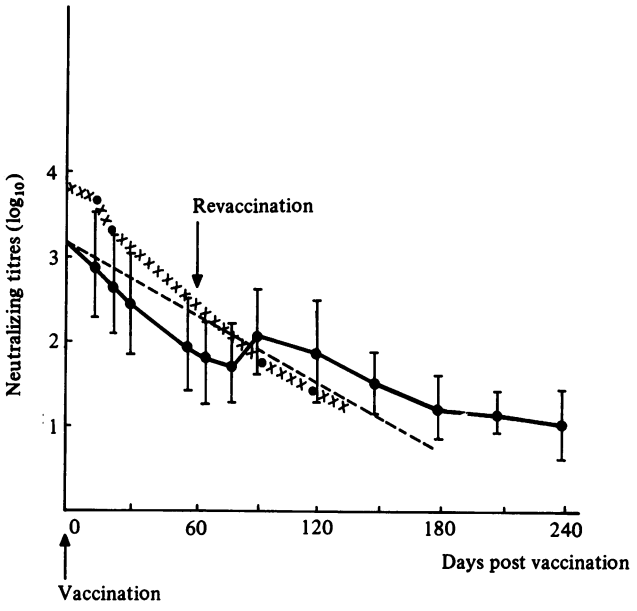

Fig. 2. Response to an oil-emulsified foot-and-mouth disease vaccine in calves immunized 1–21 days post partum in the presence of colostral antibodies. Calves born to vaccinated mothers were vaccinated 1–21 days post partum and revaccinated 60 days later, and their neutralizing antibodies tested as described in Materials and Methods. (---): theoretical half-life slope of passively acquired antibodies; (—): actual titres in vaccinated and revaccinated calves; (+ + +): actual titres in singly vaccinated calves.

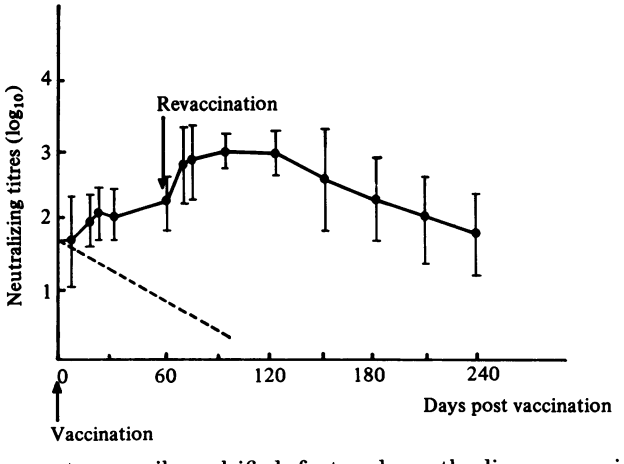

Fig. 3. Response to an oil-emulsified foot-and-mouth disease vaccine in calves immunized 30–90 days post partum, in the presence of colostral antibodies. Calves born to immunized dams were vaccinated 30 or more days post partum, revaccinated 60 days later and their neutralizing antibodies tested as described in Materials and Methods. (---): theoretical half-life slope of colostral antibodies. (—): actual titres of vaccinated calves.

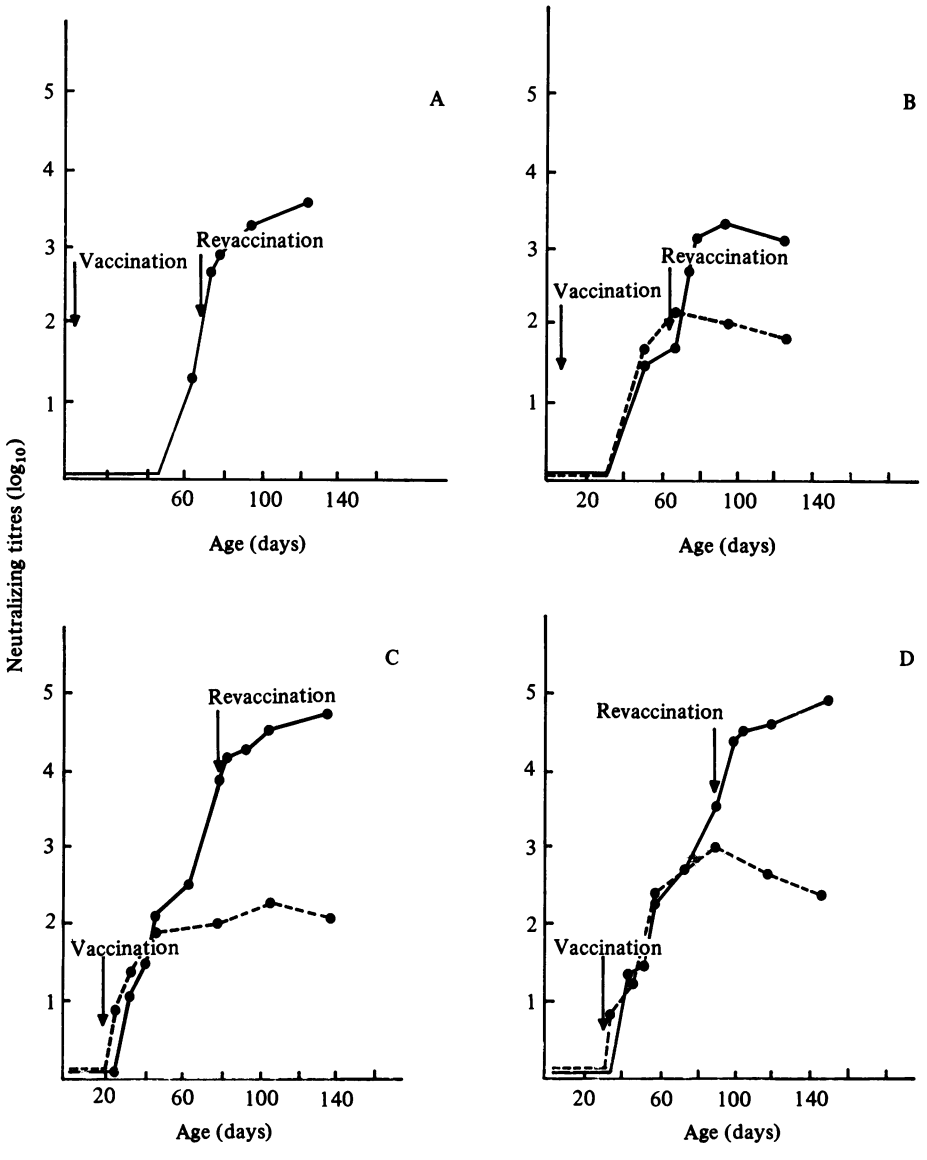

Fig. 4. Response to an oil-emulsified foot-and-mouth disease virus vaccine in calves born to nonvaccinated dams. Calves born in a disease-free area were vaccinated with an oil-emulsified vaccine at 3 (A), 7 (B), 21 (C) and 30 (D) days post partum; some of them (—) were revaccinated 60 days later; others (– – –) were not revaccinated.

showed persistent antibody titres up to 120 dpv; revaccination (Fig. 4) always led to enhanced responses.

Protection against viral challenge

Four calves were challenged 180 dpr (Table 3). The youngest developed local lesions, followed by production of virus infection-associated (VIA) antibodies but was protected against generalized infection. All the others were totally protected, and remained VIA-negative.

Table 3. *Protection against viral challenge*

Calf	Days post partum: Vaccination	Revaccination	Challenge	Titre*	VIA†	Lesions: Local	Podal
e	1	61	241	1·2	+	+	–
l	30	90	270	1·90	–	–	–
n	60	120	300	3·30	–	–	–
o	90	150	330	1·70	–	–	–

* Neutralizing antibodies on day of challenge

† VIA: virus infection-associated antigen. All animals were negative before challenge and were tested at 7, 21 and 30 days post challenge.

DISCUSSION

When studying the immune response of fetal and newborn calves against several antigens, Smith & Ingram (1965) showed that it depends both on the kind of antigen used and on the presence or absence of CAb. Husband & Lascelles (1975) showed that the most important cause of immunological unresponsiveness in neonates is not so much the immaturity of the lymphoid system as the effects of CAb, and the lack of response of young calves to FMDV vaccination has been attributed to the presence of CAb (Graves 1963; Nicholls *et al.* 1984).

Nicholls *et al.* (1985) reported that 1-week-old newborn calves responded as well as adult cattle to Aq-vaccines in the abscence of CAb. However, using a trivalent commercial Aq-vaccine, we have observed very poor or no responses in CAb-free calves from Patagonia up to 30 days of age (Sadir *et al.* to be published). Other authors have also reported that young cattle need higher doses than adults to produce comparable antibody responses (Brooksby, 1974). The reason for this difference in results is unknown but may be due to differences in the vaccine formulation or content, in the cattle population used or in other factors. Moreover, we here show that, although vaccination with OE-FMDV vaccines 3 and 7 dpp did elicit antibody responses, these were detectable only after a considerable period of time. In fact, the calves that were vaccinated 14 dpp had detectable antibodies before those that had been immunized 3 and 7 dpp. Although the number of animals is small, these and the data referred to above suggest that, at least in our conditions and during the first days of life, calves may be poor responders to FMDV vaccines in the absence of CAb; this unresponsiveness would have to be overcome before a positive reaction may be elicited. Using OE vaccines, the antigen is slowly released (Osebold, 1982) and may be presented to the immune system once it is able to respond. It should be noted that vaccination of 1-week-old pigs devoid of CAb with OE-FMDV vaccine led to poor immunity against challenge at 6 or 7 months of age (Francis & Black, 1986).

Although newborn calves are able to react to many antigens, they respond poorly to others such as polysaccharides, even if deprived of CAb (Smith & Ingram, 1965). In an experimental murine model, it has been reported that immune responses to FMDV infection may be elicited in the absence of T cells (Borca *et al.* 1986) and that in congenitally athymic (nu/nu) mice the course of infection is identical to that in their normal nu/+ littermates (Fernández *et al.*

1986). This suggests that, in certain conditions, FMDV may induce non T-dependent reactions in mice, even if indirect evidence, for example the IgM to IgG switch, does point to helper T-cell involvement in anti-FMDV reactions. The delay in response to FMDV vaccines in newborn calves may also be due to an atypical response to these virus antigens in young cattle. It should be noted that functional studies at birth show that the stimulation of lymphocytes with mitogens is depressed (Fossum, 1985) – an effect which may be explained by the low numbers of T and B lymphocytes of very young calves. Finally, high perinatal levels of cortical hormones may have immunosuppressive effects in newborn calves (Roth, 1982) and different levels in these hormones may explain the differences between the results obtained by Nicholls *et al.* (1985) and ourselves.

Our data also show that CAb interfere with the immune response to FMDV vaccines; in fact calves born to vaccinated dams gave no detectable responses to OE vaccines until 21 dpp, but after 30 dpp, the responses to this vaccine are comparable to those of adult cattle. Nicholls *et al.* (1984) have shown that Aq vaccines should not be used before 5 months of age, since they do not induce antibody responses in calves with high CAb titres. Nevertheless, when very young calves from immunized dams were vaccinated and revaccinated with OE vaccine, good protection was observed, as shown by virus challenge 180 dpr. Once again, this protection may be due to a persistent stimulation of the immune system given by the slow release of antigen from the OE vaccine.

From a practical point of view, in endemic areas, where cattle are vaccinated periodically, CAb can confer a good protection to newborn calves for at least 60 days (Sadir *et al.* 1984). During this period, Aq-FMDV vaccines should not be used, since they lead to a reduction in CAb titre (Nicholls *et al.* 1984), while OE-FMDV vaccines are highly efficient from 30 dpp on, even in the presence of CAb. Vaccination of calves 30–60-days old with OE-FMDV vaccines will then lead to high levels of herd protection.

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