

RESPONSE OF CATTLE TO FOOT-AND-MOUTH DISEASE (FMD) VIRUS EXPOSURE ONE YEAR AFTER IMMUNIZATION WITH OIL-ADJUVANTED FMD VACCINE

Ivo Gomes¹; P. Sutmüller¹; R. Casas Olascoaga¹

SUMMARY

Thirty cows that had been vaccinated 3 times at 6-month intervals with oil-adjuvanted foot-and-mouth (FMD) vaccine were exposed to virus type O₁ by intradermolingual inoculation and exposure to infected cattle and pigs 13 months after their last vaccination.

According to the pre-challenge antibody levels a high protection was expected which was confirmed by the challenge test. Only one of the 30 cattle developed generalized FMD. Of the 29 protected cattle only 6 had a lesion on the inoculation site.

These observations confirm, in general, that uniform high antibody levels of cattle populations vaccinated with oil-adjuvanted FMD vaccine indicate solid population immunity.

INTRODUCTION

Field studies to evaluate the efficacy of foot-and-mouth disease (FMD) vaccines are mainly based on the results of virus neutralizing antibody tests. Most commonly used for this purpose by the Pan American Foot-and-Mouth Disease Center (PAFMDC) are the microneutralization test (MNT) (8) and the mouse protection test (MPT) (7). For the MPT the relationship between the serum values of some 700 vaccinated cattle and their protection at 21-30 days post-vaccination (DPV) against intradermolingual (IDL) virus exposure were established (10). Of cattle vaccinated with inactivated vaccine with a mouse protection index (MPI) in the range 0.1-1.0, 25% were protected. In the intermediate range (1.0-2.0) approximately 60% of vaccinated cattle did not develop foot lesions after tongue inoculation. Based on that study an expected

percentage of protection (EPP) for MPI values was established (10), indicating that more than 90% of cattle with an MPI \geq 2.5 would be expected to be protected at FMD virus challenge. Most of this data was derived from cattle exposed to virus by tongue inoculation at 21-30 DPV.

In a joint experiment (6) of the PAFMDC and the FMD Control Directorate (Dirección de Lucha contra la Fiebre Aftosa, DILFA), Uruguay, cattle vaccinated with dilutions of inactivated oil-adjuvanted vaccine were challenged by tongue inoculation 3 months later. Cattle of the groups with protective levels of \geq 90%, generally, had an MPI \geq 2.5 and MNT \geq 3.0.

In a collaborative experiment (1) with the National Institute of Agricultural Technology (Instituto Nacional de Tecnología Agropecuaria, INTA), Argentina, cattle vaccinated with oil-adjuvanted vaccine were protected when inoculated IDL at 6 months after vaccination if their MPI or MNT was \geq 2.5.

An experiment (12) of the PAFMDC and the Plum Island Animal Disease Center (PIADC) showed that groups of cattle with a mean MPI of 2.0-3.0 at 6-12 months after revaccination with inactivated oil-adjuvanted FMD vaccine had a protection against tongue challenge on the order of 60-90%.

Serum surveys in areas where oil-adjuvanted FMD vaccines have been used showed very satisfactory antibody levels (4, 5). Such populations appear to be well protected against FMD virus exposure; however, experimental data to substantiate this assumption is scarce.

We were given an opportunity to make such a study when a farm situated in an oil-adjuvanted vaccine pilot area had to dispose of 30 cattle which had been vaccinated three times with oil-adjuvanted vaccine; the last vaccination had occurred 13 months before they were brought to the PAFMDC and exposed to virus.

¹Pan American Foot-and-Mouth Disease Center, PAHO/WHO, Caixa Postal 589, 20000 Rio de Janeiro-RJ, Brazil.

MATERIALS AND METHODS

Vaccinated cattle

The farm from which the 30 crossbred cows originated (Fazenda Santa Monica, Juparana, Rio de Janeiro) belongs to the Brazilian Agency for Agricultural Research (Empresa Brasileira de Pesquisas Agropecuarias, EMBRAPA) of the Ministry of Agriculture. All cattle were over 4 years old.

Four of the 30 cows had been affected by FMD virus type A during an FMD outbreak in 1976. Oil-adjuvanted vaccine was used in January and July 1977 and in January 1978. The cows arrived at the PAFMDC at the end of January 1979 and were exposed to virus early in February 1979, slightly more than one year after their last vaccination.

Unvaccinated cattle and pigs

Four susceptible cattle and 6 pigs without vaccination history and free of antibodies were added to the group of the 30 vaccinated cows as controls and additional virus donors.

Vaccine

The oil-adjuvanted vaccines were from routine production batches of the PAFMDC and have been used in field trials and demonstration areas (5).

Virus

FMD virus subtype O₁, strain Campos of cattle origin was used for virus exposure of the cattle and pigs.

Virus exposure

All cattle were inoculated with 10⁴ bovine ID₅₀ in the tongue epithelium. Four unvaccinated cattle were similarly inoculated as well as 3 pigs which were inoculated with 10⁴ bovine ID₅₀ in one foot. All animals plus 3 uninoculated pigs were housed together in intimate contact until one week after virus inoculation when they were separated into small groups in loose boxes in the same barn.

Cattle examination

Cattle were examined for tongue lesions two days after inoculation and checked for generaliza-

tion 9 days after exposure.

Sera and oesophageal-pharyngeal (OP) fluid were collected before virus exposure and at 15 and 36 days after virus exposure.

Test procedures

Circulating antibodies were assayed by the MNT (8) against virus strains used in the vaccine: O₁ Campos, A Bage, A Venceslau and C Indaial. Virus strain O₁ Campos was used in the mouse protection test (7).

VIA antibodies were assayed in the double diffusion agar precipitation (DDAP) test as described (3).

Virus isolation from OP fluid was carried out as described (13) using TTE emulsification and 3 serial passages of 10 ml volumes in IB-RS-2 cell monolayer cultures in Roux flasks. All cultures with cytopathic effect were assayed by the complement fixation test for type confirmation.

RESULTS

All control cattle and pigs developed generalized FMD. One of the control cattle died as did 2 of the pigs. Only one of the vaccinated cattle developed generalized lesions on the feet. Of the 29 protected cattle only six had a lesion on the site of virus inoculation.

Table 1 summarizes these results as well as those of the various antibody and virus isolation tests.

In the MNT most titers were ≥ 3.0 . For type C only one animal showed titers below this value. For the two A strains Bage and Venceslau there were 4 and 9 cattle respectively, with values less than 3.0. For O₁ Campos, 4 cattle were below 3.0.

The MPT values varied over a wider range than the MNT. The EPP of the group based on the MPI was 92.3 ± 4.4 . The one unprotected animal (No. 667) had an MNT of 2.6 and an MPI of 1.0. In the MPT the animals with a tongue lesion at the inoculation site had values in the 1.5-2.8 range. Animals with higher values did not show even this type of lesion. However, a few cattle with an MPI in the intermediate range were also completely negative. This type of possible relationship

TABLE 1. Response of cattle exposed to foot-and-mouth disease virus O₁ one year after vaccination with oil-adjuvanted vaccine

Cattle No.	Before challenge				MPI O ₁	VIA	Virus isol.	Challenge Lesions	15 DPC ^a			36 DPC			
	Microneutralization			O ₁					MPI O ₁	VIA	Virus isol.	VIA	Virus isol.	VIA	Virus isol.
	C Ind.	A Bage	A Venc.												
651	≥3.2	3.2	≥3.5	≥3.6	3.7	-	-	Neg. ^b	>5.0	-	+ 1st	-	+ 1st		
652	≥3.6	≥3.5	2.9	3.3	2.2	-	-	T ^c	>5.0	+	+ 2nd	+	Neg.		
653	≥3.6	≥3.5	3.3	3.2	4.9	-	-	Neg.	>5.2	-	+ 2nd	-	+ 3rd		
654	≥3.6	≥3.5	≥3.6	≥3.6	>4.5	-	-	Neg.	>5.0	-	+ 2nd	+	+ 2nd		
655	3.3	≥3.6	3.0	≥3.5	2.1	-	-	Neg.	>5.0	-	Neg.	-	+ 1st		
656	≥3.6	≥3.6	3.2	≥3.5	4.8	+	-	Neg.	>5.0	+	+ 1st	-	+ 1st		
657	≥3.5	3.2	3.2	2.7	4.8	-	-	Neg.	>5.0	+	+ 2nd	+	Neg.		
658	3.3	≥3.5	2.7	3.2	2.8	+	-	T	4.9	+	+ 2nd	+	+ 1st		
659	≥3.6	3.3	3.2	≥3.5	3.0	-	-	Neg.	4.2	+	+ 1st	-	+ 1st		
660	≥3.6	2.9	2.9	3.3	>4.5	-	-	Neg.	>5.2	+	+ 2nd	+	+ 1st		
661	≥3.5	≥3.6	3.0	3.3	2.5	-	-	T	5.2	+	+ 2nd	+	+ 1st		
662	≥3.6	≥3.6	≥3.5	≥3.6	4.0	-	-	Neg.	5.0	-	+ 2nd	-	+ 2nd		
663	≥3.6	≥3.6	≥3.6	≥3.6	>4.5	-	-	Neg.	5.0	-	+ 2nd	-	+ 2nd		
664	≥3.6	≥3.6	≥3.5	≥3.6	>4.5	+	-	Neg.	>5.0	+	+ 2nd	+	Neg.		
665	≥3.5	3.3	≥3.5	2.9	2.0	-	-	Neg.	>5.0	+	+ 2nd	-	+ 1st		
666	≥3.6	≥3.6	≥3.6	≥3.6	4.7	+	-	Neg.	>5.0	-	+ 2nd	+	Neg.		
667	2.7	2.7	2.3	2.6	1.0	-	-	T4F ^d	>5.0	+	+ 2nd	+	+ 1st		
668	≥3.6	≥3.6	2.9	≥3.5	>4.3	+	-	Neg.	>5.0	+	+ 2nd	+	+ 2nd		
669	≥3.6	≥3.5	2.9	≥3.5	>4.3	+	-	Neg.	>5.0	+	+ 2nd	+	+ 2nd		
670	≥3.6	≥3.5	≥3.6	≥3.5	>4.3	+	-	Neg.	>5.0	+	+ 2nd	+	+ 2nd		
671	3.3	2.9	3.2	3.0	3.3	+	-	Neg.	>5.0	+	+ 2nd	+	+ 1st		
672	≥3.6	≥3.5	≥3.6	3.3	>4.3	-	-	Neg.	>5.0	-	Neg.	-	+ 3rd		
673	≥3.6	≥3.5	3.0	3.3	3.6	-	-	Neg.	>5.0	+	+ 2nd	+	+ 1st		
674	≥3.5	≥3.5	2.7	3.3	2.7	-	-	T	>5.0	+	Neg.	+	+ 2nd		
675	≥3.6	≥3.5	3.0	≥3.5	4.7	-	-	Neg.	>5.0	-	Neg.	-	+ 3rd		
676	3.3	2.9	2.4	2.6	1.5	-	-	T	5.0	+	+ 1st	+	Neg.		
677	≥3.6	3.3	2.4	3.0	1.5	-	-	T	>5.0	+	+ 2nd	+	Neg.		
678	≥3.6	3.3	≥3.5	≥3.6	>4.3	-	-	Neg.	>5.0	-	+ 2nd	-	+ 3rd		
679	3.3	≥3.6	3.3	3.2	3.0	+	-	Neg.	>5.0	+	+ 1st	+	+ 1st		
680	≥3.5	≥3.5	≥3.5	3.3	1.9	+	-	Neg.	5.0	+	+ 2nd	+	+ 1st		

^aDPC = Days post-challenge.

^bNegative for tongue and feet.

^cT = tongue.

^dF = feet.

between primary tongue lesions and the MPI was not apparent with the MNT. All MPI increased to convalescent values (7, 9) following virus exposure. Ten of the 30 cows had VIA antibody before virus inoculation probably as a result of the type A, FMD outbreak in 1976. At 15 days post-challenge 20 of the 30 were positive. At 36 days post-challenge 3 of the positive cattle became negative and 2 of the negative became positive.

No virus could be isolated from any of the cattle before challenge. At 15 days post-challenge virus type O₁ was isolated from 26 cattle. Five cattle were positive at first passage in IB-RS-2 cell culture; the remaining 21 were positive at second passage.

At 36 days 13 cattle were virus positive at first passage and 11 were at second or third passage, making a total of 24 cattle from which type O₁ virus was isolated.

All four control cattle became VIA positive and virus was isolated from the OP fluid of each. As expected their antibodies reached convalescent levels.

DISCUSSION

According to pre-challenge antibody levels the cattle used in this test were expected to be well protected against FMD. This assumption proved correct as all but one animal withstood an extremely severe challenge of virus O₁: inoculation in the tongue epithelium of all cattle with 10⁴ virus units; intimate contact with 4 similar inoculated unvaccinated cattle; exposure to 3 inoculated donor and 3 contact pigs all with generalizing disease. Indeed, all cattle became infected as evidenced by antibody response and virus isolations. It is noteworthy that not all cattle became VIA positive (DDAP) even though virus had replicated in their pharyngeal area. Similar observations were made by others (2, 11).

This experiment confirms the assumption that a cattle population with an EPP of ≥ 90 is well protected against FMD even if the last vaccination was more than a year ago. The same is true when a high percentage of the herd has an MNT ≥ 3.0 . A more severe challenge of immunity than in this experiment would be difficult to en-

vision under farm conditions or even at auctions or cattle shows.

ACKNOWLEDGMENTS

The authors wish to acknowledge the cooperation of the staff of the Fazenda Santa Monica, Juparana, Rio de Janeiro, and of the National Dairy Cattle Center (Centro Nacional de Gado de Leite) of EMBRAPA for making available the cattle used in this experiment.

The authors would also like to thank the staff of the Diagnostic and Reference Laboratory of the PAFMDC for the performance of the VIA tests and virus typing. The microneutralization tests were made under the supervision of Dra. Kleise de Freitas Costa.

REFERENCES

1. ABARACON, D.; MAGALLANES, N.; CHARLES, E.G.; DURINI, L.A.; FRICK, E.; ALBARRACIN, G. F. de; BURGHI, E.D. de; RADISICH, T. Vida útil de una vacuna antiaftosa inactivada con adyuvante oleoso. (Shelf life of inactivated oil-adjuvanted foot-and-mouth disease vaccine). *Bltn Centro Panamericano Fiebre Aftosa 37-38*: 17-20, 21-24, 1980.
2. ALONSO FERNANDEZ, A.; SONDAHL, M.S. Pre-GOMES, I.; ROSENBERG, F. El uso del antígeno asociado a la infección viral (VIA) en la detección de ganado expuesto al virus de la fiebre aftosa. (The use of virus-infection-associated antigen (VIA) in the detection of cattle exposed to foot-and-mouth disease virus). *Bltn Centro Panamericano Fiebre Aftosa 17-18*: 17-22, 1975.
3. ALONSO FERNANDEZ, A.; SONDAHL, M.S. Preparación y concentración de los antígenos 140S, 12S y VIA del virus de la fiebre aftosa. *Bltn Centro Panamericano Fiebre Aftosa 17-18*: 1-8, 1975.
4. AUGÉ DE MELLO, P.; ASTUDILLO, V.; GOMES, I.; CAMPOS GARCIA, J.T. Respuesta inmunitaria de bovinos adultos vacunados contra la fiebre aftosa con vacuna oleosa. (Immune response of adult cattle vaccinated with oil adjuvanted foot-and-mouth disease vaccines). *Bltn Centro Panamericano Fiebre Aftosa 26*: 23-25, 27-29, 1977.
5. CASAS OLASCOAGA, R. Summary of current research of the Pan American Foot-and-Mouth Disease Center on oil adjuvanted vaccines. *Bull. Off. int. Epizoot. 89* (11-12): 1015-1054, 1978.

6. CENTRO PANAMERICANO DE FIEBRE AFTOSA; DIRECCION DE LUCHA CONTRA LA FIEBRE AFTOSA. Prueba de potencia para vacunas contra la fiebre aftosa de adyuvante oleoso: ensayos de DP_{50} en cobayos y en bovinos de una vacuna preparada en forma semi-industrial con una emulsión del tipo agua en aceite. (Potency testing of oil adjuvanted foot-and-mouth disease vaccine: PD_{50} assays of a semi-industrial water-in-oil type emulsion in guinea pigs and cattle). *Bltn Centro Panamericano Fiebre Aftosa* 29-30: 55-59, 61-65, 1978.
7. CUNHA, R.G.; BAPTISTA, Jr., J.A.; SERRÃO, U.M.; TORTURELLA, I. El uso de los ratones lactantes en la evaluación de los anticuerpos contra el virus de la fiebre aftosa y su significación inmunológica. *Gac. Vet.*, B. Aires, 19 (110): 243-267, 1957.
8. FERREIRA, M.E.V. Prueba de microneutralización para estudios de anticuerpos de la fiebre aftosa. (Microtiter neutralization test for the study of foot-and-mouth disease antibodies). *Bltn Centro Panamericano Fiebre Aftosa* 21-22: 17-20, 21-24, 1976.
9. GOMES, I.; ALONSO FERNANDEZ, A.; AUGÉ DE MELLO, P. Foot-and-mouth disease circulating antibodies in convalescent cattle. *Bull. Off. int. Épizoot.* 77 (5-6): 731-741, 1972.
10. GOMES, I.; ASTUDILLO, V. Foot-and-mouth disease: evaluation of mouse protection test results in relation to cattle immunity. *Bltn Centro Panamericano Fiebre Aftosa* 17-18: 9-16, 1976.
11. McVICAR, J.W.; SUTMÖLLER, P. Foot-and-mouth disease: the agar gel diffusion precipitin test for antibody to virus-infection-associated (VIA) antigen as a tool for epizootiologic surveys. *Am. J. Epidem.* 92 (4): 273-278, 1970.
12. PLUM ISLAND ANIMAL DISEASE CENTER; PAN AMERICAN FOOT-AND-MOUTH DISEASE CENTER. Vacunas contra la fiebre aftosa. II. Estudios sobre la duración de la inmunidad en bovinos y porcinos. (Foot-and-mouth disease vaccines. II. Studies on the duration of immunity in cattle and pigs). *Bltn Centro Panamericano Fiebre Aftosa* 19-20: 17-23, 24-30, 1975.
13. SUTMÖLLER, P.; COTTRAL, G.E. Improved techniques for the detection of foot-and-mouth disease virus in carrier cattle. *Arch. ges. Virusforsch.* 21: 170-177, 1967.