THE RELATIONSHIP OF NEUTRALIZING ANTIBODY TITERS FOR FOOT-AND-MOUTH DISEASE VIRUS AND THE PROTECTION OF CATTLE

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SUMMARY

A total of 791 sera from vaccinated cattle exposed to foot-and-mouth disease (FMD) virus were tested at the Pan American Foot-and-Mouth Disease Center (PAFMDC) by the tube serum neutralization test using the variable serum-constant virus technique with BHK_{21} cell monolayers as the indicator system to detect non-neutralized virus. The cattle were exposed to FMD virus at three different facilities by the intradermalingual route. Important differences in the relationship antibody titer and protection at challenge were observed between the three testing facilities and between the different virus types used. Since all sera were tested at the PAFMDC these differences point to the difficulty in standardizing tests involving the exposure of cattle. Neutralization titers above 1:64 would indicate a high level of protection. Titers in the range between 1:8 to 1:32 are difficult to interpret in terms of protection at challenge.

INTRODUCTION

The Pan American Foot-and-Mouth Disease Center (PAFMDC) has for several years used a neutralization test according to the constant virus-variable serum technique for the assay of neutralizing foot-and-mouth disease (FMD) virus antibodies in cattle serum (*1*).

The indicator system for non-neutralized virus consisted of baby hamster kidney cell (BHK_{21} Clone 13) (*4*) monolayers grown in regular cell culture tubes. The present paper analyzes the neutralization titers obtained by this technique relative to the protection of the vaccinated cattle when exposed to virulent virus.

MATERIALS AND METHODS

Cattle

PAFMDC. A total of 273 crossbred Zebu steers were used which originated from farms that had been free of FMD for several years. The cattle were tested for the absence of protective or neutralizing antibodies prior to vaccination with inactivated aluminum-gel or oil-adjuvanted FMD vaccine. The vaccines were prepared at the PAFMDC from the following FMD virus strains: O_1 Campos, A_{24} Cruzeiro and C_3 Resende. The cattle were challenged 21-28 days after vaccination with the homologous virus strains.

UCV[2]. 179 non-immunized cattle were vaccinated with inactivated aluminum-gel-formalin FMD vaccine and challenged at 21 days. The same strains as above were used for vaccine production, challenge and the serum neutralization tests.

SELAB[3]. 339 cattle originating from a FMD-free region of the country were vaccinated with inactivated aluminum-gel-saponin FMD vaccine and challenged at 21 days. The vaccines were prepared from strains from commercial production laboratories. The strains A_{24} Argentina/68 (8345) was used for challenge and A_{24} Cruzeiro was used for the serum neutralization test. Strain O_1 Caseros was used for challenge and strain O_1 Campos for the serum neutralization tests. C_3 Resende was used for challenge of the cattle as well as for the neutralization tests.

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Challenge of cattle

Cattle were inoculated by the intradermalingual route with 10^4 ID_{50} of virulent virus. They were considered to be protected when no foot lesions developed.

Sera

Blood samples were collected prior to challenge of the cattle and the sera stored at -20° C until tested.

Virus neutralization

The neutralization test was performed at the PAFMDC as described (*1*). Briefly the method was as follows:

After inactivation of the serum at 60° C for 20 minutes two-fold dilutions from 1:2 to 1:256 were made in modified Eagle's medium (MEM). A stock virus suspension containing 10^3 ID_{50}/ml was prepared in MEM. To each 1 ml of serum dilution 1 ml of the stock virus was added and the mixture held at 37° C for 1 hour followed by 30 minutes at 4° C.

Confluent BHK cell monolayers grown for 48 hours in Pyrex tubes (16 x 150 mm) with freshly changed 0.8 ml of MEM were inoculated with 0.2 ml of the virus-serum dilution. Six tubes were used per dilution. All tubes were read after 72 hours of incubation at 37° C and the endpoints calculated according to the method of Reed and Muench (*5*). The neutralization titer was expressed as the $\log_{10}$ of the reciprocal of the dilution protecting 50 percent of the cell cultures against 100 ($CCID_{50}$) of virus. This value is often referred to as the "S" index (*1*).

RESULTS

The sera were classified according to their neutralization titer "S" index, by their origin (PAFMDC, SELAB or UCV) and by virus type used (*Tables 1* and *2*).

TABLE 1. *Cattle classified according to their serum neutralization titer, protection at challenge, virus type used and testing facility*

	PAFMDC[a]				SELAB[b]				UCV[c]			
	O	A	C	Total	O	A	C	Total	O	A	C	Total
≤0.5	0/20[d]	1/23	0/25	1/68	2/27	3/32	2/23	7/82	0/17	2/18	0/22	2/57
0.6 - 1.0	5/20	6/14	5/16	16/50	6/28	16/28	32/44	54/100	1/24	3/10	4/17	8/51
1.1 - 1.5	9/12	10/16	10/11	29/39	17/32	14/21	30/33	61/86	3/10	4/10	6/8	13/28
1.6 - 2.0	15/15	15/15	15/15	45/45	25/37	2/5	7/7	34/39	3/5	8/8	7/7	18/20
2.1 - 2.5	12/12	11/11	15/15	38/38	18/18	3/3		30/30	0/1	10/10	2/2	12/13
2.5 - 3.0	5/5	12/12		17/17	2/2			2/2	2/2	4/4	3/3	9/9
>3.0	8/8	8/8		16/16						1/1		
Totals	54/92	63/99	45/82	162/273	70/134	38/89	80/116	188/339	9/59	32/61	22/59	63/179

[a] Pan American Foot-and-Mouth Disease Center, Rio de Janeiro, Brazil.
[b] Laboratory Service-National Animal Health Service (SELAB-SENASA), Buenos Aires, Argentina.
[c] Vaccine Control Unit, Executive Group for FMD Control(UCV-GECOFA), Rio Grande do Sul, Brazil.
[d] Number protected/number exposed.

TABLE 2. *Cattle classified according to their serum neutralization titer, protection at challenge and virus type used*

Neutralization titer class	Virus			
	O	A	C	Total
⩽0.5	2/64[a]	6/73	2/70	10/207
0.6 - 1.0	12/72	25/52	41/77	78/201
1.1 - 1.5	29/54	28/47	46/52	91/173
1.6 - 2.0	43/47	25/28	29/29	98/102
2.1 - 2.5	30/31	24/24	26/26	77/78
2.6 - 3.0	9/9	16/16	3/3	28/28
>3.0	8/8	9/9		17/17
Total	133/285	133/249	147/257	413/791

[a]Number protected/number exposed.

Dose response curves were established similar to the method used by Gomes and Astudillo for the mouse protection test (*3*). Briefly, the percentages of protection of each class were transformed into probits. A regression line of these probits and the antibody titer class midpoints was computed[4] by means of the weighted least square method (*2*). This relationship between percentage of protection and serum dilution was used to establish response curves for the sera collected at the PAFMDC, SELAB and UCV (*Fig. 1*).

Curves of the relationship between the percentage protection and the neutralization titers for the 3 virus types are shown in *Fig. 2*.

In *Figs. 3, 4* and *5* the response of each of the virus types in relation to the origin of the sera is plotted.

[4]PDP11/34 computer (DIGITAL). BASIC program available upon request.

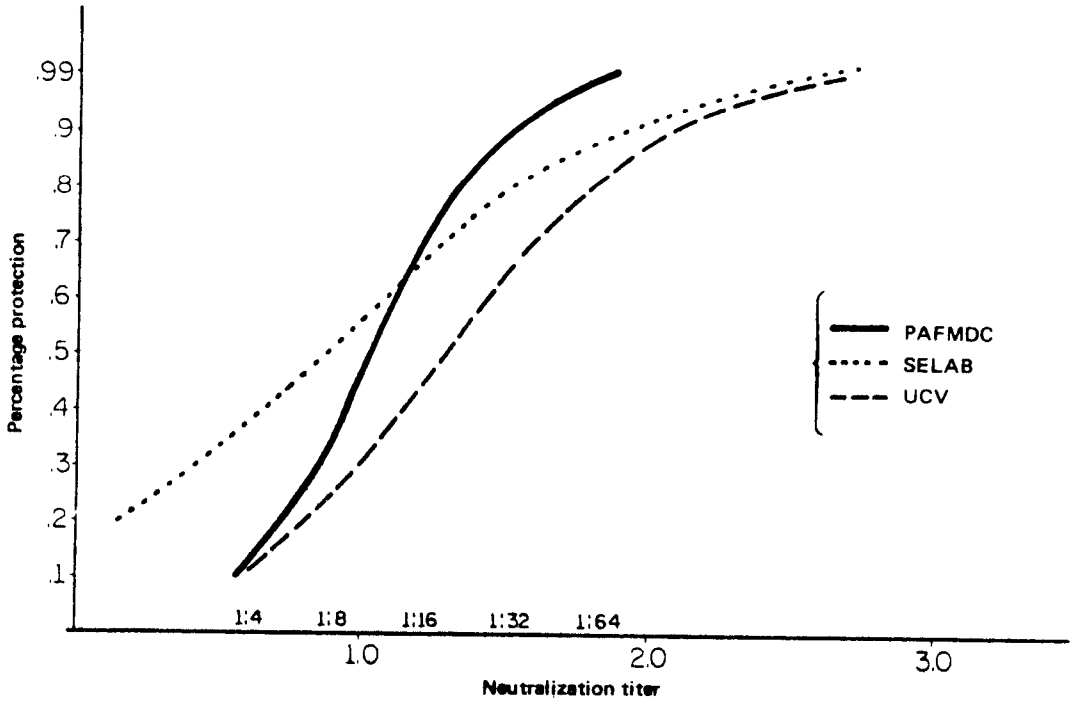

FIGURE 1. *Relationship of neutralization titers and percentage of protection of cattle vaccinated with inactivated FMD vaccine at 3 different locations (PAFMDC, Rio de Janeiro, Brazil; SELAB, Buenos Aires, Argentina; UCV, Rio Grande do Sul, Brazil).*

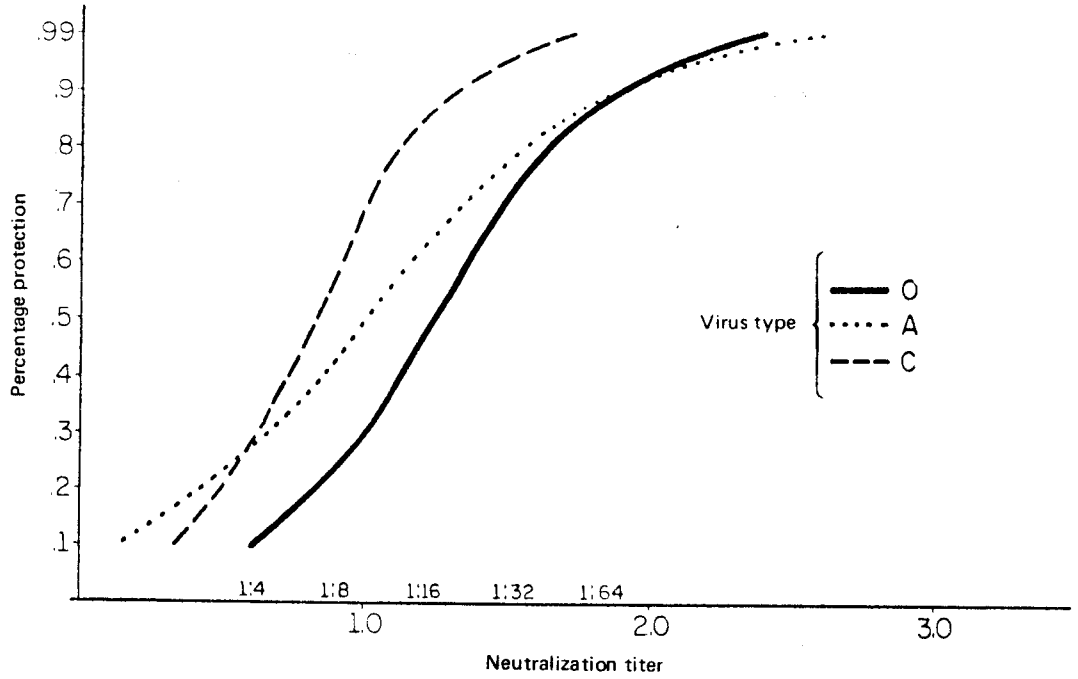

FIGURE 2. *Relationship of neutralization titers against different FMD virus strains and protection of cattle vaccinated with inactivated FMD vaccine.*

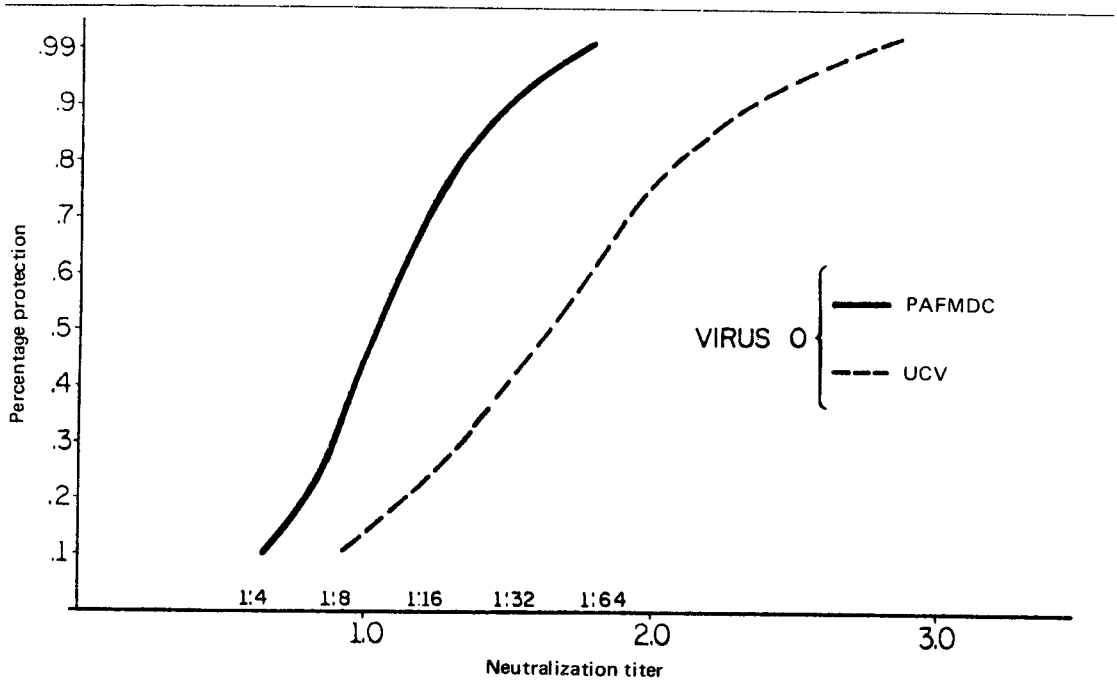

FIGURE 3. *Relationship of neutralization titers against FMD virus type O and protection of cattle vaccinated at 2 different locations (PAFMDC, Rio de Janeiro, Brazil; UCV, Rio Grande do Sul, Brazil).*

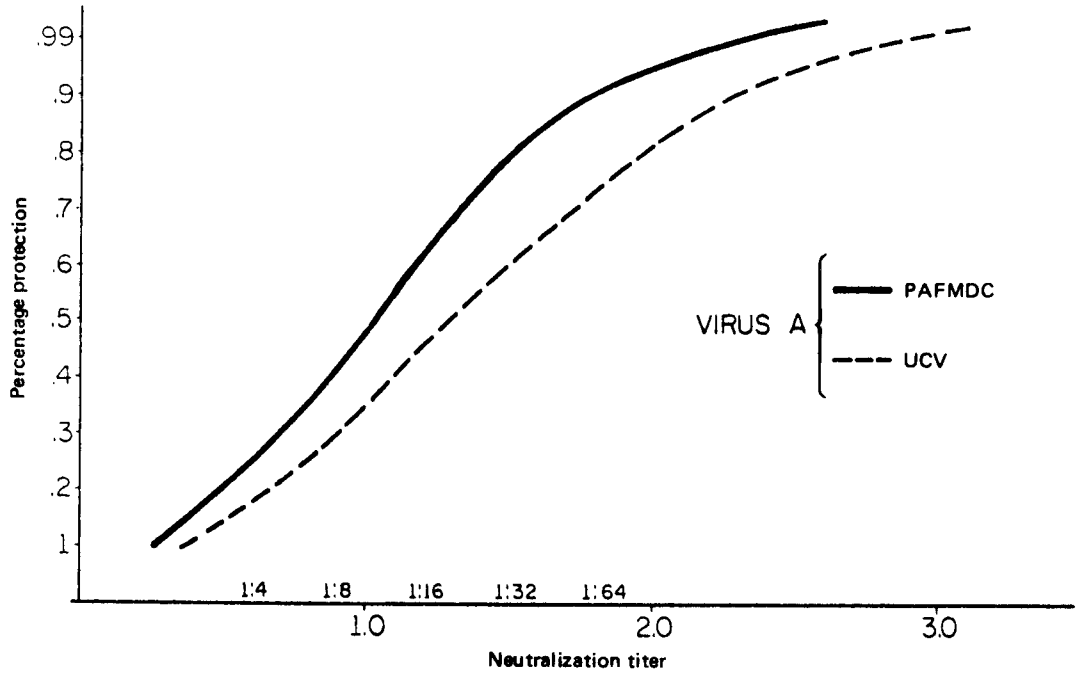

FIGURE 4. *Relationship of neutralization titers against FMD virus type A and protection of cattle vaccinated at 2 different locations (PAFMDC, Rio de Janeiro, Brazil; UCV, Rio Grande do Sul, Brazil).*

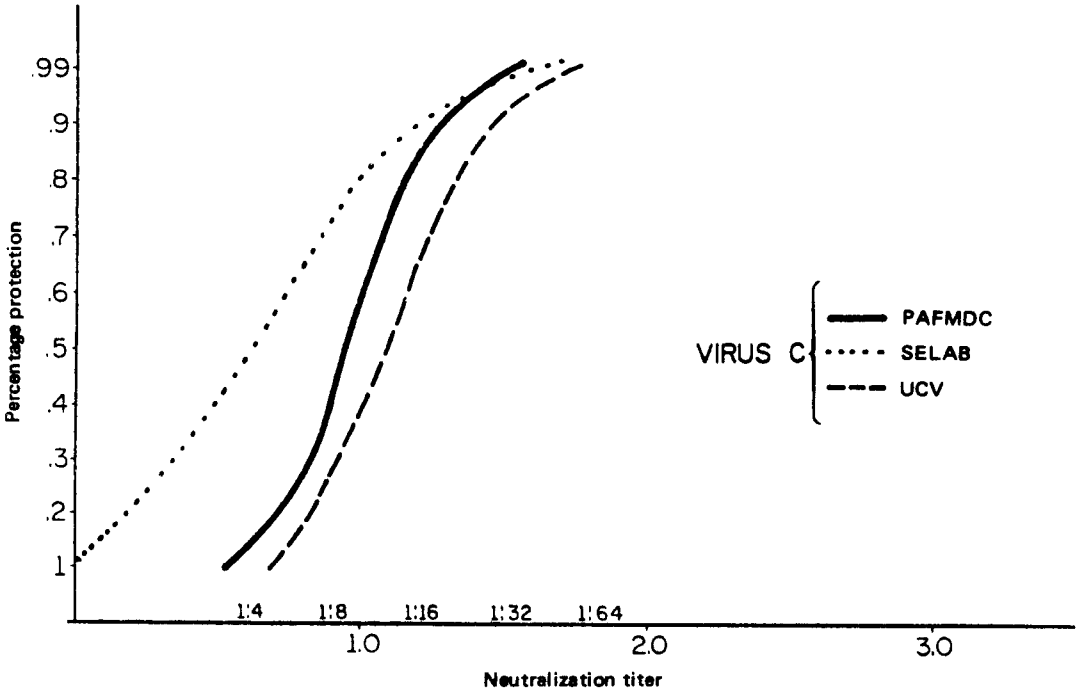

FIGURE 5. *Relationship of neutralization titers against FMD virus type C and protection of cattle vaccinated at 3 different locations (PAFMDC, Rio de Janeiro, Brazil; SELAB, Buenos Aires, Argentina; UCV, Rio Grande do Sul, Brazil).*

DISCUSSION

The FMD vaccine potency control manual of the PAFMDC (*1*) states: "A serial of vaccine is approved when all valencies give and NT_{50} of 1.5 or higher in six of eight vaccinated cattle". This value was based on the data accumulated at the PAFMDC. In *Fig. 1* it is shown that at the 1:32 dilution the protection level of the cattle of the PAFMDC is 90%.

However, large differences were observed between sera of different origin (*Fig. 1*). For instance, cattle challenged at the UCV and SELAB installations with a neutralization titer of 1:32 had protection levels of only 65% and 80%, respectively. It should be noted that the valencies that were tested were quite uniformly distributed among the three facilities and that all neutralization tests were done at the PAFMDC. Thus the differences between the response at the three facilities most likely should be attributed to differences in factors such as age and conditions of the cattle, management of the cattle during the test or the challenge technique. For the Argentina sera heterologous O and A strains were used which also may have contributed to some of the differences.

The curves for the 3 virus types were quite different. At the 1:32 dilution 70-75% of cattle challenged with types O and A, respectively, were protected (*Fig. 2*). The curve for type C was displaced to the left and lower titers related to a higher percentage of cattle protected against type C virus. The differences in response of the different viruses for each of the facilitites are shown in *Figs. 3, 4* and *5*. It can be observed that the differences due to different virus types used are even more pronounced at each individual facility; with the O and A types at the SELAB facility some of this difference may be due to the different strains used for challenge and the neutralization test. These figures show that the cattle of the UCV required higher antibody titers for all viruses in order to be protected. It is not quite clear whether these differences in the response curve for different virus types are a result of differences of virus behavior in the cell cultures, in the cattle or in both. These observations point to the need to uniformize procedures among laboratories, but it will most likely be easier to standardize laboratory techniques such as a neutralization test than the tests involving the exposure of cattle to virus.

With the virus strains used in the present material it appears that a passing titer of 1:32 for types O and A vaccines is on the low side while that value likely is adequate with regard to the C valency. Thus, it probably will be necessary to set a rather arbitrary standard which guarantees vaccines of adequate potency based on the virus strain which requires the higher antibody titers. At 1:64 a high level of protection can be expected at least for the strains of virus used.

Caution must be exercized in judging the protection level of cattle with serum titers in the steep linear part of the curve between 1:8 and 1:32. Small differences in titer which could be due to normal test variation would make a large difference in the estimated level of protection.

ACKNOWLEDGMENT

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REFERENCES

1. CENTRO PANAMERICANO DE FIEBRE AFTOSA. Manual de procedimientos para el control de vacuna antiaftosa. *Ser. Man. Téc. 2*, 1980.
2. FINNEY, D.J. Statistical method in biological assay. Griffin & Co. Ltd., London 1952 (page. 524).
3. GOMES, I.; ASTUDILLO, V. Foot-and-mouth disease evaluation of mouse protection test results in relation to cattle immunity. *Boln Centr. Panam. Fiebre Aftosa 17-18*: 9-16, 1976.
4. MACPHERSON, I.; STOKER, M. Polyoma transformation of hamster cell clones-an investigation of genetic factors affecting cell competence. *Virology 16*: 147-151, 1962.
5. REED, L.J.; MUENCH, H.A. Simple method of estimating fifty percent endpoints. *Am. J. Hyg. 27:* 493-497, 1938.