THE SERUM MICRONEUTRALIZATION TEST FOR FOOT-AND-MOUTH DISEASE: ESTABLISHMENT OF AN EXPECTED PERCENTAGE OF PROTECTION

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SUMMARY

A total of 532 sera from vaccinated cattle were assayed by the microneutralization test as used routinely at the Pan American Foot-and-Mouth Disease Center. The sera were from cattle used in vaccine potency tests and were challenged by the intradermolingual route of infection. An expected percentage of protection (EPP) was established for the serum neutralization titers. It is proposed that the mean EPP of a group of cattle is a meaningful measurement of the immune status.

INTRODUCTION

This study attempts to establish an expected percentage of protection (EPP) (*6*) for the microneutralization test (MNT) as described by Ferreira (*5*). At the Pan American Foot-and-Mouth Disease Center (PAFMDC) this test has been used routinely for several years: (1) to evaluate serum antibody titers of cattle from vaccine potency control tests; (2) for evaluating antibody titers of cattle in field trials of vaccines, and (3) for serological surveillance.

In order to correlate the degree of protection of cattle with a certain serum antibody titer a study was made of the relationship between antibody levels as measured by the MNT of vaccinated cattle and the lesions developed after intradermolingual (IDL) inoculation of virus.

MATERIALS AND METHODS

Cattle

The challenge data and the sera were obtained from cattle used in routine vaccine potency tests in Argentina and in cooperative experiments of the PAFMDC with the National Institute of Agricultural Technology (Instituto Nacional de Tecnología Agropecuaria, INTA), Argentina, Laboratory Service (Servicio de Laboratorios, SELAB) of the National Animal Health Service (Servicio Nacional de Sanidad Animal, SENASA), Argentina (*1, 2*); and the Foot-and-Mouth Disease Control Directorate (Dirección de Lucha contra la Fiebre Aftosa, DILFA), Uruguay (*3, 4*).

Table 1 lists the number of cattle, divided by the type of vaccine and virus strains used.

TABLE 1. *Number of cattle by type of vaccine and virus strain used*

Virus strains	Vaccines: Saponin hydroxide	Vaccines: Oil adjuvanted	Total
O_1 Campos–Br/58	8	269	277
O_1 Caseros–Arg/67	42	0	42
A_{24} Cruzeiro–Br/55	8	48	56
A_{24} (8345) Arg/68	42	45	87
C_3 Resende–Br/55	20	50	70
Total	120	412	532

Protection of cattle

Cattle were considered unprotected when lesions developed on one or more feet after IDL inoculation.

Sera

Blood samples were collected from cattle 3-4 weeks after vaccination prior to challenge and kept at −20° C until tested.

Vaccine dilutions were used to vaccinate the cattle in some experiments. The higher dilutions of such vaccines produced the majority

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of the low titered sera. Sera from unvaccinated cattle were not included.

Classification of sera

The sera were classified according to the following antibody titer classes ≤1.49, 1.50–1.99, 2.00–2.49, 2.50–2.99, 3.00–3.49, ≥3.5 (*Table 2*). Arbitrarily, the midpoint for the ≤1.49 group was taken as 1.0. The other midpoints were 1.7, 2.2, 2.7 and 3.2, respectively. The value 4.0 was taken as the midpoint for the ≥3.5 group.

Data processing

For each antibody class the number of protected and unprotected cattle were determined according to type of vaccine and virus types (*Table 2*). Using the percentage of cattle protected in each class and the above-mentioned class midpoints for the dilution, computer[2] calculations provided the probit/dose correlation using a weighted least square calculation (*6*). Only the last dilution giving full protection was included in the computation. Next, serum dilutions for the 1–99% protection range were computed and dose response curves were developed from these values (*Figs. 1* and *2*).

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

RESULTS

The probit/dose relationship is shown in *Table 3* for: (1) all of the cattle; (2) cattle vaccinated with oil-adjuvanted vaccine or saponin-hydroxide vaccine; (3) cattle challenged with foot-and-mouth disease (FMD) O_1 virus (strain Campos) and those challenged with other strains.

TABLE 3. *Relationship between Probit (Y) and log serum dilution*

	Slope	Standard error	Y-intercept	Standard error
All cattle	.909	.178	3.645	.438
Saponin-hydroxide vaccine	1.570	.080	2.587	.168
Oil-adjuvanted vaccine	.800	.190	3.550	.472
Virus O_1 Campos	.725	.286	3.858	.741
Other strains	1.402	.096	3.012	.197

TABLE 2. *Classification of sera according to the neutralization titer*

Neutralization titer	All cattle	Vaccines		Strains	
		Saponin hydroxide	Oil adjuvanted	O_1 Campos	Others
≤1.49	5/27	2/11	3/16	0/9	5/18
1.50 – 1.99	43/66	13/21	30/45	13/21	30/45
2.00 – 2.49	71/97	26/29	45/68	20/40	51/57
2.50 – 2.99	145/159	34/35	111/124	71/83	74/76
3.00 – 3.49	114/121	19/19	95/102	71/78	43/43
≥3.5	62/62	5/5	57/57	46/46	16/16
Totals	440/532	99/120	341/412	221/277	219/255

The percentages of protection (1, 10, 20, . . . 90, 99%) were plotted against the dilution as shown in *Figs. 1* and *2*.

In *Fig. 1* the response curve of the cattle vaccinated with oil-adjuvanted vaccine is very similar to the curve representing all cattle, because of the predominance of the number of cattle vaccinated with oil vaccine among the total number of cattle studied. The value for the slope of the curve of cattle vaccinated with saponin-hydroxide vaccine is larger. According to *Fig. 2*, higher serum titers are needed to protect cattle against challenge with the O_1 Campos strain of FMD virus than against the other strains used. Since the majority of cattle used in the oil-adjuvanted vaccine experiments were challenged with O_1 Campos virus, the difference between oil-adjuvanted vaccine and saponin-hydroxide vaccine may, at least in part, be more related to the challenge virus than to the differences between those types of vaccine.

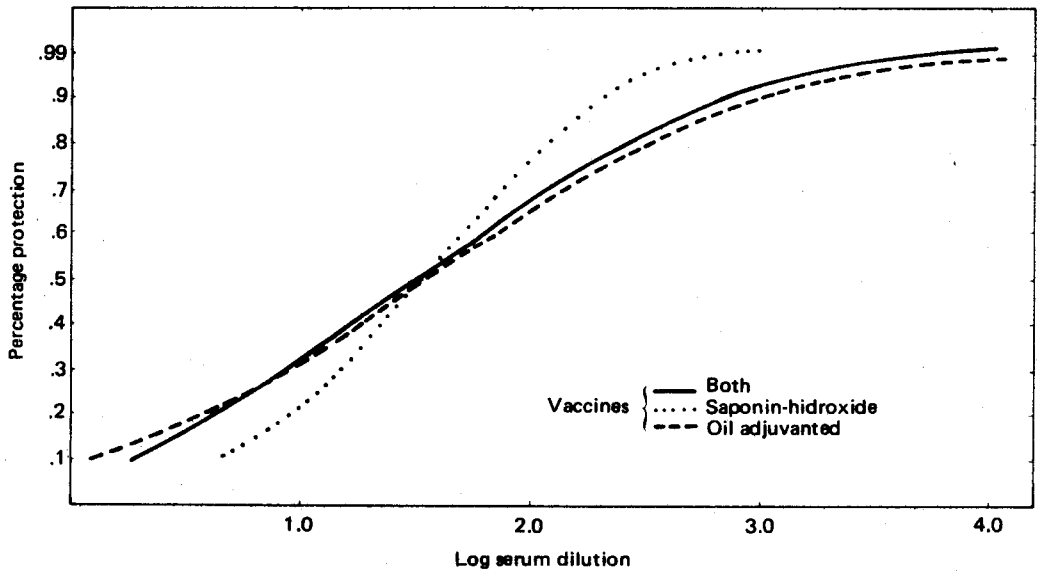

FIGURE 1. *Relationship of the serum neutralization titer and percentage of protected cattle vaccinated with inactivated FMD vaccine.*

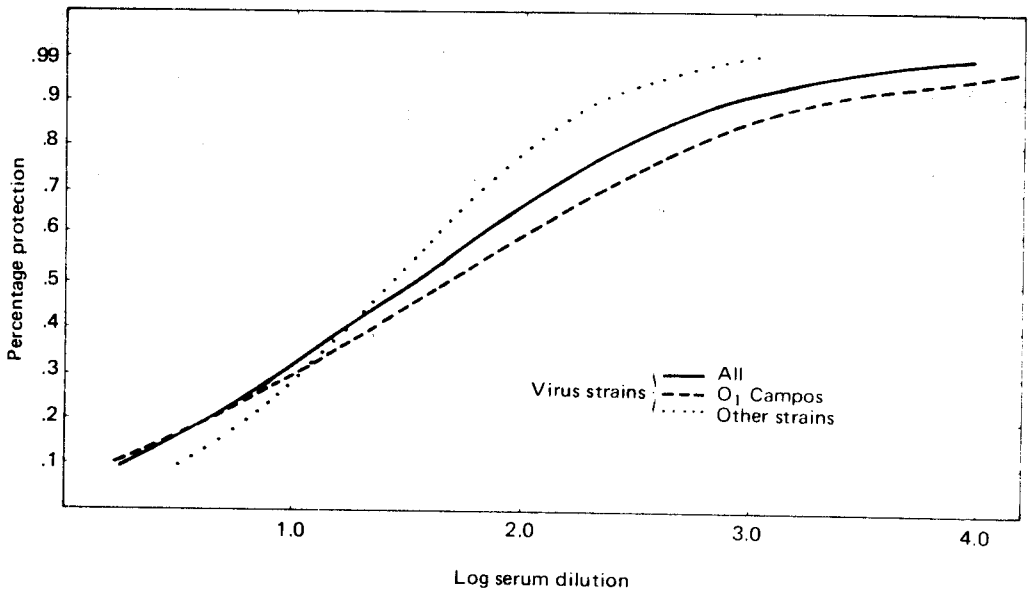

FIGURE 2. *Relationship of the serum neutralization titer against different strains of virus and the percentage of protected cattle, vaccinated with inactivated FMD vaccine.*

DISCUSION

It is likely that for different strains of virus used in challenge, different levels of antibody are required to protect equal percentages of these cattle against the disease. It also is probable that conditions of challenge, management of the cattle during the test, age of the cattle, etc., are rather important factors which may influence the outcome of such tests (*8*).

Purposely, two sets of data were eliminated from the original material because the dose response curve was very different from the one developed in this study. One of these sets concerned the data reported earlier on a group of cattle challenged by contact exposure (*4*). The other set was of a heterogeneous group of 60 cattle vaccinated with commercial saponin-hydroxide vaccines of low potency. In that test the majority of the cattle developed severe lesions and the few cattle with significant levels of antibody most likely were exposed to unusually high levels of virus, which probably caused a much higher number of reactors than expected in that category. The slope of the regression line of that group was 2.664 and the Y-intercept - 1.489. Protection of cattle in that group started only with an antibody level of >2.0 but was over 90% with values >2.8.

It is not practical to have a different measuring unit for antibody against different strains of virus or different types of vaccine. Therefore, all data were pooled with the exception of the abovementioned sets. From the resulting curve an EPP was established for each decimal MNT serum titer (*Table 4*). Group immunity could then be expressed as the mean EPP similar to the method proposed for the mouse protection test (*7*).

TABLE 4. *Expected percentage of protection*

MNT[a]	%[b]	MNT	%	MNT	%	MNT	%
0.1	8	1.1	30	2.1	71	3.1	92
0.2	9	1.2	34	2.2	74	3.2	93
0.3	10	1.3	38	2.3	77	3.3	94
0.4	11	1.4	42	2.4	80	3.4	95
0.5	13	1.5	46	2.5	82	3.5	95
0.6	15	1.6	50	2.6	84	3.6	96
0.7	17	1.7	56	2.7	86	3.7	96
0.8	19	1.8	60	2.8	88	3.8	98
0.9	22	1.9	64	2.9	90	3.9	98
1.0	26	2.0	68	3.0	91	4.0	99

[a] Microneutralization titer.
[b] Expected percentage of protection.

The EPP, like most antibody expressions, should not be interpreted in absolute terms because of the differences of protection which are likely to exist when different strains are used for challenge. Also the EPP is derived from challenge results after IDL exposure under laboratory conditions and the infection by this route may have little resemblance to infection under field conditions. As long as one recognizes these limitations the mean EPP can be a convenient way to compare the immune status of groups of cattle. It may be an advantage that a few animals with very high titers will have less influence on the mean EPP of a group than on the mean antibody titer.

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