FOOT-AND-MOUTH DISEASE: EVALUATION OF MOUSE PROTECTION TEST RESULTS IN RELATION TO CATTLE IMMUNITY

Drs. Ivo Gomes and Vicente Astudillo*

INTRODUCTION

A mouse protection test (MPT) in suckling mice for the assay of foot-and-mouth disease (FMD) antibodies was described in detail by Cunha *et al.* (2). The results of this test are expressed as a mouse protection index (MPI). These same authors found that cattle could be classified according to their immune status as follows: an MPI ≤ 1.0 would normally indicate no resistance to the virus, while cattle with a serum MPI ≥ 2.0 would for the most part be protected.

Later, Cunha and Honigman compared the MPT with a neutralization test in suckling mice in which they used standard amounts of serum and dilutions of FMD virus (3). They indicated that the results of the mouse protection test better expressed the degree of protection against FMD than those of the neutralization test.

For many years the MPT has been extensively used by the Pan-American Foot-and-Mouth Disease Center (PAFMDC), and a vast quantity of additional information has been accumulated with sera obtained from immunized or non-immunized and from recovered cattle that were subsequently exposed to the homologous virus.

This paper establishes a more quantitative relationship between the resistance of cattle upon exposure to FMD virus and the results of the MPI than was possible with the limited data of Cunha *et al.* (2). Furthermore, an attempt is made to find an MPI that could be used as a "screening" value for the classification of protected and non-protected cattle, with optimum specificity and sensitivity (6), and to develop additional methods to analyze and present the results of the test.

MATERIAL AND METHODS

In the MPT, 6-7 day old mice are inoculated subcutaneously with 0.1 ml of undiluted serum. Approximately 1 hour later FMD virus is titrated in these mice and also in untreated mice. The results of the test are expressed as the mouse protection index (MPI), the difference between titers ($\log_{10}$ mouse ID_{50}) obtained in both titrations.

The MPIs of 161 unvaccinated cattle were determined. These cattle were crossbred Zebu, 15-24 months old, originating on farms where FMD had not occurred for several years. The MPI was also determined for prechallenge sera from 701 similar cattle which had been vaccinated with inactivated virus aluminum hydroxide vaccine (IV) (1) or with a modified live virus vaccine (MLV) (5). Cattle immunity was challenged by inoculation of 10^4 ID_{50} homologous field virus into the tongue epithelium 21-28 days after vaccination. The criterion of protection was the absence of development of lesions in one or more feet. Oral lesions were considered to be only local.

* Pan American Foot-and-Mouth Disease Center - Caixa Postal 589, ZC-00, Rio de Janeiro, RJ, Brasil.

RESULTS

Only 9 of the sera of the 161 unvaccinated cattle without history of exposure to FMD had an MPI $\geq$2.0. Of the 152 remaining cattle with an MPI $<$2.0, 98% developed foot lesions of FMD after exposure to the virus by tongue inoculation. Also, 7 of the 9 with MPI $\geq$2.0 developed foot lesions (Table 1).

Table 2 presents the MPI of vaccinated cattle classified according to the results of homologous virus exposure. As expected, there is a very significant positive correlation ($r = 0.96$, $p < 0.01$)* between MPI and percentage of cattle protected at challenge. However, with both the inactivated virus and the modified live virus vaccines, more animals developed foot lesions with type 0 virus than in the corresponding classes with the two other types of virus. No differences were demonstrated between the two types of vaccines, except that a slightly higher percentage of cattle with foot lesions was observed with MLV than IV, in the MPI $<$ 2 interval.

In the lower interval (0.01 - 1.0) the vaccine showed some protective effect, as nearly 30% of the cattle were protected versus virtually none of the unvaccinated cattle. This effect is even more pronounced in the 1,0 - 2,0 interval in which approximately 70% of the cattle were protected. Only 5% of the cattle with MPI $\geq$2 developed foot lesions, but this figure is mainly determined by the relatively large number of cattle with an MPI $\geq$3.

* In the t - Student distribution with N-2 degrees of freedom.

TABLE 1 - *Relationship between mouse protection index of unvaccinated cattle and protection against foot lesions at challenge.*

TABLA 1 - *Relación entre el índice de protección en ratón de bovinos no vacunados y la protección frente a la descarga de virus.*

MPI ISP	Virus types Tipos de virus			All cattle Todos los bovinos	
	O	A	C		
0 ⊢— 1	42/44* (95%)**	19/19 (100%)	63/63 (100%)	124/126 (98%)	149/152 (98%)
1 ⊢— 2	5/5	6/6	14/15	25/26 (96%)	
2 ⊢— 3	2/2	2/2	2/2	6/6	7/9
3 ⊢— 4	0/0	0/0	0/1	0/1	
4 ⊢— 5	0/0	0/0	1/2	1/2	

* No. of cattle with foot lesions/No. of cattle.
* Número de bovinos con lesiones podales/Número de bovinos.
** Percentage with foot lesions.
** Porcentaje con lesiones podales.

TABLE 2 - *Relationship between mouse protection index of vaccinated cattle and protection against foot lesions at challenge.*

TABLA 2 - *Relación entre el índice de protección en ratón, de bovinos vacunados y la protección frente a la descarga de virus.*

MPI ISP	Virus types Tipos de virus			Vaccines Vacunas		All cattle Todos los bovinos	
	O	A	C	IV VI	MLV VVM		
0 ⊢— 1	51/65*	9/17	28/40	24/32	64/90	88/122	129/253
	78%**	53%	70%	75%	71%	72%	51%
1 ⊢— 2	22/48	2/20	17/63	13/33	28/98	41/131	
	46%	10%	27%	39%	29%	31%	
2 ⊢— 3	9/26	3/17	0/33	4/27	8/49	12/76	
	25%	18%	–	15%	16%	16%	
3 ⊢— 4	0/8	1/20	1/38	1/32	1/34	2/66	23/448
	–	5%	3%	3%	3%	3%	5%
4 ⊢— 5	0/18	1/31	1/38	1/43	1/44	2/87	
	–	3%	3%	2%	2%	2%	
⩾ 5	7/65	0/43	0/111	1/58	6/161	7/219	
	11%	–	–	2%	4%	3%	

* Number of cattle with foot lesions/No. of cattle.
* Número de bovinos con lesiones podales/Número de bovinos.
** Percentage with foot lesions.
** Porcentaje con lesiones podales.

OAC = Foot-and-mouth disease virus types O, A and C used for challenge independent of type of vaccine.
OAC = Virus de fiebre aftosa tipos O, A, y C utilizados para la descarga de virus independiente del tipo de la vacuna.
IV = Inactivated Vaccine.
VI = Vacuna inactivada.
MLV = Modified Live Virus Vaccine; independent of challenge virus.
VVM = Vacuna de virus vivo modificado, independiente de la descarga de virus.

DISCUSSION

The mouse protection index is frequently used as a screening value to divide cattle in those likely to be protected and those likely not to resist the disease. The results confirm the preliminary conclusions of Cunha *et al.* (2) that an MPI ⩾2.0 would be indicative of a high degree of protection upon exposure to the virus by tongue inoculation. If the cattle

had been classified according to a screening value of 2.0 and the challenge results, we would have obtained the following four-way table:

MPI	Challenge results	
	Without foot lesions	With foot lesions
≥2	425[a]	23[b]
<2	124[c]	129[d]
Total	549	152

The sensitivity of the mouse protection test is expressed by the relation [a/(a + c)] = 0.77 or 77%, and the specificity by the relation [d/(b + d)] = 0.85 or 85%, where:

a= Truly protected cattle, with an MPI ≥2.

b= False protected, with an MPI ≥ 2 but which developed foot lesions.

c= False unprotected with an MPI <2, but without the development of foot lesions.

d= Truly unprotected cattle with an MPI < 2.

The sensitivity of the MPI thus is the ability of the test to classify an animal as protected (positive) when exposed to FMD virus and the specificity as the ability to classify an animal as not protected (negative) under the challenge condition. In this manner the sensitivity and specifity for 3 screening values were computed for Table 3.

TABLE 3 - *Sensitivity and specificity of cattle screening according to different mouse protection index values, vaccine types and virus types.*

TABLA 3 - *Sensibilidad y especificidad de muestreo de bovinos de acuerdo con los diferentes valores de índice de seroprotección en ratón, tipos de vacuna y virus.*

MPI ISP	Virus types Tipos de virus						Vaccines Vacunas				All cattle Todos los bovinos	
	O		A		C		IV VI		MLV VVM			
	S %	E %	S %	E %	S %	E %	S %	E %	S %	E %	S %	E %
1.5	82	71	85	69	86	83	89	73	83	75	85	74
2.0	72	82	80	69	79	96	84	84	74	85	77	85
2.5	64	88	76	81	72	96	77	93	68	88	71	89

S = Sensitivity/Sensibilidad.
E = Specificity/Especificidad.
OAC = Foot-and-mouth disease virus types O, A and C used for challenge independent of type of vaccine.
OAC = Virus de aftosa tipos O, A y C utilizados para la descarga de virus, independiente del tipo de la vacuna.
IV = Inactivated Vaccine, independent of challenge virus.
VI = Vacuna inactivada, independiente de la descarga de virus.
MLV = Modified Live Virus Vaccine, independent of challenge virus.
VVM = Vacuna de virus vivo modificado, independiente de la descarga de virus.

It can be observed that the sensitivity decreases but that the specificity increases when the screening values increase. The modified live virus vaccines gave the largest percentage of false protected and false unprotected cattle. The same was true for type O in relation to the two other virus types.

The screening value of 2.0 is associated with a rather high specificity (85% ± 4%) but a rather low sensitivity (77% ± 4%) in the test. High specificity reduces the risk of having unprotected cattle with an MPI $\geq$ 2, but because of the low sensitivity valuable experimental data (protected cattle with MPI < 2) may be wasted. Direct use of the arithmetic mean MPI of a group of cattle also is rather unsatisfactory to express the immune status of the group because of the weight of high MPIs. Therefore, we propose the use of an expected percentage of protection (EPP) associated with each individual MPI and the expression of a group's immune status as the mean of the individual EPP. In the following example six cattle with MPIs of 0.7, 1.4, 1.6, 4.1, 4.3 and 5.5, respectively, were classified according to the intervals of their MPI;and the mean EPP of the group was calculated.

MPI	Expected percentage of protection*	Frequency f	f (EPP)**
0.0 ⊢— 1.0	28	1	28
1.0 ⊢— 1.5	66	1	66
1.5 ⊢— 2.0	73	1	73
> 2.0	95	3	285
		$\Sigma f = 6$	$452 = \Sigma f (EPP)$

$$\overline{EPP} = \frac{\Sigma f (EPP)}{\Sigma f} = \frac{452}{6} = 75\%$$

* According to Table 2.

** EPP = Expected percentage of protection.

The reliability of this estimate is approximately ± 30% at the 95% confidence interval using the t-Student distribution with N-1 degree of freedom. However, the calculation of the mean EPP using the values of Table 2 has the disadvantage that animals with values close to the interval class limits may still cause considerable variation in the mean

EPP when tests are repeated. To obtain more precision a response curve was calculated* based on the results from the approximately 700 cattle used in the vaccine efficacy tests at the PAFMDC. From this curve an EPP was established for each individual MPI (Table 4). The immunity of a group of cattle can thus be expressed as the mean of the individual EPP which are obtained from this response curve.

Under different challenge conditions (type of cattle strains of virus, etc.) a somewhat different relationship between the MPI and protection can be expected. It should also be noted that protection was defined as the prevention of foot lesions after tongue inoculation, which could be quite different from virus exposure under field conditions. However, in the absence of reliable data needed to establish the relationship between antibody titers and resistance to disease after a more natural exposure, it appears that the EPP as proposed in this paper is a workable alternative. Whether of not to accept the response curve and consequently the relationships as noted in Table 4 as a standard is a decision which each laboratory using this method will have to face. The same is also true for the acceptance of a screening value.

TABLE 4 - *Mouse protection index of cattle (MPI) and expected percentage of protection (EPP).*

TABLA 4 - *Indice de seroprotección en sueros de bovinos (ISP) y porcentaje de expectativa de protección (PEP).*

MPI ISP	EPP PEP	MPI ISP	EPP PEP	MPI ISP	EPP PEP	MPI ISP	EPP PEP
0.0	20	1.0	51	2.0	81	3.0	96
0.1	23	1.1	55	2.1	84	3.1	97
0.2	25	1.2	58	2.2	86	3.2	97
0.3	28	1.3	61	2.3	87	3.3	98
0.4	31	1.4	65	2.4	89	3.4	98
0.5	34	1.5	68	2.5	91	3.5	98
0.6	38	1.6	71	2.6	92	3.6	99
0.7	41	1.7	74	2.7	93	3.7	99
0.8	44	1.8	76	2.8	94	3.8	99
0.9	48	1.9	79	2.9	95	3.9	99

* Cattle were classified according to 0.5 log MPI intervals. The percentages of protection for each class were transformed to probits because plotting of the quantal response suggested similarity with a normal accumulative sigmoid curve. A regression line was calculated by means of the weighted least square method (4) and was characterized by the function $Y = 4.17 \times 0.86 X$, where Y is the probit of each protection percentage and X the MPI. From this line, expected percentages of protection were estimated for 0.1 log MPI intervals as stated in Table 4.

SUMMARY

This study attempts to further determine the relationship between the resistance of cattle upon exposure to foot-and-mouth disease virus and the mouse protection index (MPI).

Results indicate that by simply dividing cattle into those with MPI < 2 and those with MPI > 2 valuable experimental data may be wasted. The authors propose the use of an expected percentage of protection (EPP) calculated from the percentages associated with each individual MPI.

These EPP values can be read from a table or curve, and the EPP of the group may be expressed as the mean of the individual percentages.

FIEBRE AFTOSA: EVALUACION DE LOS RESULTADOS DE LAS PRUEBAS DE SEROPROTECCION EN RATON LACTANTE Y SUS RELACIONES CON LA INMUNIDAD DEL GANADO

RESUMEN

Este estudio intenta determinar las relaciones entre la resistencia del ganado que ha estado expuesto al virus de la fiebre aftosa y los índices de seroprotección en ratón lactante (ISP).

Fueron determinados los ISP de 161 bovinos no vacunados. Estos animales eran de cruza de cebú, de 15-24 meses de edad, originarios de granjas donde la fiebre aftosa (FA) no había sido diagnosticada desde hacía varios años. Los ISP fueron determinados también en sueros de 701 animales semejantes que habían sido vacunados con vacunas inactivadas y adicionadas de hidróxido de aluminio o con vacunas de virus vivo modificado. La inmunidad de estos animales fue determinada por la inoculación por vía intradermolingual de 10^4 DI_{50} dosis de virus homólogo del campo, de 21-28 días después de la vacunación. El criterio para determinar la protección fue la ausencia del desarrollo de lesiones en una o más patas. Las lesiones de la boca fueron consideradas como reacciones locales.

Sólamente 9 de los sueros de los 161 animales no vacunados y sin historia de haber estado expuestos al virus de la FA tenían un ISP $> 2,0$. De los 152 animales restantes con un ISP $< 2,0$, el 98% desarrollaron lesiones de FA en las patas después de haber estado expuestos al virus por inoculación en la lengua.

Entre los bovinos vacunados la vacuna mostró algún efecto protector en un intervalo bajo (0,0 - 1,0), porque el 30% de los animales estaban protegidos, mientras que prácticamente ninguno de los animales no vacunados estaba protegido. Este efecto es aún más pronunciado en el intervalo que va de 1,0 a 2,0 en el que aproximadamente el 70% de los animales estaban protegidos. Sólamente el 5% de los animales que tenían un ISP > 2 desarrollaron lesiones en las patas.

Estos resultados indican que dividiendo simplemente a los animales en dos grupos, uno con un ISP < 2 y otro con un ISP > 2 los datos experimentales pueden ser útiles. Los autores proponen utilizar lo que denominan

el porcentaje esperado de protección (PEP) calculado de los porcentajes asociados con cada ISP individual.

Estos valores de PEP pueden leerse en una tabla o curva y el PEP del grupo puede ser expresado como la media de los porcentajes individuales.

Con respecto a la aceptación o no de la curva de respuestas y consecuentemente las relaciones señaladas en la Tabla 4 como standard, es una decisión que deberá tomar cada uno de los laboratorios que usen este método. Lo mismo se aplica para la aceptación de un valor límite (screening).

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