POTENCY ESTIMATION OF FOOT-AND-MOUTH DISEASE VACCINES ACCORDING TO ANTIBODY ASSAY RESULTS

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SHORT COMUNICATION

The mouse protection test (MPT) for the assay of foot-and-mouth disease (FMD) antibodies has been used by the Pan-American Foot-and-Mouth Disease Center (PAFMDC) since the early fifties (*1*). In this test six to seven day old mice are given 0.1 ml heat inactivated bovine serum subcutaneously, followed by a virus inoculation approximately one hour later. The mouse protection index (MPI) is the log difference of the virus titer in the serum treated mice and in untreated control mice. In 1975, Gomes and Astudillo (*2*) published a table of MPI values and their corresponding expected percentage of protection (EPP) based on the analysis of clinical signs in 161 unvaccinated and 701 vaccinated cattle challenged by tongue inoculation 21-28 days after vaccination as shown in Table 1.

Later a similar study was made by Sutmöller *et al.* (*5*) using the results of the FMD virus neutralization test (NT) in microtiter plates as described by Ferreira (*3*) of over 500 sera of vaccinated cattle. The results of the test are expressed as the reciprocal of the log serum dilution which neutralizes approximately 100 ID_{50} of virus. The EPP of the neutralization titers are listed in Table 2.

TABLE 1. *Expected percentage of protection (Mouse protection test)*

MPI[a]	%	MPI	%	MPI	%	MPI	%
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

[a]MPI = Mouse protection index (Gomes and Astudillo *2*).

TABLE 2. *Expected percentage of protection (Neutralization test)*

NT[a]	%	NT	%	NT	%	NT	%
0.1	08	1.1	30	2.1	71	3.1	92
0.2	09	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	99
1.0	26	2.0	68	3.0	91	4.0	99

[a]NT =Neutralization titer (Sutmöller *et al., 5).*

In the present study a total of 62 batches of vaccine or dilutions of vaccines were classified according to the mean EPP of the cattle at 21-28 days post vaccination (DPV) for each vaccine as determined by both assay methods. The results were compared with the observed protection at challenge by tongue inoculation at 21-28 DPV (Table 3). Graphically these results are shown in Figure 1.

It can be noted that there is a good agreement between the classification and the observed protection with vaccines having a mean EPP higher than 60%, but that the neutralization test slightly underestimates the observed protection. In the groups of cattle with a mean EPP of less than 60% there usually are several sera without endpoint

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TABLE 3. *Classification of FMD vaccines according to the mean expected percentage of protection (EPP) and the observed protection at cattle challenge*

Range of Mean EPP	Number of vaccines	Observed protection	
		No. Prot./tested	%
Neutralization Test (NT)			
<55	6	3/30	7
55-65	6	26/43	60
66-75	8	38/53	71
76-80	10	70/81	86
81-85	14	92/105	88
86-90	13	95/105	91
91-95	5	33/34	97
Totals	62	357/451	
Mouse Protection Test (MPT)			
<40	5	4/25	16
41-65	7	18/42	43
66-75	7	30/48	63
76-80	3	20/25	80
81-85	4	33/40	83
86-90	11	62/74	84
91-95	8	55/59	93
96-99	17	135/138	98
Totals	62	357/451	

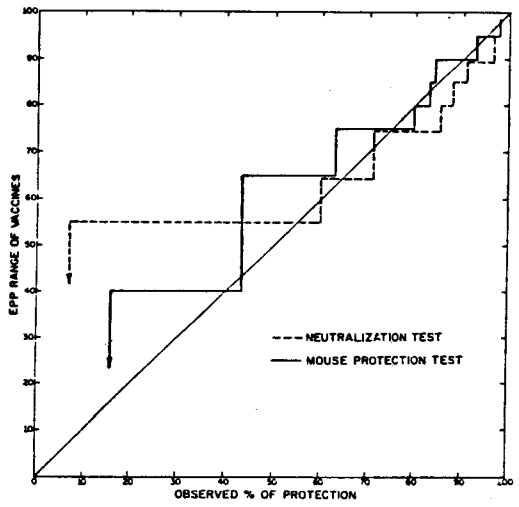

FIGURE 1. *Classification of FMD vaccines according to the mean expected percentage protection (EPP) and the observed protection at cattle challenge. Graphical presentation of Table 3.*

titers, because of the way the test is done routinely; therefore causing these groups to be classified higher than they really are.

According to Table 3 both the neutralization test and the mouse protection test results classified a nearly equal number of poor vaccines with an EPP of 75% or less. With the mouse protection test there are more vaccines with an EPP of 85% or higher than with the neutralization test. In the intermediate range the situation was reversed, probably as a consequence of the underestimation of vaccine potency by the neutralization test.

The question is: "Can the mean EPP be used meaningfully in vaccine control as an alternative for cattle challenge once the relationship between the individual EPP for a particular test system is established?"

Suppose the passmark for approval of an FMD vaccine is 75% protection of vaccinated cattle against challenge or any other comparable method at a confidence level of 95%. In that case a vaccine with a unilateral interval (mean EPP $-t_{.05}(n-1)\ SE_{EPP}$) of equal to or more than 75% would meet this requirement, where $t_{.05}(n-1)$ is the value t'Student, n is the number of animals and SE the standard error of the mean EPP. In other words in 20 tests using the same cattle population this vaccine is expected to pass the test 19 times.

We have grouped the serum results of vaccines (Table 3) with an EPP in the similar range to simulate vaccine tests with a uniform number of 12 cattle, in order to eliminate the influence of the variation of the group size. The mean EPP and standard error of these simulated vaccines were calculated and are shown in Table 4. It can be seen that 20 of the vaccines would be approved with the neutralization test against 24 with the mouse protection test. The SE of the EPP of the neutralization test in the range of 70-90% is considerable smaller than the SE of the EPP of the mouse protection test, thus with equal EPP vaccines assessed by serum neutralization test have a better chance to pass.

There are basically two factors which determine the size of the standard error of the mean. One is the number of animals in the test and the other is

TABLE 4. *Mean Expected Percentage of Protection (EPP) of groups of 12 cattle and the pass value of the vaccine*

Neutralization test (NT)					Mouse Protection Test (MPT)				
No. of vaccine	Mean EPP	SE[a]	LCL[b]	Diff.PM[c] LCL	No. of vaccine	Mean EPP	SE	LCL	Diff.PM LCL
01	74.8	4.1	67.4	−7.6	29	73.8	6.7	61.8	−13.2
02	77.3	3.6	70.8	−4.2	30	79.8	3.5	73.5	−1.5
03	78.3	2.8	73.3	−1.7	31	81.3	4.2	73.8	−1.2
04	78.4	2.7	73.6	−1.4					
05	78.8	2.7	74.0	−1.0	32	84.3	4.5	76.2	1.2
06	79.2	3.4	73.1	−1.9	33	86.5	4.6	78.2	3.2
07	79.3	3.1	73.7	−1.3	34	87.1	4.0	79.9	4.9
08	80.4	4.1	73.0	−2.0	35	87.4	4.2	79.9	4.9
					36	87.7	5.9	77.1	2.1
09	80.4	2.6	75.7	0.7	37	87.8	4.0	80.6	5.6
10	81.4	1.4	78.9	3.9	38	91.4	3.5	85.1	10.1
11	81.9	3.7	75.3	0.3	39	91.8	3.1	86.2	11.2
12	83.7	2.6	79.0	4.0	40	92.6	3.2	86.9	11.9
13	83.8	1.7	80.7	5.7	41	93.6	2.3	89.5	14.5
14	84.1	2.9	78.9	3.9	42	93.9	1.7	90.8	15.8
15	84.9	2.8	79.9	4.9	43	95.0	1.2	92.8	17.8
16	85.0	1.9	81.6	6.6	44	95.0	1.9	91.6	16.6
17	85.0	3.3	79.1	4.1	45	95.4	1.9	92.0	17.0
18	85.1	2.3	81.0	6.0	46	95.6	0.7	94.3	19.3
19	87.7	2.5	83.2	8.2	47	96.1	2.1	92.3	17.3
20	87.0	2.2	83.0	8.0	48	97.0	1.7	93.9	18.9
21	87.9	2.8	82.9	7.9	49	97.3	0.6	96.2	21.2
22	88.9	1.3	86.6	11.6	50	97.3	0.9	95.7	20.7
23	89.8	1.5	87.1	12.1	51	97.6	1.1	95.6	20.6
24	90.2	1.2	88.0	13.0	52	97.8	1.0	96.0	21.0
25	90.2	1.0	88.4	13.4	53	98.3	0.7	97.0	22.0
26	92.2	1.4	89.7	14.7	54	98.4	0.6	97.3	22.3
27	92.3	1.3	90.0	15.0	55	98.8	0.1	98.6	23.6
28	94.8	1.2	92.6	17.6					

[b] SE = Standard error.

[a] Lower Confidence Limit = Mean EPP $-t_{.05} \times SE$. For 11 degrees of freedom $t_{.05} = 1.796$ (*4*).

[c] Difference between the passmark (75%) and the lower confidence limit. If this value is negative the vaccine does not meet the minimum requirements.

the variance of the response. Larger groups and more homogenous responses result in a smaller standard error of the mean and consequently, vaccines within the critical range have a better chance to pass. Also, a vaccine with an adequate mean EPP - but with a very variable response of the animals - with good reason runs a higher risk of being rejected. Vaccines 8 and 9 of Table 4 illustrate these points. When these vaccines are combined in a total group of 24 cattle then the mean EPP of course remains 80.4, but the standard error of the mean decreases to 2.4 and the vaccine passes the test. The same is true for vaccines 31 and 32 in Table 4, which when combined have a mean EPP of 82.8% and a standard error of 3.

Thus the influence of group size and variability of response of the animals are two important

considerations for any type of potency test system. It is quite difficult to obtain cattle for potency tests which are uniform regarding age, nutritional status or genetic background and consequently may respond differently to an immune stimulus. However, the high cost of cattle in challenge tests has created a tendency to use increasingly smaller groups. Antibody assays have the same problem with regard to uniformity of the vaccinated cattle, but larger groups can be used at a much reduced cost and each animal can be tested for antibodies against each of the strains used in the vaccine.

Challenge tests require rather costly isolation stables, which unfortunately not always have been succesfull in containing the large amount of virus generated by the diseased cattle. Moreover the disposal of the cattle at the end of the tests also poses an important sanitary problem. In contrast, with serum antibodies assays, virus is handled only in the laboratory which lowers the risk of virus escape.

Finally, an antibody assay can be easily repeated at practically no extra costs and other strains of virus can be included in the test, if necessary.

Therefore, it appears that antibody assays with results expressed as mean EPP and their standard error is a valuable alternative for the estimation of vacine potency with a higher statistical value than of other potency test systems, provided of course that the relationship between protection of the vaccinated cattle and the serum assay results are well established.

SUMMARY

A comparison was made of the expected percentage of protection according to the results of the serum antibody studies by the neutralization and the mouse protection tests and the observed protection at challenge. It was concluded that antibody assays with results expressed as the mean expected percentage of protection and their standard error is an useful alternative for the estimation of potency of foot-and-mouth disease vaccine.

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