

EFFICIENCY TEST FOR FOOT AND MOUTH DISEASE VACCINES. I. REDUCTION IN C INDEX VARIATION BY INCREASING THE NUMBER OF GUINEA PIGS (*Cavia cavia*)

TESTE DE EFICIÊNCIA DE VACINAS ANTIAFTOSA. I. REDUÇÃO DA VARIAÇÃO DO ÍNDICE C PELO AUMENTO DO NÚMERO DE COBAIAS (*Cavia cavia*)

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SUMMARY

Two foot and mouth disease vaccines were submitted to the "C Index" efficiency test with six replicates each using four guinea pigs per viral dilution for titration. The values obtained, when transformed into quality of the vaccine, demonstrated that the same vaccine could be scored as "rejected regular", "approved good" or "approved very good", indicating that the random variation in the results may prevent a classification of the immunogen. To determine whether these variations are due to the small number of guinea pigs used, one vaccine was submitted to six replicates using five guinea pigs per viral dilution in the "C Index" test. Analyses of the results using 2 by 2, 3 by 3, 4 by 4 and 5 by 5 arrangements from the data corresponding to all possible combinations when 5, 10, 20, 25 or 30 guinea pigs were used per viral dilution demonstrated that the plus or minus (\pm) 0.5 \log_{10} variation with 95% confidence limits corresponds to 15 guinea pigs.

UNITERMS: Foot and mouth disease vaccine; C Index; Guinea pigs

INTRODUCTION

In countries in which foot and mouth disease (FMD) is endemic, the quality of each lot of FMD vaccine produced is determined by direct and indirect methods for immunogenicity control. The direct control methods of FMD vaccines in cattle present serious limitations of precision and sensitivity, due to the impossibility of using an adequate number of sensitive animals and due to the high cost of these tests, a fact that prevents their application in the control of all vaccine lots needed for programs of disease control. Furthermore, the precision of the tests can only be improved with the use of a larger number of animals (FERNANDEZ et al.⁷, 1972; MOWAT et al.¹⁴, 1973; GARLAND et al.⁹, 1977).

The indirect methods, when they present an appropriate relationship with direct methods, are the only ones which permit valuable and less costly routine procedures to determine the efficiency of vaccines against foot and mouth disease.

This paper describes experiments carried out in order to determine whether the number of guinea pigs per viral dilution used for titration affects the results of the C Index test, and to clarify the variations observed in the titrations when these tests are performed.

MATERIAL AND METHOD

Vaccines

Three lots of commercial foot and mouth disease vaccines were tested. The following FMD virus (FMDV) strains were used for the preparation of the vaccine: O₁ Campos, A₂₄

Cruzeiro, A Venceslau and C₃ Indaial. The viruses were grown in BHK₂₁ cells (MACPHERSON; STOKER¹³, 1962) inactivated with acetylethyleneimine (AEI) and containing aluminium hydroxide and saponin as adjuvants. These vaccines had been previously tested for efficiency by the official laboratory of the Ministry of Agriculture and Agrarian Reform, Brazil.

Challenge viruses

Challenges were performed with the O₁ Campos and A Venceslau strains of the FMDV, which are homologous to those used in the manufacturing of the vaccines, previously adapted to guinea pigs by two or more passages until lesions developed within 24 hours after inoculation into the hind footpads. Viral dilutions of 10⁻⁴ to 10⁻⁸ in phosphatebuffered saline (PBS, 0.5 M NaCl/0.01 M PO₄⁻, pH 7.4-7.6) were used to inoculate the control group and dilutions of 10⁻¹ to 10⁻⁵ were used to inoculate the vaccinated group. Guinea pigs were then observed daily for seven days for the occurrence of viral generalization. The infectious titre of the virus expressed as 50% infectious unit or dose (IT₅₀), was calculated by the REED; MUENCH¹⁵ (1938) method and the specificity of these viral strains was determined by the complement fixation test according to the technique described by CAMARGO et al.¹ (1950).

C Index in guinea pigs (*Cavia cavia*)

The C Index (CI) method for FMD vaccine testing of LUCAM et al.¹² (1964) was used. A group of albino guinea pigs weighing 450 to 550 g was injected subcutaneously with 0.1 ml dose of vaccine to be tested, and an unvaccinated group was used as control. Twenty-one days after vaccination, the FMDV

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previously adapted to this species was inoculated into one of the hind footpads at the dose of 0.1 ml. Guinea pigs with no lesions or lesions only at the inoculation site were regarded as protected and those with more extensive lesions as unprotected. The quotient between the FMDV titres obtained for the control and vaccinated animals provided the CI value.

Four guinea pigs per viral dilution were used to test the effect of number of guinea pigs on the variations in the results of the test, and five guinea pigs were used when an attempt was made to determine the minimum number of animals needed to reduce variation.

RESULTS AND DISCUSSION

The results of the test carried out to determine the efficiency of two vaccines against FMDV (vaccines I and II) and obtained by six replications of each vaccine (C Index test) using four animals per viral dilution are presented in Tab. 1. The indices calculated from the titres for control group A and for control group B and from the mean for the two groups (A and B) indicate the disparity of the results obtained. The C indices obtained varied statistically both when calculated from different control groups as well as within the same control group.

The variations in the CI results demonstrate that the quality of a vaccine being tested depends on chance when only one quality test is performed using a small number of guinea pigs.

TABLE 1

Results of the C Indices for guinea pigs, of vaccines I and II with six replications of the test, calculated from control groups A and B and from the mean (X) for the two control groups inoculated with "01" Campos of FMDV using four animals per viral dilution. Campinas - SP, 1990.

Number of replic.	Vaccine I				Vaccine II			
	Vacc. titre	*A CI	*B CI	*X CI	Vacc. titre	*A CI	*B CI	*X CI
1	3.50	1.38	2.30	1.84	2.87	1.96	2.63	2.30
2	3.00	1.88	2.80	2.34	3.81	1.02	1.69	1.36
3	2.54	2.34	3.26	2.80	2.54	2.29	2.96	2.63
4	3.40	1.48	2.40	1.94	2.89	1.94	2.61	2.28
5	3.53	1.35	2.27	1.81	2.50	2.33	3.00	2.67
6	2.58	2.30	3.22	2.76	3.37	1.46	2.13	1.80

IT₅₀ values obtained for control groups A: vaccine I, 4.88; vaccine II, 4.83
 IT₅₀ values obtained for control groups B: vaccine I, 5.80; vaccine II, 5.50
 IT₅₀ obtained for control groups A and B: vaccine I, 5.34; vaccine II, 5.17
 Vacc. titre - IT₅₀ of the vaccinated group
 * A CI, B CI and X CI - C indices obtained from control groups A and B and from their mean, respectively
 IT₅₀ - 50% infectious titre (infecting units) virus per ml log₁₀

On the basis of quality levels adopted by CUNHA et al.² (1957) in the Seroprotection Index for mice and of those detected by GOMES; ASTUDILLO¹⁰ (1975), the results for the two vaccines were transformed into "levels of vaccine quality" and classified as P (rejected poor) for vaccines which obtained a CI of less than one (CI<1), R (rejected regular) for vaccines with a CI greater or equal to one and less than two (1<CI<2), B (approved good) for vaccines with a CI greater or equal to two and less than three (2<CI<3) and M (approved very good) for vaccines reaching a CI value of three or greater (CI>3), as described in Tab. 2.

TABLE 2

Results of the quality levels of two lots of FMD vaccines (I and II) considering the levels starting from the C indices in guinea pigs, with six test replications, calculated from control groups A and B and from the means (X) for the two groups inoculated with strain "O₁" Campos of FMDV. Campinas - SP, 1990.

Number of replic.	Vaccine I			Vaccine II		
	TA	TB	TX	TA	TB	TX
1	R*	G	R	R	G	G
2	R	G	G	R	R	R
3	G	V	G	G	G	G
4	R	G	R	R	G	G
5	R	G	R	B	V	B
6	G	V	G	R	G	R
totals:						
P	0	0	0	0	0	0
R	4	0	3	4	1	2
G	2	4	3	2	4	4
V	0	2	0	0	1	0

TA and TB - Values transformed from the C Indices obtained for control groups A and B, respectively
 TX - Values transformed from the C Indices obtained from the mean of control groups A and B

* P = Poor (rejected vaccine)
 R = Regular (rejected vaccine)
 G = Good (approved vaccine)
 V = Very good (approved vaccine)

These data indicate the wide variation that can occur when four guinea pigs are used per viral dilution for the calculation of the 50% infectious titre and of the respective C Indices, since the same vaccine can be classified as regular or good, good or very good and regular, good or very good depending on chance.

For this reason, an attempt was made to determine the smallest number of guinea pigs per viral dilution needed to obtain an acceptable maximum variation, such as that proposed by GONÇALVES¹¹ (1980), who demonstrated that at least 55 suckling mice are needed per viral dilution for the variation in Mouse Protection Index to be approximately 0.5, with 95% confidence limits.

This maximum value of one (1) is fully justifiable since because of chance, the result obtained may fall within a classification range for better or for worse, i.e., it may reach a maximum of two ranges. However, if a value higher than one is used, the result, owing to chance, may be included in as many as two ranges of classification for better or for worse, and may even reach three ranges, which would be an undesirable type of evaluation.

As demonstrated in the present experiment, the number of guinea pigs may be the decisive factor for the final result of the C Index test. Indeed, LUCAM et al.¹² (1964), when proposing the C Index test using seven guinea pigs per viral dilution, considered the variations detected to be statistically acceptable. Similarly, FÉDIDA⁵ (1971) detected the existence of a correlation, which considered highly significant, between the C Index and the K Index in cattle (correlation coefficient "r" = 0.67). The variation of the values agreed with those obtained in the present study, but were statistically discordant. However, EISSNER; BOHM³ (1976) were unable

to reduce the variation of the test by increasing the number of guinea pigs when they used a virus adapted to the guinea pig myocardium for challenge.

The literature about the number of guinea pigs used in the efficiency tests of FMD vaccines is scarce, whereas studies carried out on cattle are available. The reduced number of cattle used in efficiency tests due to the high cost of the procedure leads to limitations of precision and sensitivity (FECHNER⁴, 1966; FÉDIDA⁵, 1971; FERNANDEZ et al.⁷, 1972; FÉDIDA et al.⁶, 1977; GARLAND et al.⁹, 1977; FERNANDEZ et al.⁸, 1985). The International Epizootic Office (OIE) recommends that the efficiency tests of vaccines against FMD should be expressed with a 95% confidence limit and the percentage of protection should be at least 70% in cattle (FÉDIDA et al.⁶, 1977).

With respect to the determination of the smallest number of guinea pigs utilized in the C Index test with 95% confidence limits, it was demonstrated that the variation in C Index decreases with increasing number of animals for titration. Tab. 3 presents the titres (in log₁₀) obtained for the control groups and for the vaccinated groups and the corresponding C Indices with their respective arithmetic mean, standard deviation, amplitude of variation and Pearson coefficient of variation (%).

TABLE 3

C Indices for guinea pigs, with six test replications, calculated from the vaccinated groups (vaccine III) and from the control group inoculated with the "A" Venceslau strain of FMDV, using five guinea pigs per viral dilution. Campinas - SP, 1990.

Number of replic.	* control titre	vaccinated titre	C Index
1	7.98	2.05	5.93
2	8.13	2.30	5.83
3	8.13	2.13	6.00
4	8.47	2.08	6.39
5	6.85	1.80	5.05
6	8.55	1.80	6.75
Arithm. mean	8.02	2.03	5.99
Standard dev.	0.61	0.20	0.57
Amplit. variat.	1.70	0.50	1.70
Coeffic. variat. %	7.63	9.66	9.59

* - 50% infectious viral titre per ml in log₁₀ for guinea pigs in the control and vaccinated groups

Arithm. mean - Arithmetic mean
Standard dev. - Standard deviation
Amplit. variat. - Amplitude variation
Coeffic. variat. % - Coefficient variation %

The results obtained, when submitted to 2 by 2, 3 by 3, 4 by 4 and 5 by 5 arrangements, covered all possible combinations representative of the values detected, as if 5, 10, 15, 20 and 25 guinea pigs were used, respectively, and consequently all of these results correspond to 30 guinea pigs per viral dilution. The viral titre was 10^{8.02} in the control group and 10^{2.03} in the vaccinated group, and the C Index value was 5.99.

Tab. 4 presents the values of the statistical parameters of the C Indices for the 1 by 1, 2 by 2, 3 by 3, 4 by 4, 5 by 5 and 6 by 6 arrangements corresponding to six replications. As expected, the arithmetic mean of the C Indices was 5.99 in all

cases. The variation measured by the standard deviations and by the Pearson coefficient of variation (%), as well as the amplitude of variation decreased with increasing number of guinea pigs per viral dilution (corresponding to 1, 2, 3, 4, 5 and 6 guinea pig groups).

TABLE 4

Values of the statistical parameters for the C Indices corresponding to all possible combinations in the six test replications. Campinas - SP, 1990

	Combination of the six test replications					
	1 by 1	2 by 2	3 by 3	4 by 4	5 by 5	6 by 6
N	6	15	20	15	6	1
Arithm. mean	5.99	5.99	5.99	5.99	5.99	5.99
Standard dev.	0.57	0.34	0.24	0.17	0.11	0.00
Amplit. variat.	1.70	1.13	0.78	0.57	0.34	0.00
Coeffic. variat.	9.59	5.73	0.02	2.89	0.19	0.00%

Arithm. mean - Arithmetic means
Standard dev. - Standard deviation
Amplit. variat. - Amplitude variation
Coeffic. variat. - Coefficient variation
N - Number of combinations

From deviations of plus or minus 2 (±2) standard deviations (initial proposition, α equal to 0.05), it is possible to determine both graphically or by calculation the site where variation is one (1) logarithm. This point corresponded to the 2.91 value. Since the objective was to find the number of animals, the number obtained was 14.55 (rounded to 15), meaning that 15 guinea pigs per viral dilution are needed for titration to have a maximum variation in C Index of 1 ± 0.5 at the 95% level of probability.

RESUMO

Duas vacinas antiaftosa foram submetidas a seis repetições cada, à prova de eficiência, "Índice C", usando-se para titulação, quatro cobaias por diluição de vírus. Os valores encontrados, quando transformados em qualidade de vacina, demonstraram que uma mesma vacina poderia ser enquadrada como sendo "reprovada regular", "aprovada boa" ou "aprovada muito boa", indicando que a variação dos resultados, dependendo do caso, pode indefinir a classificação do imunógeno. Para verificar se tais variações são devidas ao pequeno número de cobaias, uma vacina foi submetida a seis repetições, usando-se cinco cobaias por diluição do vírus na prova "Índice C". Os arranjos 2 a 2, 3 a 3, 4 a 4, 5 a 5, realizados a partir dos resultados correspondentes a todas as combinações possíveis quando são usadas 5, 10, 15, 20, 25 ou 30 cobaias por diluição viral, demonstraram que a variação de mais ou menos 0,5 logaritmo com 95% de segurança, corresponde a 15 cobaias.

UNITERMOS: Vacina antiaftosa; Índice C; Cobaias

REFERENCES

- 01-CAMARGO, N.F.; ELCHORN, E.A.; LEVINE, J.M.; TELLEZ, G.A. A complement fixation technique for foot and mouth disease and vesicular stomatitis. In: ANNUAL MEETING OF THE AMERICAN VETERINARY MEDICAL ASSOCIATION, 870, México, 1950. *Proceedings*, p. 207- 11.

- 02-CUNHA, R.G.; BAPTISTA JUNIOR, J.A.; SERRÃO, U.M.; TORTURELLA, I. El uso de los ratones lactentes en la evaluación de los anticuerpos contra el virus de la fiebre aftosa e su significación inmunológica. *Gac. Vet.*, v.19, p. 243-67, 1957.
- 03-EISSNER, G.; BOHM, H.O. L'épreuve de efficacité des vaccins antiaftoux sur cobayes à l'aide des souches myotropes. *Develop. Biolog. Stand.*, v. 35, p. 279-83, 1976.
- 04-FECHNER, J. Vacunas y vacunación de los animales domésticos. Zaragoza, Acribia, 1966.
- 05-FÉDIDA, M. Étude quantitative de l'état immunitaire post-vaccinal et des interrelations entre ses divers aspects dans une vireose animale, la fièvre aphteuse. Lyon, 1971. These (Docteur) - Université Claude Bernard de Lyon.
- 06-FÉDIDA, M.; DANNACHER, G.; COUBERT, M.; PERRIN, M.; MARTEL, J.L. Le contrôle d'activité des vaccins antiaphteux. *Internacional Symposium of foot and mouth disease*, Lyon, 1976. *Develop. Biolog. Stand.*, v. 35, p. 243-70, 1977.
- 07-FERNANDEZ, A.; GOMES, I.; VIEIRA, A. Control de vacunas antiaftosas. Relación entre el índice K y los índices de seroprotección y seroneutralización. *Bol. Cent. Panamer. Fiebre Aftosa*, v. 6, p. 1-16, 1972.
- 08-FERNANDEZ, A.A.; OLASCOAGA, R.C.; BAHNEMANN, H.G.; ASTUDILLO, V.M.; SONDAHL, M.S.; GOMES, I.; BALTAR, J.; FERNANDEZ, G. Producción y control de calidad de la vacuna antiaftosa en América del Sur. *Bol. Cent. Panamer. Fiebre Aftosa*, v. 51, p. 3-12, 1985.
- 09-GARLAND, A.J.M.; MOWAT, G.N.; FLETTON, B. An evaluation of some methods of assay of foot-and-mouth disease antigen for vaccines. *Intern. Sympos. foot-and-mouth disease*, Lyon, 1976. *Develop. Biolog. Stand.*, v. 35, p. 323-32, 1977.
- 10-GOMES, I.; ASTUDILLO, V. Foot-and-mouth disease: evaluation of mouse protection test results in relation to cattle immunity. *Bol. Cent. Panamer. Fiebre Aftosa*, v. 17/18, p. 9-16, 1975.
- 11-GONÇALVES, E.I. Utilização de camundongos adultos e lactentes na avaliação da eficiência da vacina antiaftosa. Belo Horizonte, 1980. Dissertação (Mestrado) - Faculdade de Veterinária, Universidade Federal de Minas Gerais.
- 12-LUCAM, F.; FÉDIDA, M.; DANNACHER, G. Mesure de l'immunité anti-aphteuse du boeuf, par épreuve sur le cobaye. *Rev. med. vet.*, v. 115, p. 225-45, 1964.
- 13-MACPHERSON, I.; STOCKER, M. Polyoma transformation of hamster cell clones. An investigation of genetic factors affecting cell competence. *Virology*, v. 16, p.147-61, 1962.
- 14-MOWAT, G.N.; MASTERS, R.C.; PRINCE, M.J. Enhancement of immunizing potency of foot-and-mouth disease vaccine for cattle by treatment of the antigen with formaldehyde. *Archiv für die gesamte virusforschung*, v. 41, p. 365-70, 1973.
- 15-REED, L.; MÜNCH, H. A simple method of estimating fifty percent endpoint. *Amer. J. Hyg.*, v. 27, p. 493-7, 1938.

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