EFFICIENCY TEST FOR FOOT AND MOUTH DISEASE VACCINE. II. RELATIONSHIP BETWEEN THE C INDEX IN GUINEA PIGS AND THE MOUSE PROTECTION INDEX

TESTE DE EFICIÊNCIA DE VACINAS ANTIAFTOSA. II. RELAÇÃO ENTRE O ÍNDICE C EM COBAIAS E O ÍNDICE PROTEÇÃO CAMUNDONGOS

Ernani Ibirá GONÇALVES1; Aramis Augusto PINTO2

SUMMARY

The efficiency of six foot and mouth disease vaccines was examined by the Mouse Protection Index and C Index tests using 15 or more guinea pigs per viral dilution for titration. A good correlation and high significance were obtained between the tests. The value of the Mouse Protection Index was approximately double the C Index value. Thus, in view of its low cost and easy execution due to the exclusive use of mice, the Mouse Protection Index can be recommended for testing the efficiency of foot and mouth disease vaccines, especially when a large number of tests is needed.

UNITERMS: Foot and mouth disease; Vaccine; C Index; Mouse Protection Index

INTRODUCTION

With the advent of the first vaccines against foot and mouth disease (VALLÉE et al., 1926; SCHMIDT; HANSEN, 1936; WALDMANN et al., 1937), the difficulty in testing the efficiency of these immunogens was immediately felt (MANNINGER; MOCSY, 1968), as demonstrated by the large number of tests proposed for this evaluation.

Thus, tests using newborn mice were developed (GARCIA MATA et al., 1952; CUNHA et al., 1957; UHLMANN; TRAUB, 1959; CUNHA, 1960; BÜLOW, 1962; EISSNER; KADEN, 1972; TOTH, 1971; SOLYON; DEAK, 1975; GONÇALVES, 1980) to quantify antibodies in the serum of cattle vaccinated against foot and mouth disease.

The C Index (LUCAM et al., 1964) was compared with the K Index (LUCAM; FÉDIDA, 1958) in cattle by FÉDIDA (1971), who detected a highly significant correlation between the two indices. In the present investigation we carried out a comparative study between the C Index performed with a number of guinea pigs per viral dilution capable of reducing the variation of the results to an acceptable level (± 0.5 logarithm) with a 95% probability, and the Mouse Protection Index, with a test based on the exclusive use of mice.

MATERIAL AND METHOD

Mice

White Swiss mice of the P strain were used. The animals, originated from the Institute for Animal Health, Pirbright, England, were reared in the Laboratory of the Ministry of Agriculture and Agrarian Reform (LARA), Campinas.

Vaccines

The six lots of commercial FMD vaccines used were routine batches from different laboratories and had already been tested for efficiency by Ministry of Agriculture and Agrarian Reform. They were prepared with “O”, Campos, “A”, Cruzeiro, “A” Venceslau and “C”, Indaiá strains grown on cells of the BHK21 line (MACPHerson; STOKER, 1962) inactivated with acetylthielenimine (AEI) and containing aluminium hydroxide and saponin as adjuvants.

Challenge virus

Challenges were performed with FMDV strains homologous to those used for vaccine manufacturing, previously adapted to guinea pigs by two or more passages until lesions developed within 24 hours after inoculating into the hind footpads or

---

1 PhD - Faculty of Agrarian and Veterinary Sciences - UNESP - Jaboticabal, São Paulo, Brazil
2 Professor - Faculty of Agrarian and Veterinary Sciences - UNESP - Jaboticabal, São Paulo, Brazil
submitted to passage through mice for a maximum of five times. Viral dilutions of $10^4$ to $10^6$ in phosphate buffered saline (PBS, 0.5 M NaCl, 0.01 M $\text{PO}_4$, pH 7.4-7.6) were used to inoculate the control group and dilutions of $10^3$ 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 and mice for the occurrence of death. The infecting titre of the virus expressed as 50% infecting unit or dose (ID$_{50}$), was calculated by the REED; MUENCH method. The specificity of these viral strains was determined by the complement fixation test according to CAMARGO et al. (1950).

**C Index in Guinea pigs (Cavia cavia)**

The method used of the FMDV vaccine evaluation was the C Index described by LUCAM et al. (1964). Details of the method are given by GONÇALVES; PINTO (1993).

Sixteen or more guinea pigs were used per viral dilution for the titration, which permitted a maximum variation of 0.5 logarithm in the result, with 95% confidence limits.

**Mouse Protection Index (MPI)**

A group of 50 female mice aged 75 to 85 days was vaccinated subcutaneously against FMD with a 0.1 ml dose of the vaccine to be tested and another group of 50 unvaccinated females was used as control. Two days after vaccination, the two groups were mated at the proportion of one male to five females. On the 18th day after mating, the females were housed in individual cages. Those that whelped from the 20th to the 24th day after mating were used for the test together with their pups. On the 29th day after mating, when the pups were five to nine days old, titration was performed by intraperitoneal inoculation of the newborn pups of the two groups with 0.05 ml FMDV, using six females with at least 55 newborn pups per viral dilution, which gave a variation in results of ± 0.5 logarithm, with a confidence limit of 95% (GONÇALVES, 1980). The MPI was determined as the quotient between the titre obtained for the group of vaccinated mothers and the titre of the control group.

**C Index (Cl) x Mouse Protection Index ratio**

To compare the two techniques, six vaccines (I, II, III, IV, V and VI) were submitted to both tests. Vaccines I, II, III and VI were challenged with the “O”, Campos strain and vaccines IV and V with the “C”, Indaiatuba strain of FMDV.

**RESULTS AND DISCUSSION**

The results obtained with the C Index and Mouse Protection Index tests applied to 16 to 30 guinea pigs per viral dilution in the Cl, using FMDV vaccines I, II, III, IV, V and VI are described in Tab. 1.

The ratio of two indices was approximately constant, with an arithmetic mean of 0.53. The standard deviation presented small variation (0.1) and the Pearson variation quotient (18.8%) was medium, indicating that the MPI value is approximately double the CI value.

**TABLE 1**

FMD vaccines and FMDV types used, number of guinea pigs per viral dilution and results of the C Index (CI) and of the Mouse Protection Index (MPI). Cl and MPI ratio. Campinas - SP, Brazil, 1990.

<table>
<thead>
<tr>
<th>vaccine n°</th>
<th>virus</th>
<th>number of guinea pigs</th>
<th>CI</th>
<th>MPI</th>
<th>CI/MPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0</td>
<td>28</td>
<td>2.60</td>
<td>4.40</td>
<td>0.59</td>
</tr>
<tr>
<td>II</td>
<td>0</td>
<td>24</td>
<td>2.25</td>
<td>3.96</td>
<td>0.57</td>
</tr>
<tr>
<td>III</td>
<td>0</td>
<td>24</td>
<td>2.19</td>
<td>3.17</td>
<td>0.69</td>
</tr>
<tr>
<td>IV</td>
<td>C</td>
<td>30</td>
<td>1.34</td>
<td>2.96</td>
<td>0.45</td>
</tr>
<tr>
<td>V</td>
<td>C</td>
<td>30</td>
<td>1.09</td>
<td>2.71</td>
<td>0.40</td>
</tr>
<tr>
<td>VI</td>
<td>0</td>
<td>16</td>
<td>1.36</td>
<td>2.98</td>
<td>0.46</td>
</tr>
</tbody>
</table>

mean ratio: 0.53
standard deviation: 0.10
amplitude of variation: 0.29
coefficient of variation: 18.8%
The correlation between the two indices was determined by the regression line $1.618 + 0.967 \times$, yielding a correlation coefficient $r$ equal to 0.806. The significance of $r$ determined by the Student $t$ test was 4.091 for four degrees of freedom, and the tabulated critical value for 5% was 2.776 and for 1% 4.604, thus showing a good and highly significant correlation.

**CONCLUSION**

A good and highly significant correlation was detected between the C Index value when 15 or more guinea pigs were used per viral dilution in the titration, and the Mouse Protection Index value. Thus, the Mouse Protection Index can be used to test the efficiency of FMDV vaccines because of its low cost and easy execution, especially when large numbers of tests are needed.

**REFERENCES**


06-FÉDIDA, M. Étude quantitative de l'état immunitaire post-vaccinal et des interrelations entre ses divers aspects dans une virose animale, la fièvre aphteuse. Lyon, 1971. These (Docteur) - Université Claude Bernard de Lyon.


Recebido para publicação em 08/09/92
Aprovado para publicação em 10/03/93