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## EFFICIENCY TESTING OF FOOT-AND-MOUTH DISEASE VACCINES PREPARED FROM STRAIN "C" WITH DIFFERENT ADJUVANTS

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### ABSTRACT

Various oil adjuvants were tested for immunogenicity enhancing action in "C" type FMD monovaccine preparations. The following composition proved to be optimal in mouse tests: Bayol F 85%, Arlacel A 14%, Tween 80 1%.

As to the quantitative relations of antigen and adjuvant, 40% oil to 60% antigen was the best among all proportions tested. Oil-adjuvanted vaccines failed to evoke an immune response in calves, but proved to be the preparation of choice for adult cattle. Oil containing and DEAE-dextran-containing vaccines had an equal efficiency in pigs; both conferred full protection on one in three pigs treated with 2.5-fold bovine doses. The number of vaccinated pigs was too small to permit definitive conclusions, but it seems clear that only oil and dextran are efficient adjuvants of FMD vaccines for pigs, and that the antigen component of such vaccines should be increased considerably in order to obtain a greater potency.

### RESUME

Différents adjuvants huileux ajoutés au type "C" de vaccins antiaphteux monovalents ont subi un test destiné à démontrer une augmentation de l'immunogénicité. Dans le test sur souris, les compositions suivantes ont offert les résultats les meilleurs: Bayol F 85%; Arlacel A 14%; Tween 80 1%.

Les relations optimales entre antigène et adjuvant se révélèrent être 40% d'huile et 60% d'antigène. Les vaccins adjuvés huileux n'ont pas déclenché de réponse immune chez les veaux, mais se sont montrés des préparations de choix chez les bovins adultes.

Les vaccins contenant soit de l'huile, soit du DEAE-dextran possèdent chez le porc une efficacité équivalente: les deux confèrent une protection complète chez un porc sur trois traités avec 2,5 fois la dose bovine. Le nombre de porcs que nous avons examinés est trop peu nombreux pour permettre des conclusions définitives; il semble évident toutefois que seuls l'huile et le dextran sont des adjuvants utilisables pour la vaccination des porcins et que la composante antigénique de tels vaccins doit être considérablement augmentée pour que soit réalisée une efficacité valable.

The immunogenicity of foot-and-mouth disease (FMD) vaccines can be considerably increased by the use of appropriate adjuvants. In FMD vaccines prepared for cattle and sheep, the aluminum hydroxide gel adsorbent and the saponin adjuvant provide excellent potency. Preparations for pigs usually contain oil or DEAE-dextran, and oil-containing vaccines have also been recommended for the vaccination of calves. The present experiments were carried out to obtain more information about the influence of various oil-containing adjuvants on the potency of FMD vaccines, and on the role of antigen-to-oil ratio in the stimulation of immune response and finally to

prepare a vaccine with the oil adjuvant found optimal in the experiments and to evaluate it by comparison to preparations otherwise adjuvanted.

## MATERIAL AND METHODS

### *Vaccines*

The experimental vaccines were prepared from type "C" FMD virus, propagated in bovine lingual epithelium cell culture. The virus suspension was inactivated with 0.04% formaldehyde for 41 h at 26°C. After inactivation various adjuvants were added to aliquots of the antigen. The individual bovine dose was 2 ml with each vaccine.

### *Determination of antigen content*

This was carried out by the quantitative complement fixation (CF) test using virus suspensions treated with fluorocarbon (Arcton 113) before inactivation. The antigen content was expressed in terms of complement fixing units (CFU).

### *Adjuvants*

The gel-adsorbed vaccine contained 1.2%  $\text{Al}_2\text{O}_3$ . DEAE-dextran adjuvant was added in the proportion of 250 mg for each 2 ml dose.

The effect of the oil adjuvant was tested in four different compositions, as follows :

- 1) 85% Bayol F + 15% Arlacel A
- 2) 85% Bayol F + 14% Arlacel A + 1% Tween 80
- 3) 70% Bayol F + 30% Arlacel A
- 4) 70% Bayol F + 27% Arlacel A + 3% Tween 80

### *Potency tests*

Two index methods were employed for this purpose.

*E-index method.* The F<sub>1</sub> generation of inbred Balb/c x CBA mice weighing 20-22 g was used. The  $\text{Al}_2\text{O}_3$  adsorbed preparations were administered subcutaneously; those adjuvanted with DEAE-dextran or oil intramuscularly, using one tenth of the bovine dose. The vaccines showing an E-index value of 2.39 or higher were qualified as potent.

*K-index method.* Potency tests were carried out on 2-3 year old cattle and 1-2 month old calves, using a group of four animals for each type of vaccine. The vaccine was classified as potent if its K-index value was 1.2 or higher.

*Pig-tests.* Groups of five pigs, weighing 30-40 kg, were treated intramuscularly with each type of vaccine. In each group of five animals, three pigs were given a 5 ml dose, and two pigs a 2 ml dose of vaccine. 21 days after vaccination two susceptible pigs were infected with type "C" FMD virus, which was administered into the coronary cushion of all four feet. The infected pigs were then placed with the vaccinated mates, and additional two pigs, not treated with either vaccine or virus, were also placed with the group to detect infection by contact. Vaccinees developing symptoms were regarded as positive (unprotected) also in the case when only one foot was involved.

## RESULTS

In the first series of experiments one adsorbed vaccine (1.2%  $\text{Al}_2\text{O}_3$ ) and 16 other preparations, containing oil adjuvants 1-4 in proportions of 10, 20, 40 and 50%, were compared for immunogenic efficiency. The results are summarized in Table I. As can be seen, the vaccines prepared with the oil adjuvant No 2 were most immunogenic in mice. As to the quantitative relation of antigen to adjuvant the best results were always obtained with 40% adjuvant. The data of Table I also show that reduction of the antigen content by 12.34, viz. 45% did not generally depress the immunogenicity

*Table I.* Comparative studies of the effectiveness of type "C" foot-and-mouth disease vaccines containing different adjuvants with E-index method in adult mice

| Adjuvant                 | Proportions of adjuvant |      |      |      |
|--------------------------|-------------------------|------|------|------|
|                          | 10 %                    | 20 % | 40 % | 50 % |
| Oil (1)                  | 3.26                    | 3.74 | 4.80 | 4.3  |
| Oil (2)                  | 4.0                     | 4.0  | 4.80 | 4.80 |
| Oil (3)                  | 3.58                    | 4.16 | 4.53 | 4.30 |
| Oil (4)                  | 4.0                     | 3.06 | 4.80 | 4.30 |
| Al (OH) <sub>3</sub> gel | -                       | -    | 4.80 | -    |

of the vaccine, if the adjuvant content was simultaneously increased. The values of the E-index were in fact higher for those vaccines in which the antigen content was reduced by 34-45%, but the adjuvant was made up to a correspondingly higher level than for those containing more antigen but only 10% adjuvant.

In the second experiment type "C" monovalent vaccines containing 228.8 CFU antigen in each 2 ml dose, were used. After inactivation of the antigen, four different types of adjuvant were added, as follows :

- (a) aluminum hydroxide gel 40%
- (b) aluminum hydroxide gel 20% + oil (2) 20%
- (c) oil (2) 40%
- (d) DEAE-dextran 40%

*Table II.* Comparative studies of the effectiveness of type "C" foot-and-mouth disease vaccine with K-index method in calves and cows, with contact challenge in pigs and with E-index method in adult mice

| Adjuvant                           | Value of K-index |      | Response of pigs treated |           | Value of E-index |
|------------------------------------|------------------|------|--------------------------|-----------|------------------|
|                                    | Calves           | Cows | 5 ml dose                | 2 ml dose |                  |
| Al (OH) <sub>3</sub> gel           | 0.98             | 1.37 | +++                      | ++        | 2.27             |
| Al (OH) <sub>3</sub> gel + Oil (2) | 0.38             | 1.5  | +++                      | ++        | 3.8              |
| Oil (2)                            | 0.0              | 2.43 | ++-                      | ++        | 5.13             |
| DEAE-dextran                       | -                | -    | ++-                      | ++        | 1.8              |

The "C" monovaccines prepared with adjuvants (a), (b) and (c) were tested in cows, calves and pigs, while the DEAE-dextran (d) containing preparation was tested only in pigs. The results are shown in Table II. The aluminum hydroxide-adsorbed preparation proved to be most immunogenic for calves, but its K-index was still lower than 0.22, the K-index value required for the first-class qualification of FMD vaccine. All four experimental preparations conferred satisfactory immunity upon adult cattle but the oil-containing preparations were relatively superior. One pig in three treated with 2.5-fold bovine dose either of the oil-containing or dextran-containing vaccines developed immunity to FMD.