

## STUDIES ON THE RAPID PRODUCTION OF FOOT AND MOUTH DISEASE HYPERIMMUNE ANTISERA IN GUINEA PIGS

by

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### SUMMARY

Two series of experiments were set up to investigate the production of FMD guinea pig antisera, of a high titre quality and in the shortest possible period of time.

In the first experiment, type A22 hyperimmune antiserum was prepared, using various dilutions of concentrated antigen combined with Freund's complete adjuvant. The highest serum titre was obtained by inoculating 1/2 dilute virus combination; giving two inoculations in an interval of 28 days and bleeding the animals 7 days after the last inoculation.

In the second experiment adjuvant effects of Quil A, Mycobacterium tuberculosis and Pumice powder were compared in regard to their ability to enhance type 0 1 antiserum titre.

The results of these experiments indicated that, Quil A was more effective than the other adjuvants used.

### INTRODUCTION

In the past different methods had been used to prepare hyperimmune antisera against FMDV types. Despite the use of different adjuvants and the time consuming methods, the resulting antisera had been of low titres (1,2).

Ever since of their introduction in 1961(3) the oil adjuvants, as enhancing agents for immunological responses of inoculated animals, have been widely used in FMD vaccine production.

The purpose of the present studies was to investigate the effects of different adjuvants to enhance the immunological responses of guinea pigs in preparation of FMD antisera, with this in mind to find the most suitable combination for obtaining a high titre antiserum in the shortest period of time.

## MATERIALS AND METHODS

### a) *Virus production and concentration (types A22 and 0 1)*

A22 and 0 1 types of FMD virus propagated in monolayer cultures of BHK 21 cells were clarified by the centrifugation at 10000 RPM for 1/2 hour. The clarified viruses were separately mixed with 7.5g% Polyethylene glycol 6000 (PEG) and the mixtures were stirred at 4°C for 4 hours (6). The mixtures were centrifuged at 6000 RPM for 1/2 hour, and sediments were resuspended in phosphate buffered saline, at pH 7.6 containing 1% inactivated normal guinea-pig serum corresponding to 25 fold concentration, separately.

b) Freund's complete adjuvant was prepared by thoroughly mixing light paraffin oil with surface emulsifier Arlacel A. The ratio of liquid paraffin to emulsifier was 8.5 : 1. 1mg/ml of heat killed Mycobacterium Tuberculosis cells (obtained from the Department of Bacteriology, Razi Institute) were ground and added to the above emulsion and suspended homogeneously (4,7).

c) Quil A: The material was supplied through the courtesy of K. Dalsgaard from Lindholm Institute, Denmark. Quil A was the only fraction of saponin drug (Quillaja Saponaria Molina) with a high adjuvant activity in FMD vaccine (5,8,9).

d) Pumice powder was obtained from Bacteriology Department of Razi Institute.

### Preparation of inocula:

Experiment 1) Various dilutions of concentrated virus (type A22) were mixed with 5% Tween 80 respectively. Amounts of Freund's complete adjuvant equal to each antigen dilution were added and well emulsified.

### Experiment 2:

. An amount of 200 µg Quil A powder (dissolved in distilled water) per guinea pig was mixed with combined paraffin oil and concentrated virus (type 0 1) containing 5% Tween 80.

. Equal volume of virus (type 0 1) was combined with an equal volume of Freund's complete adjuvant.

. An amount of 7mg/ml Pumice powder was also mixed with the combination of paraffin oil and concentrated virus (type 0 1).

All the final combinations were homogeneously emulsified by using either Turrax grinder or two syringes connected by a narrow tubing, one syringe being filled with antigen solution and the other with an equal amount of adjuvant; the plungers were raised and depressed repeatedly for a minimum of 10 minutes (7).

Immunization procedure:

The above combinations were each inoculated in 3 guinea pigs (0.1ml per foot pad of all feet, front and rear). 3 guinea pigs were used as controls in each experiment, receiving equal volume of placebo. The placebo in the first experiment was concentrated antigen and in the second one a mixture of antigen and paraffin oil. 28 days later the animals received a booster (0.4ml) subcutaneously in the leg. They were bled out 7 days after the last inoculation.

Serum titration :

The titre of each serum was determined by Complement fixation test according to Kolmer's method (100% hemolysis) 11.

## RESULTS AND DISCUSSION

The titres of the sera obtained from the guinea pigs hyperimmunized with various adjuvanted antigens are summarized in tables 1 and 2.

Table 1- Titres of sera from guinea pigs inoculated with various dilutions of A22 strain of FMD Virus combined with equal volume of Freund's complete adjuvant.

Virus dilutions	Freund's complete adjuvant	Serum titre
Undilute	+	320
1/2	+	500
1/4	+	250
1/8	+	250
Undilute	—	60

Table 2- Titres of sera from guinea pigs inoculated with combined oil emulsion, 0 1 strain of FMD Virus and various adjuvants.

Combination	Adjuvant	Serum titre
Virus (type 0 1) + Oil	Quil A	300
» »	M. Tuberculosis	240
» »	Pumice powder	200
» »	—	180

The results indicated that Freund's complete adjuvant when mixed at a proper ratio with antigen had a high adjuvant activity.

The use of Quil A in an oil emulsion resulted also in a very satisfactory immunological response. In comparing these two adjuvants, by taking into consideration the risk of contamination with the bacteria used in Freund's complete adjuvant, the preference should be given to the Quil A combined with oil emulsions in preparation of FMD hyperimmune antisera.

The procedure was not time consuming and, relatively, a high titre antisera could be obtained within 35 days.

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## RESUME

Deux expériences sur la préparation des sérums hyperimmuns ont été présentées dans cet ouvrage.

1- La préparation d'un sérum hyperimmun anti virus aphteux type A22 à l'aide de différentes dilutions de virus concentré de l'adjuvant complet de Freund.

28 jours après la première injection on réalise la deuxième inoculation, 7 jours plus tard les animaux sont saignés.

Le meilleur titre de sérum a été donné par les animaux rechargés avec un virus à la dilution du 1/2 injecté simultanément avec l'adjuvant complet de Freund.

2- La deuxième expérience a portée sur une comparaison entre les différents adjuvants suivants: "Quil A, Mycobactérium-Tuberculosis et Poudre de pierre Ponce". Ceci dans le but d'améliorer le titre d'un sérum hyperimmun de type 0 1 de virus aphteux.

Les résultats montrent que l'adjuvant "Quil A" est plus efficace que les autre adjuvants utilisés dans cette expérience.