DEVELOPMENT OF A FOOT-AND-MOUTH DISEASE VACCINE POTENCY TEST WITHOUT CONDUCTING ANIMAL CHALLENGE EXPERIMENT

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ABSTRACT

Introduction
Vaccine manufacturers evaluate the efficiency of their vaccine according to the method which is defined in the European Pharmacopoeia. Regarding foot-and-mouth disease (FMD) vaccine there are some difficulties to find animals for potency tests in the countries like Turkey where FMD is endemic and vaccine campaigns are carried out. In addition, potency tests must be carried out in containments having high biosecurity levels. There are many publications indicating a correlation between protection from virus challenge and neutralizing antibody response. However, up to now, none of the suggested method has been found valid.

Materials and methods
An analysis was made of data from potency tests on four batches O1 Manisa and two batches of Asia-1 Tur 73 monovalent oil adjuvanted foot-and-mouth disease vaccine. Regression were calculated for the relation of protection from virus challenge versus antigenic load (Log 146S) and neutralizing antibody response (Log SN50), versus only Log 146S, versus only Log SN50.

Results
For the relation of protection from virus challenge versus Log 146S and Log SN50, R square was determined as 0,809 for O1 Manisa vaccines, 0,866 for Asia-1 Tur 73 vaccines. In addition, the amount of required antigen for % 50 protection in O1 Manisa and Asia-1 Tur 73 vaccines was found 1,15 µg and 0,75 µg respectively.

Conclusion
We have shown that Log SN50 and Log 146S can give good correlation with protection from virus challenge and can be used as an indirect indicator of potency.

1. INTRODUCTION

Foot-and-mouth disease (FMD) is highly contagious and economically important viral disease of cloven hoofed animals due to international trade restrictions and loss of productivity (OIE, 2004). European manufacturers have to perform a number of tests including potency to evaluate vaccine quality. Potency of FMD vaccines in Europe have been assessed by the method described in European Pharmacopoeia (EP) Monograph 04/2005:0063 (Ph. Eur., 2006).

Non-vaccinated and non-infected 17 cattle which were free from FMD antibodies and at least 6 months old are used for potency control of FMD vaccines (Ph. Eur., 2006). It is difficult and costly to find these animals in countries like Turkey where the disease is endemic and routine mass vaccination is operational.

Although cattle challenge method is accepted as a gold standard for potency evaluation of FMD vaccines, it has some disadvantages such as high cost, difficulties of finding suitable animals, requirements for high security laboratories and people's growing awareness on animal welfare. Therefore many researchers are trying to develop an alternative method. However no method has been accepted complete until now.
The well established test for assessing antibodies to FMD vaccines is virus neutralization test (VNT). A good correlation was reported between VNT and protection (Ahl et al., 1990, Pay and Hingley, 1992b). Another alternative test to estimate a potency of vaccine could be antigen payload of the vaccine measured by 146S density gradient procedure. The intact 146S particle is essential for efficacy of a vaccine and is one of the most important parameter for calculating antigen payload within a vaccine formulation. The test's precision and sensitivity is high and very straightforward for operators. Finally, some elements of the procedure can easily be standardised as demonstrated by the author and colleagues with an international joint project involving all of the main FMD laboratories at that time. Vaccine manufacturers routinely use it for vaccine formulation. Many manufacturers have been already set a correlation between 146S concentration and protection (Doel, 2003).

In this study it was investigated whether there was any statistical relationship between protection from virus challenge, antigenic load and neutralizing antibody response, and whether it was significant enough to omit animal challenge experiment.

2. MATERIALS AND METHODS

Vaccine
FMDV antigen was produced in BHK21 cell culture, clarified and inactivated using binary etyleneimine. Inactivated antigen concentrated by PEG 6000. Then the antigen formulated with Montanide ISA 206 (Seppic/France) 1:1 to obtain ready to use DOE vaccine. Sterility and safety tests were performed to ensure suitability for using in the field.

In this study, four series of O1 Manisa and two series of Asia-1 Tur 73 monovalent oil adjuvanted FMD vaccine with different concentrations of 146S were used (Figure 1).

Cattle
Holstein-Friesian, 8-12 months old, free from FMD antibodies, non-vaccinated cattle were provided from a state farm under strict veterinary control.

Potency Test
Cattle were divided into 3 groups, each consist of five animals for potency testing of each series. Full dose (2 ml) administered to five animals by intramuscular route. Five animals received 1/4 dose (0, 5 ml) and other five animals 1/16 dose (0,125 ml) of vaccine. 2 control animals remained non-vaccinated. Animals were challenged by two separate intradermolingual inoculation with 10^4 bovine infective dose 50 of fmdv on day 28th post vaccination. They were monitored for 8 days after challenge with daily records of rectal temperature and clinical examinations for specific lesions.

146S Antigen assay
The concentration of 146S of each series was measured by method described by Doel et al. (1981).

Virus neutralizing antibody assay
The cattle were bled at 28. Days after vaccination for serum neutralising antibody assay. Virus neutralisation test were performed against O1 Manisa and Asia-1 Tur 73 homologue viruses by using BHK 21 cell culture as described in World Organisation for Animal Health (OIE) Manuel of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE, 2004).

Statistical analysis
Linear regression models were used to predict protection by 146S and SN50. Pearson correlation analysis was performed to relationship between 146S and SN50.

3. RESULTS

Neutralising antibody titers and the results of potency tests against four series of O1 Manisa and two series of Asia-1 Tur 73 at the day 28 after vaccination were shown on Figure 1.

Relationship between protection and Log 146S
The relationship between protection and Log 146S value was shown at Figure 2 and 3. Both type O1 Manisa and Asia-1 Tur 73 regressions was statistically significant (p<0.01 for both types). However R square was determined as 0.866 for Asia-1 Tur 73 whereas type O1 Manisa was 0.613. It was concluded that to achieve a 50% protection level was needed 1, 15 µg O1 Manisa and 0.75 µg Asia-1 Tur 73 146S antigen.

Interrelationship between protection, 146S and Log SN50

Interrelationship between protection and two variables Log 146 S and Log SN50 were analysed. R square was calculated 0.809 for type O1 Manisa and 0.866 for Asia-1 Tur 73 according to analysis. This has shown that there was a strong correlation between protection, Log 146S and Log SN50 (O1 Manisa p<0.01, 01, Asia-1 Tur 73 p<0.05).

From the data in Fig. 1, the protection rate can be determined according to the following formula

for O1 Manisa

\[ \text{Protection} = -0.077 + 0.177 \text{Log}146S + 0.487 \bar{x} \]

for Asia-1 Tur 73

\[ \text{Protection} = 0.521 + 0.554 \text{Log}146S + 0.036 \bar{x} \]

where \( \bar{x} = \frac{\sum_{i=1}^{n} \text{Log}SN50_i}{n} \)

in which 146S is defined as measured antigen concentration of each batch vaccine by the density gradient procedure and \( \bar{x} \) is defined as arithmetic mean of neutralizing antibody response of animals which vaccinated with each batch vaccine. As a result, formula 1 means 100% protection and 0 means 0% protection.

4. DISCUSSION

FMD vaccine potency testing is a highly important, but expensive matter. Due to its high variability and low repeatability it only yields an approximate estimate of the PD50 vaccine content. A number of researchers have tried to develop alternative approaches which use both animal models and in vitro methods (Barnett, 2003a). Furthermore according to Pay and Hingley (1987) a correlation could be found between antigen load in vaccine (146S) and protection. In this study we design a strategy seeking a correlation among SN50, antigen payload and protection in order to omit animal challenge experiment.

One promising alternative of potency test is VNT. According to homologous challenge study of Ahl et al. (1990) the BHK21-CT cell used titres was low compared to the IBRS2 cells used, but correlated well with protection. Indeed, only 2 of the 85 were protected had low challenge virus specific neutralizing antibodies in this study. Many factors can effect of VNT titres of a serum: the cell substrate; the maturity of cells, medium and pH, the virus dose; the antigenic relationship of the assay virus to the vaccine virus; whether serum dilutions are encountered before or after with the virus inoculum; etc. Therefore, different laboratories may have different antibody pass-levels or log_{10} serum titers. (Barnett, 2003b). Pay and Hingley (1992a) announced that there is a big difference existed in the correlation of antibody to protection between laboratories, particularly in the case of the O serotype. Hence every laboratory should set their own correlation if VNT was thought as the alternative method. Moreover 146S density gradient procedure is extremely susceptible, reliable, reproducible and straightforward test. By setting the correlation, Log 146S only, or combine with SN50 value, may assess the potency indirectly.

Nevertheless as the VP1 polypeptide composes only \( \approx 20\% \) of total 146S virion and as the neutralizing epitopes most likely compose less than 10% of total VP1 polypeptide, it can be deduced that a few hundred picograms of epitope of some strains in vaccine can be anticipated to protect 50% of cattle (Pay and Hingley, 1987). Therefore, same amount of 146S can not be given same percentage of protection because of stably of 146S or portion of VP1 in 146S concentration.
Numerous authors and manufacturers accepted that more antigen is needed for protection against type O than other types (Doel, 2003, Pay and Hingley, 1987) Same results were obtained in this study, for 50% protection, 1.15 µg and 0.75 µg of antigen of O1 Manisa and Asia-1 Tur 73 type were needed respectively (Figure 2 and 3). A few previous publications which have given estimates for the 140S concentrations which will supply 50% protection of cattle were produced that by Pay and Hingley (1987) who reported an estimate 220 ng for type O, BFS strain vaccine, and that Mowat (1972) gave estimate 6 ng for a type Asia-1 Israel 3/63 strain vaccine. These differences could come from strain variations or different process of manufacturers used in production.

Manuel of Diagnostic Tests and Vaccines for Terrestrial Animals points out that the expected percentage of protection in indirect tests should be equal to or greater than 75% when 16 animals are used or 70% when 30 animals are used in the experimental group (OIE, 2004). In this study 3, 55 µg and 2, 03 µg of 146S of O1 Manisa and Asia-1 Tur 73 strain were needed respectively for 75% protection. If Log 146S and Log SN50 are used together to predict the potency, expected percentage of protection can be used as 70% when 30 animals are used.

We strongly recommend using 146S assay only or some serological tests which together increase the reliance on estimating potency of specific vaccine batch. They can also be assembled with an interconnected method which is based on the 146S concentration of the final vaccine batch.

5. CONCLUSIONS

Log SN50 and Log 146S can give good correlation with protection from virus challenge and can be used as an indirect indicator of potency.
To release FMD vaccine batch by an alternative test of European Pharmacopoeia vaccine PD50 test is recommended.
Only 146S test or combined with VNT as an alternative of potency is enough to omit animal challenge experiment.

6. RECOMMENDATION

Researches on other types are needed.
An in-vitro method should be developed and standardized for monitoring and measuring the antigenic integrity of 146S.

7. ACKNOWLEDGEMENTS

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8. REFERENCES


Figure 1: Potency test data for O1 Manisa (A) and Asia-1 Tur 73 (B)

Figure 2: Relation of protection from virus challenge versus Log146S for O1 Manisa
Figure 3: Relation of protection from virus challenge versus Log146S for Asia-1 Tur 73