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# THE EFFECT OF PURIFICATION AND CONCENTRATION OF FMD VIRUS USING POLYETHYLENE GLYCOL ON ITS ANTIGENIC AND IMMUNOGENIC PROPERTIES

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## SUMMARY

Foot and mouth disease virus was propagated on BHK21 and purified by polyethylene glycol (PEG) precipitation. The antigenic properties of the virus before and after purification were studied by infectivity, complement fixing activity and protein assay tests. Inactivated FMD vaccines were prepared from the purified antigen and the crude one. The potency of both vaccines was tested in guinea pigs. The results revealed that the process of PEG precipitation of the virus will improve the quality of antigen used in vaccine formulation to produce a good quality FMD vaccine.

## INTRODUCTION

Foot and mouth disease is a highly contagious viral disease of cloven-hoofed animals, which has considerable socio-economic impact on the country income (Zhang et al., 2002). Seven types of Foot and Mouth Disease virus in the genus aphthovirus, family Picornaviridae (Davies 2002), cause the disease. There are about 65 subtypes within the seven types (Pereira 1977). The disease has occurred in Egypt in all susceptible livestock causing drastic losses in milk and meat production with deaths, especially among young animals (Daoud et al., 1988).

FMD virus harvest is extremely impure and contains large quantities of cellular proteins and media constituents relative to the concentration of 146S particles. These impurities can be determinal to the safety, quality and efficacy of FMD vaccines in numbers of ways. First: cellular proteases may cleave a number of sites in VP1 protein of the virus capsid reducing considerably the ability of the vaccine to stimulate neutralizing an-



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tibodies. Second: there is some evidence to suggest that the quality of FMD specific antibodies induced by a vaccine is adversely affected by the presence cellular proteins derived from the production system. Finally, impurities in a vaccine may cause local and systemic adverse reactions in the target species. Therefore, significant benefits to purify FMD virus harvests are highly needed (Doel 1999). FMD virus is poorly immunogenic. So, there is a need to improve the quality of the vaccine by incorporating purified virus antigen to prevent sporadic breakdown of immunity in regularly vaccinated organized herds (Sen and Saha 1994).

Binary ethylenimine inactivated aluminum hydroxide gel vaccine was prepared with purified ten fold concentrated virus antigen. The potency test conducted in cattle gave 100% protection with 1 ml concentrated vaccine (Sen et al., 1985).

Precipitation of macromolecular proteins such as viruses by high molecular weight polyethylene glycol 6000 (PEG) is an effective concentration method because the viruses are slowly precipitated in a cold, high - salt environment which protects them from chemical and physical denaturation, PEG is more effective than ammonium sulfate precipitation (Killington et al., 1996).

Concentration and purification of antigen has many advantages as follows (1) large quantities of monovalent product must be stored at 4°c until the results of bacteriological and safety tests become available and the vaccine can be formulated, (2) Concentrated vaccine can be stored in smaller cool units, (3) If the vaccine contains purified antigen, adverse reactions after vaccination will be reduced (Barteling and Vreeswijk 1991).

The purpose of this work is to separate the virus in a purified and concentrated from the host cells in which it has grown and studying the antigenic and immunological activity of FMD virus before and after purification by PEG 6000. Infectivity, complement-fixing activity, protein content of the PEG purified FMD virus are described. FMD vaccine has been prepared with PEG purified antigen and its potency in guinea pigs were compared with a vaccine prepared form crude virus.

## MATERIAL AND METHODS

### 1. Virus: -

Locally isolated Foot and Mouth Disease virus type O1/1993was propagated in BHK-21cell monolayer and grown in roller bottles as described by (Ubertini et al., 1967).

### 2. Tissue culture: -

Baby hamster kidney (BHK) cells clones 13 were propagated in FMD department, Veterinary Serum and Vaccine Institute, Cairo using Eagleís MEM with 10% newborn calf serum.

### 3. Animals: -

Healthy adult guinea pigs of 400-500 gm body

weight each, were used for vaccine potency and obtaining fresh complement used for complement fixation test.

**4. PEG 6000:-**

Polyethylene glycol 6000, Fluka chemie GmbH; No.81260.

**5. Elution buffer:-**

2.42 g TRIS

22.5 g KCL

Add demi-water up to 1 liter.

**6. Micro BCA protein Assay Reagent Kit:-**

No.23235 Pierce, USA.

**7.Virus Purification and concentration: - according to (Killington et al., 1996).** The harvested fluid from the infected BHK-21 cell cultures was centrifuged by cooling centrifuge at 3000 rpm for 20 minutes to remove cell debris. PEG 6000 was added with stirring to 7% final (w/v) concentration and the mixture was placed at 4°C for 2 hours to ensure complete solubilization of the PEG then transfer the mixture to a refrigerator and allow the virus to precipitate overnight at 4 °C. The mixture was centrifuged at 6000 r.p.m. for 20 minutes then the supernatant was discarded. Then the precipitate was resuspended to the original volume in elution buffer. Insoluble debris was removed by centrifugation at 3000 r.p.m. for 20 minutes.

**8. Vaccines:** - according to ( Sen and Rao 1990). Crude and PEG purified FMD virus was inactivated 0.1% M bromoethylein amine hydrochloride (BEA; Sigma) at 37 oc for 24 hours at pH 8.0.At the end of inactivation period, residual BEA was neutralized by 2% sodium thiosulphate and 100 ml of the inactivated virus were added to 30 ml of aluminum hydroxide gel. The pH of the vaccine was adjusted to 8.0.Saponin was added to both vaccines to give a final concentration 2 mg / dose.

**9. Assay procedure:-**

Infectivity assay for both crude and purified virus was performed in BHK21 cell culture according to (Mahy and Kangaro 1996).

Complement fixing activity of both viruses was estimated according to (Traub and Manso 1944) to determine the antigenicity of the viruses.

Protein content of FMD virus before and after treatment with PEG 6000 was estimated by using Micro BCA protein Assay Reagent Kit.

The potency for both vaccines was tested in guinea pigs according to (Terpestra 1974). The prepared vaccines were diluted in four-fold dilution (undiluted, 1/4, 1/16, 1/64) and 0.5 ml from each dilution was inoculated into each group of guinea pigs by subcutaneous route. 21days post inoculation, the animals were challenged by 10,000 MLD50 guinea pig-adapted virus. All animals checked daily for signs of generalization for 3-5 days. Guinea pig protective dose 50 (GPPD50) was calculated according to ( Reed and Muench 1938).



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## RESULTS

**Table (1):** Assays for crude and PEG purified virus preparations.

| Assays | Crude virus | PEG purified virus |
|---|---|---|
| Infectivity $Log_{10}$ $TCID_{50}$/ml | 8.2 | 7.9 |
| CF activity* | 1/8 | 1/4 |
| Protein mg/ml | 16.8 | 2.1 |

* CF: Complement fixing activity = antigen Dilution giving 50% heamolysis

**Table (2):** Potency test of vaccines prepared with crude and purified FMD virus

| Vaccine | Dilution of vaccine** | | | | | VaccineCalculated $GPPD_{50}$ in $log_{10}$ | Number of $GPPD_{50}$ |
|---|---|---|---|---|---|---|---|
| | Undiluted | 1/4 | 1/16 | 1/64 | control | | |
| Crude vaccine | 0/3 | 1/3 | 2/3 | 2/3 | 3/3 | 1.05 | 11.22 |
| Purified vacine | 0/3 | 0/3 | 1/3 | 2/3 | 3/3 | 1.5 | 31.6 |

** Numbers of guinea pigs showed generalization over total number of challenged animals.

## DISCUSSION

Vaccination is considered to be an important control policy for foot and mouth disease in endemic areas with advanced eradication programs, as well as, in free regions that decide to use immunization as control measure after recent introduction of the disease ( Bergmann et al., 2003).

One of the crucial requirements for production of inactivated virus vaccines is the viral antigen, which must be available in high concentration and in large enough quantities (Barteling and Vreeswijk 1991).

The results obtained in table (1) revealed that recovery of infective virus and complement fixing activity of the PEG purified preparations was approximately 100% and 50% respectively of that in crude antigen. These results were agreed with



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(Panina and De Simone 1973) who stated that about 100% of infectivity and 60% of CF were recovered. The protein content was reduced to approximately 90% of that originally present and this is agreed with (Panina and De Simone 1973) and who stated that the protein content was reduced to10% of the origin. Barteling and Vreeswijk (1991) reported that one precipitation step with PEG is sufficient to remove allergenic components from BHK vaccines where they found that 95% of the proteins from the virus culture harvest are removed.

In table (2) potency of the vaccine prepared from PEG purified FMD virus was greater than that prepared from crude one and this is agreed with (Iyer et al., 2001) who found that vaccines formulated with virus purified with 8% polyethylene glycol were more immunogenic than the vaccines formulated with untreated harvest virus. These results are also supported by (Black et al., 1985) who stated that when oil emulsion FMD vaccines were tested by GPPD50 ,results giving 15 PD50 per dose or more are considered good results while results giving less than 5 PD50 per dose are considered low. Such data is also agreed with (Nair and Sen 1993) who found that the PEG concentrated gel vaccine was of comparable immunogenicity to oil adjuvanated vaccine.

Finally, the process of PEG precipitation of the virus will improve the quality of antigen used in vaccine formulation to produce a good quality FMD vaccine which is the most significant factor in the eradication of foot and mouth disease.

## REFERENCES


Barteling S.J and Vreeswijk J. (1991):Developments in Foot and mouth disease vaccines.Vaccine vol. 9,February, 75-88.

Bergmann IE,Malirat V,Neitzert E and Correa Melo E (2003):Vaccines and companion diagnostic tests for foot and mouth disease virus, an overview of the experience in South America.Dev Bio (Basel). 114:59-65.

Black,L., Pullen,A.and Boge,A., (1985): Guinea pig protection test as indicator of potency of oil emulsion FMD vaccine Res. VET. Sci. 39,207-211.

Daoud A;OmarA; El bakry M ;Metwally N; El Mekkawi M and El Kilany, S (1988):Strains of FMD virus recovered from 1987 outbreak in Egypt. J. Egypt Vet. Med. Assoc. 48(1) 69-71

Davies G.(2002): Foot and Mouth Disease.Res. Vet. Sci. Dec; 73(3): 195-9.

Doel T.R. (1999): Optimisation of the immune response to foot and mouth disease vaccines.Vaccine 17 . 1767-1771.

Iyer A.V; Ghosh S;Singh S.N and Deshmukh R.A. (2001):Evaluation of three ready to formulate oil adjutants for FMD vaccine production.Vaccine 19 1097-1105.

Killington RA,Stokes A and Hierolzer (1996):Virology methods manual; chapter 4 ;72-89 Academic press Ltd;ISBN 0-12-465330-8.

Mahy,B.w.J.and Kangaro,H.o.(1996): Virology methods manual (textbook), Academic press, Harcourt




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Brace,London,New York.

Nair-SP and Sen-AK (1993):A comparative study of the immune responses of sheep against foot and mouth disease virus types Asia1 and O PEG-concentrated aluminum hydroxide gel and oil adjuvanated vaccines.Vaccine 11:7,782-786:23ref.

Panina G.F.and De Simone F. (1973): Imnological activity of FMD virus purified by polethylene glycol precipitation. Zbl. Vet. Med B, 20 733-782.

Pereira HG (1977): Subtyping of FMD virus International Symposium on FMD; Lyon, France, 5-8 October pp. 167-174.

Reed,L.J and Muench ,H (1938):A simple method of estimating fifty percent end points. Am.J. Hyg.27: 493-497.

Sen-AK, Negi-BS and Rao-BU (1985):A simple method for dose volume reduction of Foot and Mouth Disease vaccine in cattle. Indian Vet. Journal 62:5,362-364.

Sen-A.K. and Rao B.O.(1990):Large scale production of foot and mouth disease type A22 vaccine with partially purified and concentrated virus antigen. Indian Vet.J. 67,March,281-282.

Sen-AK; and Saha-AN (1994):Development of an effective vaccine against foot and mouth disease with partially purified and concentrated virus antigen. Acta-Virol Feb; 89(1) 17-9

Terpestra, C; (1974):Comparison of laboratory techniques for the evaluation of foot and mouth disease vaccines. Research group of European commission for control of FMD, Lelystad, Netherlands, pp22-24.

Traub,E and Manso,I.R.(1944):Uber die Herstellung Komplement Bindender Meersch-Weinchonsera Fur die Typendiagnose die Maul und Klauenseuche.Zbi.Bakt. lorig, 151:380-381.

Zhang ZD;Hutching G;Kitching P and Alexandersen S (2002): The effects of gamma interferon on replication of FMD virus in persistently infected bovine cells. Arch. Virol. Nov; 147(11) 2157-67.

Ubertini,B; L.Nardell, A.Dal Prato G and S. Barei, (1967): BHK21 cell cultures for the large-scale production of foot and mouth disease virus.Zbl. Vet. Med. B, 14, 432-411.



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