DEVELOPMENT OF AN EFFECTIVE VACCINE AGAINST FOOT-AND-MOUTH DISEASE WITH PARTIALLY PURIFIED AND CONCENTRATED VIRUS ANTIGEN

A. K. SEN, S. N. SAHA

Indian Veterinary Research Institute, Bangalore Campus, Bangalore 560 024, India

Received May 14, 1993; revised July 30, 1993

Summary. – Foot-and-mouth disease (FMD) virus is poorly immunogenic. There is need to improve the quality of the vaccine by incorporating enhanced quantity of purified virus antigen to prevent sporadic breakdown of immunity in regularly vaccinated organized herds. A technique has been standardized for virus purification and concentration by polyethylene glycol (PEG) treatment for large scale production of concentrated FMD vaccine. The vaccine prepared with tenfold concentrated antigen was given field trial in an organised farm with a reduced dose as compared to the conventional vaccine. High level serum neutralizing antibody in cattle was observed throughout the entire period of study. The concentrated vaccine controlled the spread of the disease when used in face of outbreak in villages adjacent to the organized farm.

Key words: foot-and-mouth disease vaccine; polyethylene glycol; virus concentration; BHK-21

Introduction

FMD is endemic in India and repeated vaccination is recommended for better protection against the disease. Even after regular vaccination there are reports of breakdown of immunity in organized farms as the virus is poorly immunogenic. There is need to improve the existing vaccine by increasing its antigen content to enhance the immune response for a reasonable period. Since it is difficult to increase the yield of FMD virus in BHK-21 cells appreciably by manipulating various factors the attempts have been made to develop an easy method for concentration of FMD virus and production of concentrated vaccine for cattle on industrial scale.

Materials and Methods

Cell and virus production. BHK-21 cells were grown in suspension in batches in 300 l fermentors using Eagle's medium (MEM-G) with 8 % PEG-treated bovine serum. The same medium with 5 % tryptose phosphate broth and 2 % PEG-treated serum was used for virus growth. A number of batches of FMD virus type O, A22, C, and Asia-1 were produced in the fermentors. These virus batches were treated with 1 % chloroform, clarified at 7 500 rpm and filtered through Seitz K5 filter pads.

Purification and concentration of virus. The filtered virus was precipitated by adding 8 % PEG (M_r 6 000) and kept at 4 °C overnight. The virus was processed for tenfold concentration with 0.05 mol/l Tris buffer. 10 l bell filter with one K5 filter pad and large Orion filter with five filter pads (40 × 40 cm) were used for filtration of 20 l and 300 l batches of precipitated virus, respectively (Sen and Rao, 1986; Sen and Rao, 1990). This procedure ensured total retention of the PEG precipitated virus on the filter pads.

Virus inactivation was carried out with binary ethyleneimine (BEI) at 37 °C for 20 hrs (Bahenemann *et al.*, 1974).

Preparation of aluminium hydroxide saponified Alhydrogel vaccine was done by the method of Sen and Rao (1990). A dose of 4 ml polyvalent vaccine incorporating FMD virus types O, A22, C and Asia-1 was recommended for cattle.

Innocuity and potency test of Alhydrogel vaccine. To test the innocuity of the vaccine for cattle two susceptible animals were inoculated intradermolingually 2 ml dose of the vaccine into the tongue and observed for 10 days. Potency tests were conducted in both guinea pigs and in cattle (Sen and Roa, 1990).

Serum neutralization (SN) test was performed in 96 well Nunc disposable plates, following the method of Rweyemamu and Hingley (1984). 50 µl of inactivated serum was added in each well starting from dilution 1:8 to 1:512. This was followed by the addition of 50 µl (100 $TCID_{50}$) of respective virus to the diluted serum in each well. The mixture was incubated for 1 hr at 37 °C. 50 µl of BHK-21 (Glasgow) cells (1 million cells per ml) with

4 % foetal calf serum was added to each well. Suitable controls were kept along with the test. The plates were incubated at 37 °C for 48 hrs in CO_2 incubator and CPE was read. The SN index (log SN_{50}) was calculated in a standard way.

Estimation of 146S particles was performed by the method described by Lei (1978) with some modifications.

Results

Experimental and pilot scale studies on purification and concentration of FMDV by PEG precipitation were conducted with 20 l (25) batches and 300 l (3) batches of FMD virus grown in fermentors, respectively. The mean results with each type of experimental batches of FMD virus type O, A22, C and Asia-1, and 3 pilot scale batches of FMD virus type A22, C and O in respect of complement fixation (CF) titer, infectivity titer ($TCID_{50}$) and quantum of infective virus particles (146S) before and after 10-fold concentration is presented in Table 1.

Discussion

The presented results show that there was high percentage of recovery of virus after PEG precipitation and filtration. Similar recovery of virus was reported earlier with small and large virus batches (Sen and Rao, 1986; Sen and Rao, 1990). This method ensured high rate of virus recovery with existing facility available in the vaccine production unit and was cheaper as commpared to the techniques reported earlier (Morrow *et al.*, 1974; Cowan *et al.*, 1974). Lei (1974) reported that filtration procedure was more efficient than the centrifugation method for concentration of virus, but he could not reduce the dose of vaccine with PEG precipitated virus obtained by filtration through Kiesselghur.

The concentration and purification of the antigen is necessary for the following reasons. Large quantitites of monovalent product can be stored at 4 °C until the results of bacteriological and safety tests are available and the vaccine can be finally formulated. With the concentrated antigen a smaller dose volume of polyvalent vaccine can be obtained. If the vaccine contains purified antigen, adverse reactions after vaccination are reduced (Bauer *et al.*, 1970). With some procedures the antigen can be both concentrated and purified (Barteling and Wreeswijk, 1990). Ultrafiltration systems for concentrating FMDV virus were successfully pioneered by Strohmaier (1967). Later, follow fibre systems were applied by Morrow (1972) and they have been introduced by many vaccine manufactures in South America to obtain high antigen concentration necessary for oil adjuvanted vaccines. However, even if filter materials with cut off values of 10 000 (M_r) are used, purification of the antigen by ultrafiltration is limited. On the other hand, one precipitation step with PEG is sufficient to remove aller-

Table 1. Purification and concentration of FMD virus by PEG precipitation

Virus type	Original volume (l)	Titer before concentration			Titer after 10-fold concentration		
		CF (CFU/ml)	Infectivity ($TCID_{50}$/ml)	146S (µg/ml)	CF (CFU/ml)	Infectivity ($TCID_{50}$/ml)	146S (µl/m1)
A22	20	80	5.43	0.60	320	6.19	9.47
C	20	80	5.93	0.52	320	6.43	9.50
Asia-1	20	80	5.43	1.2	320	6.19	6.16
O	20	80	5.43	0.34	320	5.93	4.5
A22	300	80	6.10	0.84	320	6.4	7.5
C	300	80	5.91	0.52	320	6.43	7.8
O	300	80	5.43	0.40	320	6.1	4.5

The potency of the BEI inactivated single concentration vaccines tested in quinea pigs and cattle were highly satisfactory (Table 2). The single concentrated, Alhydrogel polyvalent saponified vaccine was tested under field conditions in State Livestock Farm, Hessarghatt, Bangalore. High immune response against all the types of FMD virus was observed in vaccinated cattle by SN test (Table 3). Satisfactory efficacy of this vaccine (1 ml dose) to protect cattle under field conditions was also noted on two occasions of FMD outbreak due to type A22 and O. A total of 40 000 doses of vaccine against type O and A22 were used in the field for prophylactic vaccination. Vaccines prepared out of single PEG concentrated virus were found to be satisfactory in these conditions.

Table 2. Potency tests of vaccine prepared from single PEG concentrated and BEI inactivated FMD virus

Vaccine batch No.	Virus type	Guinea pig C index	Cattle protection (%)
1	Asia-1	2.57	ND
2	O	2.44	ND
3	O (pilot scale)	4.0	ND
4	C (pilot scale)	ND	100
5	O	ND	80
6	A22 (pilot scale)	ND	100

ND – not done.

Table 3. Immune response in cattle vaccinated with single PEG concentrated polyvalent FMD vaccine (dose 4 ml), State Livestock Farm, Hessarghatta, Bangalore

Immune response against type	SN index (log SN_{50})[+]						
	1st vaccination				2nd vaccination		
	Day						
	0	25	45	164	0	45	164
O	1.57	1.75	1.76	2.06	2.06	2.09	2.05
C	1.24	1.84	1.91	2.30	2.30	2.30	2.40
Asia-1	1.46	2.01	2.01	2.20	2.20	2.10	2.12
A22	1.44	2.16	2.16	2.06	2.06	2.25	2.06

[+]Mean value of 20 vaccinated animals.

genic components from vaccines (Bauer *et al.*, 1970). The method can be applied on a large scale and the virus can be concentrated up to 1 000 times. At least 95 % of the proteins from the virus culture harvest are removed (Barteling and Vreeswijk, 1990).

In large scale production, the concentration of FMD virus is achieved either by follow fibre system (Amicon) or by cassette system (Millipore). In both the systems, cellular products, serum and other non-specific proteins are also concentrated along with the virus. The time required to concentrate large quantity (300–600 l) of virus is also a limiting factor. Moreover, high cost involved in replacing the cartidges should be also considered.

The method of virus purification and concentration in the present study seems to be very suitable for large scale vaccine production unit. The advantage of the present method is maximum recovery of virus and minimum degradation of virus particles (146S) as 300 liters of virus harvest can be concentrated in the cold within 30 mins. Moreover, the vaccine prepared with concentrated virus proves to be very potent under field conditions.

Acknowledgement. The authors are thankful to the Director of the Veterinary Research Institute for providing necessary facilities.

References

Bahenemann, H. G. Arch. Ange De Mello, P., Abaracon, D., and Gomes, I. (1974): Immunogenicity in cattle of foot and mouth disease vaccines inactivated with binary ethyleneimine. *Bull. Off. Int. Epizoot.* **81**, 1335–1343.

Barteling, S. J., and Vreeswijk, J. (1990): Developments in foot and mouth disease vaccines. *Vaccine* **9**, 75–88.

Bauer, K., Kaaden, O. R., and Mussgay, M. (1970): Experimentelle Untersuchungen über Allergien Vom Spattyp nach der schutzimpfung von Rindern mit. Maul – und Klauensenche (MKS) – Vakzinen. *Berl. Munch. Tieraztl. Wochenschr.* **83**, 292.

Cowan, K. M., Erol, N., and Whiteland, A. P. (1974): Heterogenecity of type Asia-1 foot and mouth disease virus and BHK-21 cells and the relationship to vaccine preparation. *Bull. Off. Int. Epizoot.* **81**, 1271.

Lei, J. C. (1974): Application of polyethylene glycol in the preparation of a concentrated purified foot and mouth disease vaccine. Present status of research. *Bull. Off. Int. Epizoot.* **81**, 1169.

Morrow, A. W. (1972): Concentration of the virus of foot and mouth disease in a tangential flow ultrafiltration unit. *J. Appl. Chem. Biotechnol.* **22**, 501.

Morrow, A. W., Whittle, C. J., Eales, W. A., and Bull, I. (1974): A comparison of methods for the concentration of virus for vaccine preparation. *Bull. Off. Int. Epizoot.* **81**, 155.

Rweyemamu, M. M., and Hingley, P. J. (1984): Foot and mouth disease virus strains differentiation: Analysis of serological data. *J. Biol. Stand.* **12**. 225–229.

Sen, A. K., and Rao, B. U. (1986): Note on the concentration of foot and mouth disease virus suspension by polyethylene glycol precipitation and filtration methods. *Ind. Vet. J.* **63**, 252.

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

Strohmaier, K. (1967): Virus concentration by ultrafiltration. *Methods Virol.* **111**, 245.