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Different Methods for Inactivation, Purification and Concentration of FMD Virus during Vaccine Manufacture

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Abstract

Foot and mouth disease (FMD) is a highly infectious disease in cloven- hoofed animals, the vaccination is widely used to control, eradicate and prevent FMD infection. This current study investigates the effective methods for inactivation, purification and concentration of FMD antigens, it was found that inactivation of the virus by BEI-FA and Concentration and purification of the antigen by PEG precipitation with aid of filtration were suitable for production of inactivated polyvalent highly potent FMD vaccine while the results for evaluation of the final product showed purity of the prepared vaccine from NSP which will be useful to discriminate between infected and vaccinated animals, high protection level more than the local commercial vaccine.

Keywords: Concentration of FMD antigens, FMD vaccine, Foot and mouth disease, Inactivation, Livestock , Purification , Serological tests.

1. Introduction

Foot and mouth disease (FMD) is a highly infectious and economically devastating disease with high morbidity in cloven-hoofed animals, including important livestock species such as cattle and buffalos [8] , and causes an economically devastating disease of cloven hoofed animals. Studies estimated 25% loss of productivity of animals following infection e.g. reduction or loss of milk production in lactating animals and the deterioration in body condition. Foot and mouth disease is caused by Foot-and-mouth disease virus (FMDV), the prototype member of the genus Aphthovirus of the family Picornaviridae. FMDV occurs as seven distinct serotypes (Euroasiatic serotypes A, O, C, and Asia1 and South African Territories [SAT] serotypes SAT1, SAT2 and SAT3) . In Egypt, FMD has taken an enzootic form and many outbreaks had occurred since 1950 and onwards. FMDV type O was the most prevalent until serotype A appeared in 2006 [25,13, 9]then during April and May 2012, six outbreaks of FMD type SAT 2 were reported in Egyptian governorates [16].

The vaccination is widely used to control, eradicate and prevent FMD infection [9] .

The efficacy of this policy depends not only on appropriate vaccine application in the field, but also on the quality (purity, safety and potency) and suitability (appropriate FMDV strain selection) of the chosen vaccine, which must comply with the requirements. At the beginning of the 21st century the protocol for production of inactivated FMD vaccines allows the use of serological tests that can differentiate infected from vaccinated animals, formulation of vaccines that include multiple serotypes and subtypes and a number of adjuvants [15] However, the basic technology for vaccine production has remained the same, still requiring the growth of large volumes of virulent FMDV, subsequent virus inactivation and antigen concentration and purification. This current study investigates the effective methods for inactivation, purification and concentration of FMD antigens that are suitable for production of inactivated polyvalent highly potent FMD vaccine and allow differentiation of vaccinated and infected animals through serological tests.

2. Materials and Methods

2.1 Viruses

The Egyptian isolates O/EGY-4-2012, A/EGY/1/2012 and SAT2/EGY/2/2012 were typed and subtyped at the FMD Department, Veterinary Serum and Vaccine Research Institute, Abassia, Cairo and confirmed by the World Reference Laboratories, Pirbright, United Kingdom. These viruses were adapted and titrated on Baby Hamster kidney (BHK) cells and used in vaccine preparation and serum neutralization assays.

2.2 Cell line

Baby Hamster Kidney (BHK-21) cell line: It was supplied by FMD Department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. The cells were grown and maintained according to[24] . It was used for viruses propagation, titration, verification of inactivation and serum neutralization test (SNT).

2.1 Inactivation kinetics for FMD viruses

2.1.1 Inactivation with formaldehyde

FMDV serotypes O, A and SAT2 were inactivated as describe by [12,28] .

2.3.2 Inactivation with Binary Ethyleneimine (BEI)

FMDV serotypes O, A and SAT2 were inactivated as described by [1].

2.3.3 Inactivation with combination of Binary Ethyleneimine and formaldehyde (BEI-FA)

Clarified chloroform-treated virus culture was brought to a temperature of 30ºC and then inactivated with a combination of 1mM BEI and 0.04% FA (BEI-FA). The FMD virus (BEI-FA) suspension was mixed well by stirring at 37^{o}C for 8 hours to allow complete virus inactivation, the inactivation was stopped by the addition of Na-thiosulphate as described by [5] Finally verification of inactivation kinetics by inoculation of inactivated antigen on BHK cells.

2.4 Concentration and purification of inactivated FMD antigens

2.4.1 Precipitation by polyethylene glycol (PEG)

The inactivated virus was cooled to 4^{o}C, then polyethylene glycol 6000 (PEG-6000) was added (100 gm/1-liter virus). The mixture was placed on a stirrer (for complete mixing and solubilization) at 4^{o}C for 2 hours, and kept at 4^{o}C for overnight. The mixture was centrifuged at 6000 rpm for 20 minutes and the supernatant was discarded according to method described by [23]This procedure was repeated using 8% PEG-6000 for further concentration of the viral antigen. The volume was brought to 100 ml using the elution buffer (Tris – KCl). Then centrifugation at 10000 rpm for 15 minutes and the supernatant was collected in clean sterile tubes (concentrated purified FMDV antigen).

2.4.2 Precipitation by polyethylene glycol (PEG) with aid of filtration

This method was carried out as described above in addition Filtration of the precipitated antigen together with a filter- aid using (Centricon Plus-70 Centrifugal Filter, Ultracel-PL Membrane, 30kDa) was more convenient [3,22].

A- Sample concentration

1. Add solution to sample filter cup (maximum of 70 mL); seal with supplied cap. Place sample filter cup into filtrate collection cup.
2. Place Centricon Plus-70 assembly in centrifuge bucket.
3. Spin at 3,500 × G for 15 minutes.
4. After the concentration step, remove Centricon Plus-70 device from centrifuge and separate sample filter cup from filtrate collection cup. If retaining the filtrate, cap filtrate collection cup and store as appropriate.

B- Concentrate recovery

1. Turn the concentrate cup upside down and place on top of the sample filter cup.
2. Carefully invert device, place in centrifuge, and counterbalance with a similar device. Spin at no more than 1,000 × G for up to 2 minutes.
3. Remove the concentrate cup containing the concentrated sample from the sample filter cup. Keep the filter cup inverted during this process.
4. Remove the sample with a pipette or cap the concentrate cup and store sample for later use.

2.5 Estimation of 146s content in the produced FMDV antigens

The test was carried out for each serotype of FMD vaccinal strains A, O and SAT2 and for different ways of inactivation and concentration.as described by[6].

2.6 Vaccine formulation and animals vaccination

Vaccine formulation was done after election of the best method for inactivation, purification and concentration in regards to the results of validation for inactivation kinetics and 146s estimation, the vaccine formulation was carried out as described by [2] where the oil phase consisted of Montanide ISA 206, mixed as equal parts of an aqueous and oil phase weight/ weight, and mixed thoroughly. The FMD 146s concentration in the final vaccine formula was adjusted to be 4.8 µg viral particles/dose/ O serotype, 4.5 µg viral particles/dose/ A serotype and 5.0µg viral particles/dose/ SAT2 serotype. The pH was brought to 8.2 with glycol buffer, and the sodium thiomersal was added as a preservative at a final concentration of 0.0001 (1 ml of 10% Sod. Thio-mersal / one liter vaccine). Thirty four calves (local breed) of six to eight months old of about 200 – 300 kg body weight were used and allotted into 4 groups, group(1): 15 calves were vaccinated by prepared vaccine, group (2): 15 calves were vaccinated by commercial vaccine, group (3): 2 calves were used for detection of Ab against NSP by 3 ABC ELISA(prepared vaccine) and group (4): 2 calves were used for detection of Ab against NSP by 3ABC ELISA (commercial vaccine).

2.7 Estimation of vaccine purity

Calves were vaccinated from two to three times at 21- to 30-day intervals and then tested before each revaccination and 30–60 days after the last

vaccination for the presence or absence of antibody to NSPs using PrioCHECK® FMDV NS blocking ELISA.

2.8 Serum neutralization test

The bovine vaccinated sera for group (1) and group (2) were used to measure the variance in efficacy between prepared and commercial FMD vaccines; the test was performed by using the micro technique as described by [18].

3. Results

The inactivated antigens by different ways were inoculated on BHK cells, 25ul/well were used for each strain, and observed after 48 hrs for CPE to verify the inactivation kinetics as shown in table (1). Result of 146s content estimation in the produced FMDV antigens after concentration by PEG Precipitation and Precipitation by (PEG) with aid of filtration which is expressed by µg/ml (146s), as shown in table (2). Determination of Optical density percentage in screening for antibodies against FMDV-NS for validation of vaccine purity, showed – ve result for sera collected after 1st and 2nd vaccination from the calves vaccinated (group 3) with prepared vaccine while the sera collected from the calves vaccinated (group 4) with local commercial vaccine gave +ve result as shown in Table (3). Estimation of humeral immune response in vaccinated calves (group 1) with prepared vaccine and vaccinated calves (group 2) with commercial vaccine against A,O and SAT2 using SNT showed that protective neutralizing serum antibody titer (1.2 $\log_{10}$) started from 3rdweek post vaccination against O , A and SAT2 in vaccinated calves with commercial vaccine while the vaccinated calves with prepared vaccine, the protective neutralizing serum antibody titer started from 3rdweek post vaccination against O and A started at 2nd week against SAT2 and persisted in protective level until the 28thweek post vaccination in both groups(commercial and prepared), the highest level of antibody was recorded at 10thweek against A and SAT2 while was recorded at 8th week against O in vaccinated calves with prepared vaccine , while the vaccinated calves with commercial vaccine showed the highest level of antibody at the 10th week against O,A and SAT2, as shown in Figs (1,2,3).

Table (1) verification of the inactivation of FMD virus serotypes used in inactivated FMD vaccine preparation using BHK cell culture inoculation

FMDV Serotypes	Inactivation with formaldehyde for 48 hrs	Inactivation with binary ethyleneimine (BEI) for 24 hrs	Inactivation with BEI-FA: For 8 hrs
O/EGY/4/2012	+	-	-
A/EGY/1/2012	+	-	-
SAT2/ Egypt/2 /2012	+	-	-

*(+) the CPE observed on BHK cell culture

Table (2) Estimation of 146s content in the produced FMDV antigens after concentration by Precipitation using PEG and Precipitation using (PEG) with aid of filtration

FMDV Serotypes	146S(µg/ml)	
	Precipitation by PEG	Precipitation by (PEG) with aid of filtration:
O/EGY/4/2012	5.1	6.3
A/EGY/1/2012	5.0	6.0
SAT2/ Egypt/2 /2012	5.2	6.5

4. Discussion

Foot-and-mouth disease (FMD) is one of the most contagious diseases of animals. The causative agent, FMD virus (FMDV), is the type species of the genus Aphthovirus within the Picornaviridae family [19] Inactivated vaccines evolved several problems manifested themselves, such as the incomplete viral inactivation of formaldehyde treated antigens. This problem was solved by the introduction of BEI inactivated antigens. In early vaccine production systems concentration of the antigen was achieved through the use of aluminum hydroxide gel adsorption, or polyethylene glycol precipitation, although these methods are effective and still in use in some parts of the world, they have been largely replaced by industrial ultrafiltration and chromatography in order to remove unwanted cellular protein contaminants and viral non-structural proteins. The need to further purify vaccine antigens arose not only to prevent unwanted allergic reactions to cell proteins in animals after multiple vaccinations but also to allow differentiation of infected from vaccinated animals during control campaigns [16]. In the

present study, the preparation of polyvalent inactivated vaccine of FMD virus was carried out by different studies on vaccine production in process. Verifying the inactivation methods of FMD virus serotypes used in inactivated FMD vaccine preparation using BHK cell culture inoculation showed the complete inactivation by Inactivation with Binary Ethyleneimine (BEI) within 24 hrs and inactivation with BEI-FA within 8 hrs while the inactivation by formaldehyde within 48 hrs observed incomplete inactivation as shown in table (1), this agreed with [7,5,11].

Estimation of 146s content in the produced FMDV antigens O/EGY/4/2012, A/EGY/1/2012 and SAT2/Egypt/2/2012 after concentration by Precipitation by PEG and Precipitation by (PEG) with aid of filtration showed that the result of 146s content in case of PEG precipitation were 5.1, 5.0 and 5.2 µg/ml while in case of PEG precipitation with aid of filtration were 6.3, 6.0 and 6.5 µg/ml respectively as shown in table (2), this result agreed with[4,20].

Purity of the prepared vaccine and local commercial vaccine were detected through testing of FMD nonstructural protein (NSP) in calves vaccinated with prepared vaccine and local commercial vaccine using PrioCHECK® FMDV NS blocking ELISA (3ABC ELISA) showed –ve result for sera collected after 1st and 2nd vaccination from the calves (group 3) vaccinated with prepared vaccine while the sera collected from the calves (group 4) vaccinated with local commercial vaccine gave +ve result as shown in table No (5), this result agreed with[9, 21].

The humeral immune response in vaccinated calves (group 1) with prepared vaccine and vaccinated calves (group2) with commercial vaccine against A,O and SAT2 using SNT showed that protective neutralizing serum antibody titer (1.2 $\log_{10}$) started from 3rdweek post vaccination against O , A and SAT2 in vaccinated calves with commercial vaccine while the vaccinated calves with prepared vaccine, the protective neutralizing serum antibody titer started from 3rdweek post vaccination against O and A started at 2nd week against SAT2 and persisted in protective level until the 28thweek post vaccination in both groups(commercial and prepared), the highest level of antibody was recorded at 10thweek against A and SAT2 while was recorded at 8th week against O in vaccinated calves with prepared vaccine , while the vaccinated calves with commercial vaccine showed the highest level of antibody at the 10th week against O,A and SAT2, as shown in figs (1,2,3), this result of SNT agreed with [14,26] From the discussed results we can observe that the polyvalent inactivated FMD vaccine preparation process was carried out in cascaded steps by different ways to determine the best methods which were inactivation of the virus by BEI-FA and Concentration and purification of the antigen by PEG precipitation with aid of filtration, while the results for evaluation of the final product showed purity of the prepared vaccine from NSP which will be useful to discriminate between infected and vaccinated animals, high protection level more than the local commercial vaccine.

Table (3) Determination of Optical density percentage in screening for antibodies against FMDV- NS by using 3ABC ELISA

Vaccinated calves No.	**Optical density percentage in screening for antibodies against FMDV- NS**		
	Zero day pre vaccination	**21 days post 1st vaccination**	**21 days post 2nd vaccination**
Group 3	11.3	38.0	43.3
	6.6	31.0	40.7
Group 4	4.0	52.0	58.3
	20	54.7	61.0

- <50% (negative) Antibodies against the NS protein of FMDV are absent in the test sample.
- ≥50% (positive) Antibodies against the NS protein of FMDV are present in the test sample.

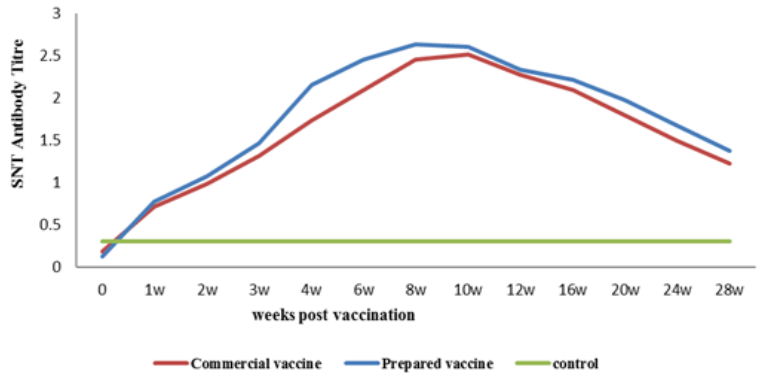

Fig (1) Neutralizing antibody titre of vaccinated calves (group1) with polyvalent inactivated FMD vaccine and calves (group2) vaccinated with FMD commercial vaccine against FMDV type O

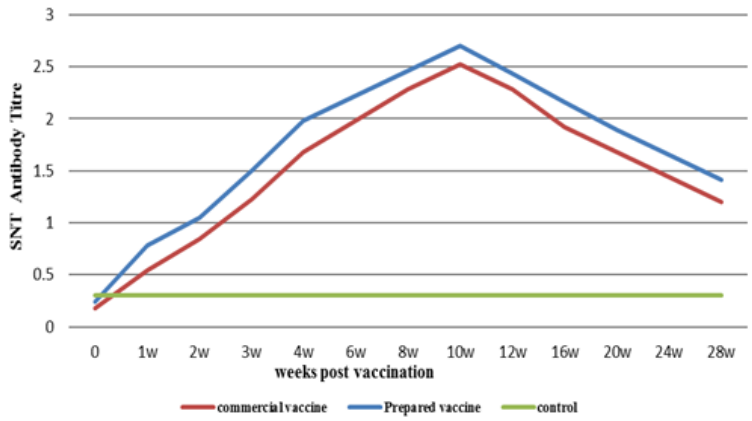

Fig (2) Neutralizing antibody titre of vaccinated calves (group1) with polyvalent inactivated FMD and calves (group2) vaccinated with FMD commercial vaccine against FMDV type A

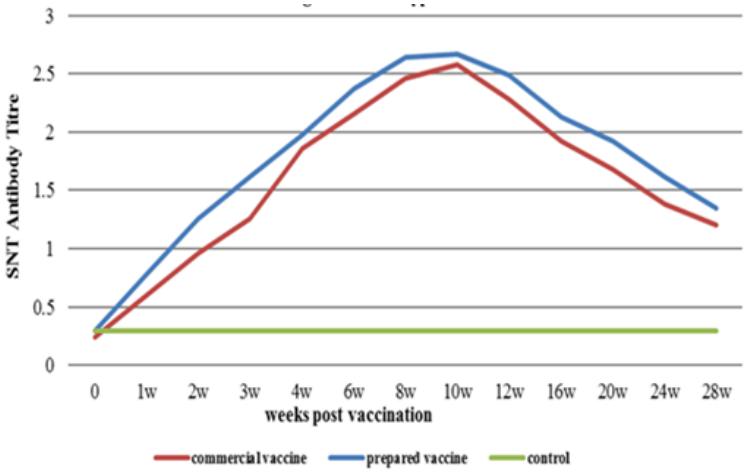

Fig (3) Neutralizing antibody titre of vaccinated calves (group1) with polyvalent inactivated FMD vaccine and calves (group2) vaccinated with FMD commercial vaccine against FMDV type SAT2

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