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CONCENTRATION, PURIFICATION AND IMMUNOGENECITY OF 146S ANTIGENIC PARTICLES OF FMDV

By

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ABSTRACT

The 146S antigenic mass load is the key success of FMD vaccines. In the current study, concentrated, ultra-filtered and quantified FMD 146S antigens were prepared for immunization of cattle via S/C (sub-cutaneous) and mucosal routes. The immunological reactivity of the prepared antigens was estimated by DOT-ELISA. The coupling of both concentration and ultrafiltration methods, showed the highest 146S quantity peak and the strongest antigen-antibody reaction in DOT-ELISA. The SNT results of the immunized cattle revealed that the protective neutralizing antibody response was successfully elicited following single S/C immunization. While, in cattle immunized via mucosal route, slight increase in antibody levels was observed. In conclusion, this study gives an insight to the importance of preparing highly purified and quantified 146S FMD antigens required for inducing potent immune response especially in mucosal vaccines.

Key words:

FMDV; 146S antigenic mass; concentration; ultrafiltration.

INTRODUCTION

Foot and mouth disease virus (FMDV) is one of the contagious diseases affecting cloven hoofed animals causing vesicle disruption in udder, foot and tongue resulting in high morbidity rates **()**. FMDV is classified into four particles according to their sedimentation coefficients following sucrose density gradient centrifugation: Whole virus particle (146S or 140S), empty capsids (75S), virus infection- related peptides (45S) and 12S protein subunits (12S). The immunogenicity of the FMD inactivated vaccines is mainly dependent on the 146S (whole virus particle) **()**. The 146S contain four structural proteins VP1, VP2, VP3 and VP4 whereas, 75S particles contain only three VP1, VP3 and VP0 (precursor of VP2 and VP4) **()**.

Purification of vaccine antigens aims to removing or reducing proteins that may induce allergic reactions. Also, nonstructural proteins (NSPs) of FMD virus are removed or their concentration is reduced**.** Since that, the integrity of the 146S virus particle is critical in vaccine production, the 146S quantitative sucrose density gradient analysis is greatly recommended to quantify virus antigen which would assure good vaccine antigenicity. The 146S preparation method was developed by Barteling and Melon **()**.The method consists of ultracentrifugation of the sample on a sucrose gradient of about 10 to 40%. The sucrose gradients are prepared by layering sucrose solutions of decreasing concentration. In the present study, we prepared concentrated, ultra-filtered FMD antigens for S/C and mucosal administration in a step for vaccine design and formulation.

MATERIAL AND METHODS

Propagation of FMD virus:

Pan-Asia/2012 seed virus, was obtained from Veterinary serum and vaccine research institute, and propagated on BHK-21 cell line for vaccine production and harvested 18-21 hours after cytopathic effect (CPE) was observed **()**.

FMDV concentration and inactivation:

The FMDV harvest was concentrated at 8% polyethylene-glycol (PEG-6000) and inactivated with binary ethyleneimine (BEI) **()**.

Ultrafiltration of FMDV:

The PEG-concentrated FMD antigen and the collected 146S fractions were ultra-filtered using the ultra-filter device**.**

Estimation of the 146S content:

The prepared FMD antigens (FMDV harvest, PEG-concentrated FMDV, ultra-filtered PEG concentrated FMDV, ultra-filtered 146S fractions) were prepared for 146S protein content quantification by sucrose density gradient centrifugation as previously reported (7).The area under the 146S peak was calculated and used to determine the concentration of the antigenic mass in the samples as previously described **().**

Evaluation of the immunogenicity of the prepared antigens using DOT ELISA:

For assaying the antigenicity of the prepared FMD antigens. It was performed as previously reported **()**, with some modifications using coating buffer (carbonate bicarbonate buffer), 5%

NFDM was used as blocking buffer to prevent any nonspecific binding. The intensity of the developed color is proportional with the antigen concentration.

Calves inoculation and serum neutralization assay:

Serologically negative calves (*n*=8) were divided into 3 groups with two unimmunized controls.Gp.1:Three calves immunized S/C (sub-cutaneous) with FMD oil based 146S antigen (4*μg/ml*). Gp.2: Three calves immunized via mucosal route with the FMD IMS based 146S antigen (30*μg/ml)*. GP.3: Two unimmunized calves as control. Sera were collected pre and at 21 days post immunization, for assessing the presence of neutralizing antibodies. The test was performed as previously described **(OIE, 2012) ()**. Titers were expressed as the final dilution of serum present in the serum/virus mixture where 50% of wells were protected.

RESULTS

Estimation of the146S antigenic content prepared FMD antigens:

The prepared FMD antigens showed varied 146S antigenic mass. FMD harvest (before PEG-concentration) showed a peak, when calculated =*1.3μg/ml*. PEG-concentrated FMD antigen increased 10x to be *13μg/ml*. Samples tested after ultrafiltration of the PEG Concentrated FMD antigen =*79μg/ml,* while the highest antigen load was observed in collected ultra-filtered 146S fractions =*115μg/ml*.Fig. (1A-1B-1C-1D).

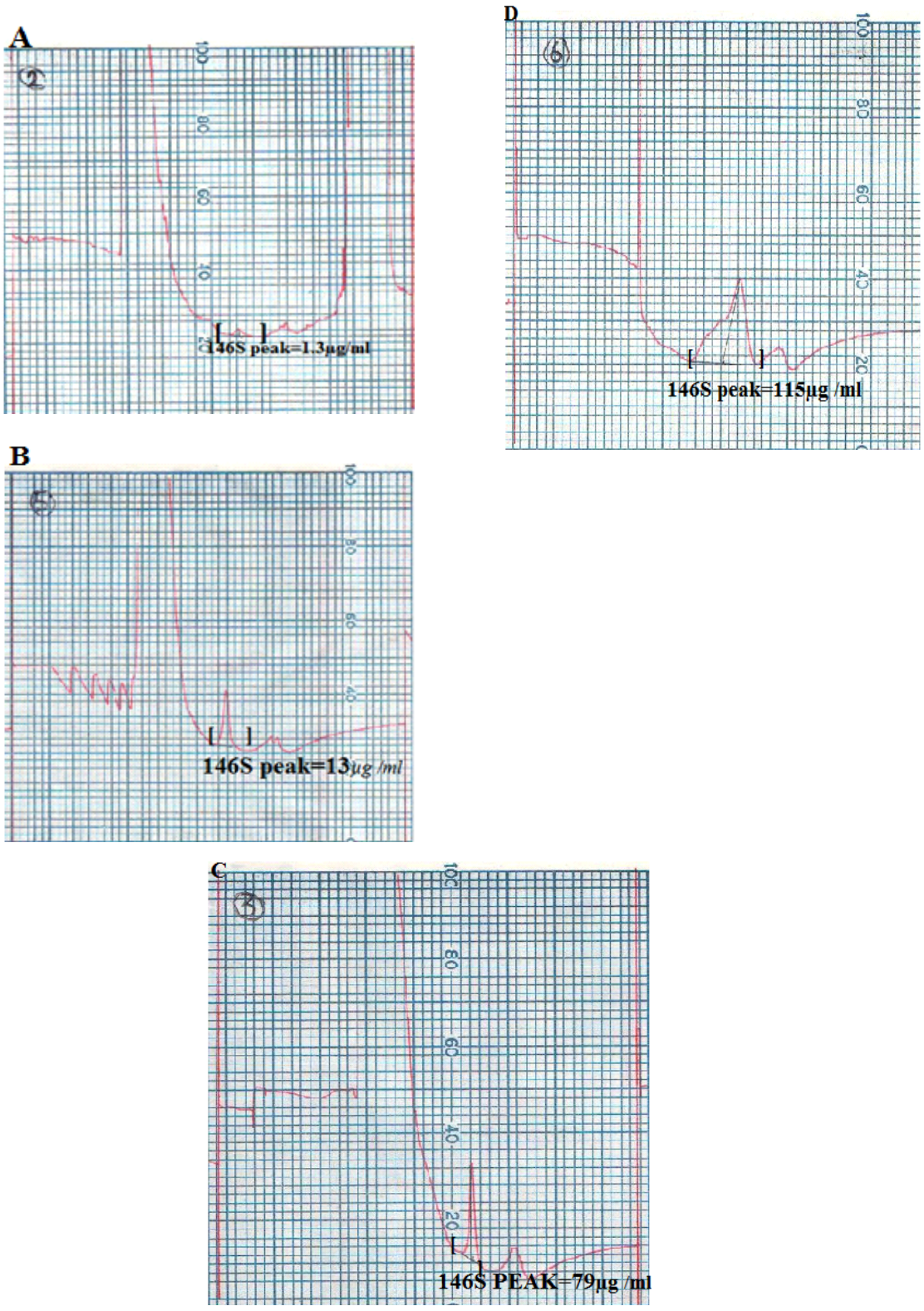

Fig. (1: UV): Scaning of FMDV fractions following Sucrose Density Gradient ultracentrifugation for 146S quantification.

Fig. (1): Showing the sedimentation of the prepared FMD antigens upon sucrose density gradient ultracentrifugation expressed in µg /ml.1A: FMD harvest sample, sample volume: 0.5 ml, 146S= 1.3*µg/ml* 1B: PEG concentrated FMD sample, sample volume: 0.4 ml, 146S= 13*µg /ml.* 1C: Ultra filtered PEG-concentrated FMD sample, sample volume: 0.1 ml, 146S= 79 *µg /ml.* 1D: Ultra-filtered 146s fractions, sample volume: 0.18ml, 146S= 115*µg /ml*.

DOT-ELISA:

The intensity of the developed color in the membrane was proportional to the antigen concentration. In comparison with the negative control which showed no blue spot, the tested FMD antigen varied in color intensity from weak positive (faint blue color) to strong positive (deep blue color) as shown in Fig. (2).

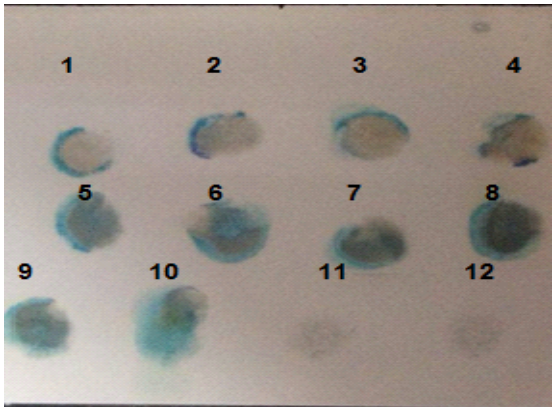

Fig. (2): DOT-ELISA of the prepared FMD antigens

Fig. (2): Showing the Prepared FMDV antigens dotted on nitrocellulose membrane for detection of their antigenic properties. Samples were added in duplicated manner. Dots 1 and 2 represented: The collected 146S fractions before ultrafiltration; Dots 3 and 4 represented: Filtrate of the collected 146S fractions after ultrafiltration. Samples no.1,2,3,4 are considered weak positive; Dots 5 and 6 represented: Ultra-filtered 146S fractions concentrate; Dots **7** and 8 represented Ultra-filtered PEG concentrated FMD concentrate; Dots 9 and10 represented: PEG concentrated FMD virus before ultra-filtration. Samples no. 5,6,7,8,9,10 are considered strong positive; Dots 11and 12 represented negative control (BHK-21non infected cells).

Serum neutralization assay:

Results showed the mean neutralizing antibody titers ±SD of the immunized cattle. Cattle immunized with the FMD oil based antigen preparation induced significant increase in the antibody titers at 21 days' post immunization (1.890 ± 0.201) with achieving protective levels (Antibody titers considered to be protective if is 1.2 or more). On the other hand, the FMD

IMS based antigen induced slight increase in the antibody titers at 21 days' post immunization (0.870 ± 0.268) which did not reach the protective levels.

DISCUSSION

The FMDV consists mainly of 146S, 75S, 45S and 12S antigenic particles from which 146S is the main immunogenic part (3). Inactivated whole-virus vaccines require evaluation of the antigen quality (146S quantification and purity) (2). Concentration of FMDV harvest using PEG-6000 is considered the simplest and the most rapid method for retaining the infective virus particle and to get rid of the virus free media **()**. Moreover, It is regarded as the best method causing less viral damage and high recovery of the infectious particle in comparison to previously used aluminum sulfate that caused change in pH and salt concentration**()**. The efficiency of the PEG-concentration method was ascertained by the 146S quantification Fig.(1A,1B) that showed 10 fold increase in the 146S antigenic mass in PEG concentrated FMD antigen (13*μg /ml)* in comparison with the FMD harvest (1.3*μg /ml)* without concentration. Ultrafiltration using ultra-filter membrane of regenerated cellulose provide the ability to retain the required proteins according to the cut off molecular weight of the used ultra-filter **()**. It was confirmed by the 146S peak Fig.(1C,1D) that increased in the ultra-filtered PEG concentrated FMD antigen which reached 79 *μg /ml* and the ultra-filtered 146S fractions with highest peak reaching 115 *μg /ml,* highlighting the critical role of ultrafiltration (11). The reactivity of the prepared FMD antigens were assessed in vitro by DOT ELISA that is considered to be inexpensive, time saving and convenient method as the nitrocellulose membrane is able to bind antigens efficiently and it does not require specialized tools to analyze the results (,). DOT ELISA results revealed strong antigen antibody reaction observed as highly intense blue spot in the PEG concentrated FMD antigens before and after ultrafiltration and the ultra-filtered 146S fractions represented which is attributed to the high antigen concentration as previously measured in 146S quantification. On the other hand, weak antigen antibody reaction represented in faint blue spot was detected in the collected 146S fractions before ultrafiltration that may be attributed to sucrose infiltration. In vivo, animals inoculated with the oil based FMD antigen (4*μg /ml)* induced increase in neutralizing antibody levels with protective levels indicating the capability of the prepared antigen to elicit protective levels of neutralizing antibody upon single dose administration of the prepared vaccine S/C. These results come in parallel to those previously demonstrated (20).On the

other hand, animals immunized via mucosal route induced slight non-protective neutralizing antibodies. Mucosal vaccine development requires the preparation of highly purified antigen due to high antigenic mass needed. In conclusion, our results show a great potential of using the concentrated ultra-filtered FMD antigen for vaccine design and formulation to be used for animal's immunization.Such vaccines will rely mainly on quantified antigenic mass rather than high injectable volumes in order to attain high quality protective vaccine.

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