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Comparative Studies of the Potency of Foot and Mouth Disease Virus Trivalent Vaccine with Different Concentration of the Antigenic Content (146S).

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Key Words: 146S, SDG, SNT, potency testing, trivalent highly potent FMDV vaccine.

ABSTRACT:

Estimation of antigenic content (146S) of FMDV serotypes (A, O, SAT2) by sucrose density gradient (SDG) ultracentrifugation by determining the absorbance at 254 nm using ISCO520C density gradient system to produce a highly potent trivalent virus vaccine. The antigenic mass 146S (µg/ml) of serotypes (O Pan Asia2, A Iran O5 and SAT2/EGY/2012) were 6.5, 6.2 and 5.9, respectively. The vaccine was injected into three groups of calves (2individuals/each group) subcutaneously in lateral part of the neck for a dose 3 ml (6.2µg/serotype/ml), a dose 1.5 ml (4.1µg/serotype/ml) and a dose 1 ml (2µg/ml), the sera samples were collected at 7th day post vaccination (dpv), 14th dpv, 21th dpv, 28th dpv and every 2wks till 40 weeks to evaluate the immune response along that period. The antibody titers/40wpv for a 3 ml dose (6.2µg/ml) of serotypes (O Pan Asia-2, A Iran O5 and SAT-2/EGY/2012) were 2.08, 2 and 1.94, respectively (over the protective titer, PT=1.5 in SNT for cattle), a dose (4.1µg/ml) of the three serotypes were 1.56, 1.62 and 1.63 (over PT), respectively, But for (2µg/ml) dose of the three serotypes, the antibodies titer were 1.25, 1.19 and 1.2 (below PT), that show the antibodies titer depend on the concentration of the antigenic mass (146S) and with increase of the 146S concentration increase of the potency of the vaccine. The potency testing of that study depend upon the correlation between 146S and the neutralizing antibody titers were measured by SNT which are the perfect alternative of other potency tests which employ the challenge of the cattle with virulent virus. The immune response of the highly potent vaccine (4.1µg/serotype/ml and 6.2µg/serotype/ml) started early after 1st wpv and the protective titer remain for more than 38 wpv (especially in 6.2µg/ml injected calves) and that confer the potency of the vaccine of that dose.

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1. INTRODUCTION

Foot and Mouth Disease (Aphtae epizooticae), is an economically devastating and highly contagious disease of livestock. FMD is an infectious and fatal viral disease that affects cloven-hoofed animals, including domestic (cattle, sheep, goats and pigs) and wild animals, which is a severe plague for animal farming due to it is a highly infectious and easy to spread. Although the vaccines were available since early 1900s, it remain the only way for eradication of FMD from parts of the world, the disease still affect the millions of animals around the globe and remain the main sanitary barrier to the commerce of animals and animal products (Depa *et al.*, 2012).

The etiological agent of FMD is a small positive sense, ssRNA virus (approx. 8.3kb) which belongs to genus *Aphthovirus* of family *Picornaviridae* (Bachrach, 1968 and Belsham, 1993). The virus exists in the form of seven serologically and genetically distinguishable types, namely O, A, C, Asia1, South African Territories (SAT1, SAT2, and SAT3), but a large number of subtype variants have evolved within each serotype. This antigenic variation creates a major problem for the control of FMD, as infection or vaccination with one serotype of FMDV does not provoke protection against other serotypes and may fail to protect fully against other subtypes within the same serotype (Paton et al., 2005).

The whole Foot and mouth disease virus harvest contain four virus specific particles (Crowther, 1986): (i) the infective 146S virus particle, comprising one molecule of ss-RNA (2.6 million m.wt.) and 60 copies of each of four polypeptides VP1,VP2,VP3(m.wts = 24,000) and VP4(m.wts = 8,000); (ii) the empty 75S particles, devoid of RNA and comprising 60 copies of each of VP1,VP3 and VP0 (precursor of VP2 and VP4); (iii) the 12S

subunit consisting of five copies(pentamer)of VP1,VP2 and VP3 but devoid of VP4;and (iv) virus infection associated antigen (VIA) with sedimentation coefficient in Sucrose gradient 3.5S, RNA polymerase associated. M.wt. 56,000.

Neutralizing antibody production is associated mainly with the I46S particles (Brown and Crick, 1959), The I2S particle is produced by mild acid disruption of the I46S particle or by heating at 56 °C. Whereas the 12S particles stimulate the production of an antibody which has low neutralizing activity but reacts in precipitin and complement fixation tests, the antigen associated with the 12S particles is not present on the surface of the I46S particles. The 12S antigen might be considered a crypto-antigen, revealed only after gentle disruption of the 146S particles so, the absorption of hyperimmune serum with excess 12S particles does not reduce the neutralizing activity of the serum, nor its ability to fix complement with the I46S particles (Cartwright, 1962), but the antigenic characters of both 146S and 75S appear to be similar if not identical that in antibodies blocking activities, plaque reduction tests and competitive ELISA (Rowlands et al., 1975). An Acetyl ethylenimine treated 75S of A61 strain not induce significant antibodies, but in other study (Cowan, 1970) was proved that 75S of A24 strain did stimulate significant levels of neutralizing antibodies with lesser extent of the 146S. So, 146S antigen is the major antigenic and immunizing part which we depend on in our study.

In Egypt FMD was enzootic and the outbreaks were reported since 1950. The FMD serotypes (O), (A), and (SAT2) were reported in years 1972, 2000 and 2012, respectively (Aidaros, 2002, Knowles et al., 2007 and FAO, 2012), but the serotypes O was incriminated in the outbreaks in the years 1987 and 1993.

Although the present conventional FMDV vaccines can prevent clinical disease, the short live protection (~6 month), frequent revaccination for prophylactic control, and vaccination does not induce rapid protection against challenge or prevent the development of the carrier state, but the vaccination still the corner stone and the only approach to control the disease, the immunogenicity of FMD is depend on the large extent on the production of the whole virus particles (virus titration mainly at 10^8 or estimation of 146S particles) in the tissue culture (BHK21) and the stability of these particles after inactivation procedures (Shawky et al., 2013).

This study was done to evaluate the potency of inactivated trivalent FMDV vaccine through measuring neutralizing antibody titers for different concentrations of the antigenic mass (146S).

2. MATERIAL AND METHODS

1- **Cattle**: seven local breed calves (6 months old) which are clinically healthy and FMDV antibodies free, tested by SNT and ELISA. Calves were allotted to three groups in addition to fourth group of control. They were used in vaccine potency testing and antibody titers estimation in SNT.

2- **Ethical approval**: The experiment was as per the protocol of Institutional Animal Ethics Committee, we took permission of animal owners of the private farm.

3- **Tissue culture**: Baby hamster kidney cell cultures (BHK_{21}), were serially passaged and maintained with Minimum Essential Medium (MEM) modified with Hank's salt solution with 1-2 % bovine serum (Huang et al., 2011) in the FMD Research Department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. The cells were obtained from the World Reference Lab. Pirbright Surrey, U.K.

4- **Tissue culture FMD virus strains**: local FMDV type O Pan Asia, A Iran O5 and SAT2/EGY/2012 were propagated in BHK21 cell line monolayer cultures for preparation of virus infected fluids in Department of Foot and Mouth Disease Virus Research, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. The titer of the three viruses expressed in $\log_{10}$ $TCID_{50}$ (Reed and Muench, 1938) and CF carried out according to (Traub and Manso, 1944 and Health protection agency, 2009).

5- **Virus purification**: Aseptically, the harvested culture medium from FMD virus infected BHK_{21} cell cultures were centrifuged in a cooling centrifuge at 7000 rpm for 20 minutes to remove cell debris (Killington et al., 1996).

6- **FMDV serotypes concentration:** The tissue culture viral fluids of the three serotypes were centrifuged at 7000 rpm/30 minutes in cooling centrifuge then concentrated by PEG-6000 to reach 1/10 of its original volume (Panina and De-Simone, 1973).

7- **Estimation of the antigenic content (146S) in the concentrated virus serotypes**: The content of 146S particles in prepared viral antigen estimated by using sucrose density gradient ultracentrifugation (SDG) by determining the

absorbance at 254nm using ISCO 520C density gradient system (Doel and Chong, 1982 and Bartelling et al., 1990).

8- **Virus inactivation:** Each FMD virus (O, A and SAT2) of the 7th passage on BHK monolayer with an infectivity titer of 10^8 TCID /dose for 2ml of the vaccine was treated with 1.5% chloroform then the inactivation occur by using combination of Binary ethyleneimine 1mM and 0.04% FA (BEI-FA) according to the method described previously (Barteling and Cassim, 2004 and Ali et al., 2009). 2% of each of sodium thiosulphate (20%) and sodium bisulphate (20%) which added after the inactivation process to neutralize the excess BEI and formaldehyde.

9- **Formulation of FMDV vaccine with Montanide ISA-206**: the oil phase (Montanide ISA-206) was mixed with the equal volume with a queos phase weight/weight and mixed thoroughly (Gamil, 2010 and El-Sayed et al., 2012). Each phase have to be heated up at 31^0C before mixing. Stable preparations are obtained by mixing the aqueous medium into the Montanide ISA206 VG under mixing at a low shear rate (to maintain temperature at 30^0C) that described by Seppic company/France.

10- **Injection of the vaccine in calves**: The 1st group of calves were injected with 3 ml of the vaccine (~6.2µg of 146S/ml) and injected a booster dose at 8 wpv, other 2 calves were injected with 1.5 ml of the vaccine (~4.1µg of 146S/ml), 2 calves were injected with 1ml of the vaccine (~2µg of 146S/ml), and one calf control with no injection.

11- **Collection of the blood samples**: The blood samples were collected at days 7th, 14th, 21th, 28th, 35th, and every 2week through a period to 40weeks post vaccination. The sera samples were inactivated by heating at 56^0c/30 minutes.

12- **Serum neutralization test (SNT):**

The sera samples were tested by SNT (Ferreira, 1976).

3. RESULTS AND DISCUSTION

FMD is a highly contagious and economically devastating disease in many countries all over the world and is a continued threat to disease free countries (Knowles and Samuel, 2003 and Ko et al., 2009). Special regarding in Egypt the disease make many outbreaks because of FMDV serotypes (O, A, SAT-2) mainly, as serotype O found in Egypt since 1972 and incriminated in outbreaks in years 1978 and 1993, serotype A recorded since 2000 and recently in 2012 recorded SAT-2 (Aidaros, 2002, Knowles et al., 2007 and FAO, 2012). So, the recent conventional vaccine used in Egypt is trivalent (containing the three present serotypes O, A, SAT-2) inactivated oil adjuvanted vaccine. Despite the vaccination is the corner stone and the only way for controlling the disease, but the antigenic variation creates a major problem in controlling of FMD, as vaccination with one serotype of FMDV does not protect against other serotypes that is the consequence for failing to protect fully against other subtypes within the same serotype (Brooksby, 1982, Cartwright et al., 1982, Mattion et al., 2004 and Paton et al., 2005), the vaccination resulted in a short term immunity up to 6 month that require frequent revaccination for prophylactic control and it does not prevent carrier state's cattle (Parida, 2009). So, in that study we planned to use of the highly potent vaccine with estimation of different concentration of major antigenic part (146S) of FMDV type (O Pan Asia-2, A Iran O5, and SAT-2/EGY/2012) in µg/ml, versus log SN in injected cattle that the indirect method for estimation of the potency of the vaccine without a burden of the animal challenge.

The antigenic payload (concentration of antigen in µg/ml) is the indirect method to determine the duration of the immunity and the initial magnitude of antibody response determine the duration of effective immunity. The 146S particles are considered as a major immunogenic component of FMDV and any degradation of 146S particles may reduce the potency of the vaccine (Doel and Chong, 1982).

In this study the basic corner is the intact 146S particles and the results revealed that the 146S antigenic content of the FMDV serotypes (O Pan Asia-2, A Iran O5 and SAT-2/EGY/2012) were 4.56, 4.17 and 3.69µg/ml before the concentration respectively and reached to 6.5, 6.2 and 5.9µg/ml respectively after concentration with PEG-6000, and the infectivity of the three FMDV types were estimated by inoculation on BHK_{21} cells, their titration obtained by $TCID_{50}$ methods were 7.8, 7.2 and 7.6 $\log_{10}$ $TCID_{50}$/ml respectively (table-1).

The experiment is performed to injected calves with the trivalent vaccine of the three FMDV types (O

Pan Asia-2, A Iran O5 and SAT-2/EGY/2012) -the present isolated serotypes in Egypt (FAO, 2012 and Shawky et al., 2013)- and performing the serum neutralization on the obtained blood of these calves, the resulted mean antibody titers (as an indirect tool for determination of the immunity and the initial magnitude of antibody response determine the duration of the effective immunity) from (6.2µg, 4.1µg and 2µg) for each serotype of FMDV for about 40 weeks post vaccination. It was found that 6.2µg/ml (~9.1µg/serotype/dose) of 146S, the antibody titers in SNT were 2.08, 2 and 1.94 for (O Pan Asia2, A Iran O5 and SAT2/EGY/2012), respectively. It was observed that the titers were over the protective titer (PT=1.5 in cattle in SNT and 1.9 in ELISA according to OIE, 2010), while 4.1µg/ml (~6µg/serotype/dose), the antibody titers were 1.56, 1.62 and 1.63 for the three serotypes, respectively and also over the protective titer (PT= 1.5), but using of 2µg/ml (~3µg/serotype/dose), the antibody titers were 1.25, 1.19 and 1.2 for the three serotypes respectively, were lower of protective titer. It was observed, the calves which were injected by a dose 3 ml (containing 6.2µg/ml), the immune response elevated from the 3rd WPV and the peak continue to the week 14th for (O Pan Asia-2), 16th WPV for (A Iran O5 and SAT2/EGY/2012) and the titer remain protective up to 38 week post vaccination, (P-value=1) for the three serotypes, while the calves which were injected with 1.5 ml of vaccine dose (4.1µg 146S/ml), the antibody titers elevated from 3rd WPV and continue protective to 28th WPV for (O Pan Asia-2 and SAT-2/EGY/2012) and to 30WPV for (A Iran O5) with P- value was 0.999857, 0.999994 and 0.999977 for serotypes (O Pan Asia2, A IranO5 and SAT-2/EGY/2012), respectively. But calves which were injected with 1 ml of the vaccine (2µg 146S/ml), the antibody titers elevated from 3th WPV and were protective up to 16th WPV for the three serotypes with P-value was 0.989591, 0.999499 and 0.99964 for (O, A and SAT-2) (table 2-5), (fig. 1-3).

Table – (1). FMD virus titer and 146S concentration.

FMD virus types	Titer (log_{10} $TCID_{50}$)	146S (µg/ml)	146S after concentration (µg/ml)*
O Pan Asia-2	7.8	4.56	6.5
A Iran O5	7.2	4.17	6.2
SAT-2/EGY/2012	7.6	3.69	5.9

*: 146S content after concentration by PEG-6000.

Fig-(1). FMD serotype (O Pan Asia-2) serum neutralizing antibodies titer in calves vaccinated with trivalent inactivated ISA 206 oil adjuvanted FMDV vaccine.

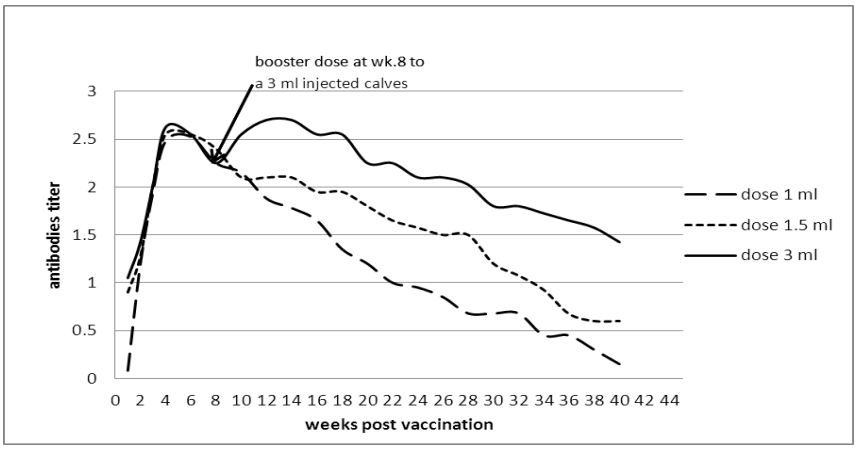

Table – (2). FMD serotype (O Pan Asia-2) serum neutralizing antibodies titer in calves vaccinated with inactivated trivalent FMD ISA-206 oil adjuvanted virus vaccine.

Weeks post vaccination (WPV)	FMD serum neutralizing antibody titer (log_{10}/ml)									
	3ml of the vaccine(6.2µg 146S/ml)			1.5ml (4.1µg 146S/ml)			1ml (2µg 146S/ml)			
	C1	C2	Mean	C3	C4	Mean	C5	C6	Mean	CC
1 WPV	1.05	1.05	**1.05**	0.9	0.9	**0.9**	0.75	0.9	**0.825**	0.0
2 WPV	1.35	1.5	**1.425**	1.35	1.2	**1.275**	1.2	1.2	**1.2**	0.0
3 WPV	1.95	2.1	**2.025**	2.1	1.95	**2.025**	1.95	1.95	**1.95**	0.0
4 WPV	2.55	2.7	**2.625**	2.7	2.4	**2.55**	2.4	2.55	**2.475**	0.0
6 WPV	2.4	2.7	**2.55**	2.7	2.4	**2.55**	2.4	2.65	**2.525**	0.0
8 WPV	2.1**	2.4**	**2.25**	2.4	2.4	**2.4**	2.1	2.4	**2.25**	0.0
10 WPV	2.7	2.4	**2.55**	2.1	2.1	**2.1**	1.95	2.35	**2.15**	0.0
12 WPV	2.7	2.7	**2.7**	2.1	2.1	**2.1**	1.65	2.1	**1.875**	0.0
14 WPV	2.7	2.7	**2.7**	2.1	2.1	**2.1**	1.65	1.9	**1.775**	0.0
16 WPV	2.4	2.7	**2.55**	2.1	1.8	**1.95**	1.65	1.65	**1.65**	0.0
18 WPV	2.4	2.7	**2.55**	2.1	1.8	**1.95**	1.35	1.35	**1.35**	0.0
20 WPV	2.1	2.4	**2.25**	1.8	1.8	**1.8**	1.05	1.35	**1.2**	0.0
22 WPV	2.1	2.4	**2.25**	1.65	1.65	**1.65**	0.95	1.05	**1**	0.0
24 WPV	2.1	2.1	**2.1**	1.65	1.5	**1.575**	0.95	0.95	**0.95**	0.0
26 WPV	2.1	2.1	**2.1**	1.5	1.5	**1.5**	0.95	0.75	**0.85**	0.0
28 WPV	1.95	2.1	**2.025**	1.5	1.5	**1.5**	0.6	0.75	**0.675**	0.0
30 WPV	1.8	1.8	**1.8**	1.2	1.2	**1.2**	0.6	0.75	**0.675**	0.0
32 WPV	1.8	1.8	**1.8**	0.95	1.2	**1.075**	0.6	0.75	**0.675**	0.0
34 WPV	1.65	1.8	**1.725**	0.95	0.9	**0.925**	0.3	0.6	**0.45**	0.0
36 WPV	1.65	1.65	**1.65**	0.75	0.6	**0.675**	0.3	0.6	**0.45**	0.0
38 WPV	1.5	1.65	**1.575**	0.6	0.6	**0.6**	0.3	0.3	**0.3**	0.0
40 WPV	1.35	1.5	**1.425**	0.6	0.6	**0.6**	0.0	0.3	**0.15**	0.0
Mean	2.02*	2.14*	2.076*	1.6*	1.56*	1.557*	1.17•	1.33•	1.246•	
SE	0.102	0.106	0.082	0.142	0.129	0.0028	0.156	0.163	0.112	0.0
SD	0.469	0.488	0.082	0.651	0.592	0.0028	0.717	0.749	0.112	
P value (Chi-test)		1 (100%)			0.999857 (99.98%)			0.989591 (98.95%)		

P value for (O Pan Asia-2) versus the mean of the three doses of the trivalent vaccine average equal 0.8974.

WPV: weeks post vaccination, C: calf, CC: calf control,**: booster dose at 8wpv to a 3ml dose injected calves, (6.2µg/serotype/ml =9.1µg/serotype/dose 146S), (4.1µg/serotype/ml =6µg/serotype/dose 146S), (2µg/serotype/ml =3µg/serotype/dose 146S),*: the titer over the PT, •: below the PT (PT=1.5).

Fig-(2). FMD serotype (A Iran O5) serum neutralizing antibodies titer in calves vaccinated with trivalent FMD inactivated ISA 206 oil adjuvanted virus vaccine.

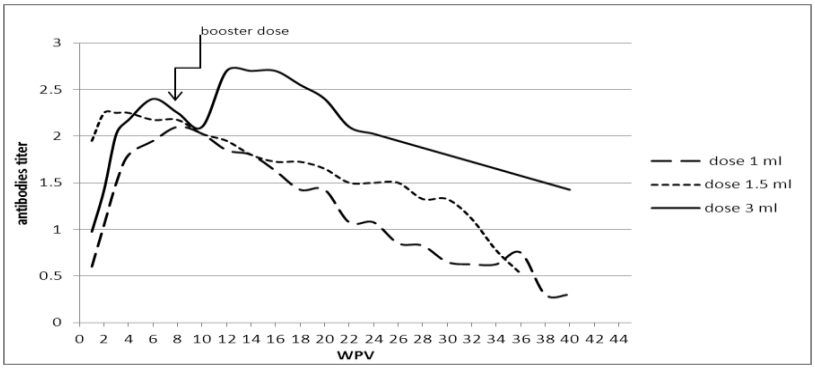

Table - (3). FMD serotype (A Iran O5) serum neutralizing antibodies titer in calves vaccinated with inactivated trivalent FMD ISA-206 oil adjuvanted virus vaccine.

Weeks post vaccination (WPV)	FMD serum neutralizing antibody titer (log_{10}/ml)									
	3ml of the vaccine (6.2µg 146S/ml)			1.5ml (4.1µg 146S/ml)			1ml (2µg 146S/ml)			
	C1	C2	Mean	C3	C4	Mean	C5	C6	Mean	CC
1 WPV	0.9	1.05	**0.975**	0.75	0.9	**0.825**	0.6	0.6	**0.6**	0.0
2 WPV	1.35	1.5	**1.425**	1.35	1.2	**1.275**	0.9	1.2	**1.05**	0.0
3 WPV	1.95	2.1	**2.025**	2.1	1.8	**1.95**	1.5	1.5	**1.5**	0.0
4 WPV	2.1	2.25	**2.175**	2.4	2.1	**2.25**	1.95	1.65	**1.8**	0.0
6 WPV	2.4	2.4	**2.4**	2.4	2.1	**2.25**	2.1	1.8	**1.95**	0.0
8 WPV	2.25**	2.25**	**2.25**	2.4	2.1	**2.25**	2.1	2.1	**2.1**	0.0
10 WPV	2.1	2.1	**2.1**	2.4	1.95	**2.175**	1.95	2.1	**2.025**	0.0
12 WPV	2.7	2.7	**2.7**	2.4	1.95	**2.175**	1.75	1.95	**1.85**	0.0
14 WPV	2.7	2.7	**2.7**	2.25	1.8	**2.025**	1.65	1.95	**1.8**	0.0
16 WPV	2.7	2.7	**2.7**	2.1	1.8	**1.95**	1.5	1.75	**1.626**	0.0
18 WPV	2.4	2.7	**2.55**	1.8	1.8	**1.8**	1.35	1.5	**1.425**	0.0
20 WPV	2.4	2.4	**2.4**	1.8	1.65	**1.725**	1.35	1.5	**1.425**	0.0
22 WPV	2.1	2.1	**2.1**	1.8	1.65	**1.725**	0.95	1.2	**1.075**	0.0
24 WPV	2.1	1.95	**2.025**	1.8	1.5	**1.65**	0.95	1.2	**1.075**	0.0
26 WPV	1.95	1.95	**1.95**	1.5	1.5	**1.5**	0.75	0.95	**0.85**	0.0
28 WPV	1.95	1.8	**1.875**	1.5	1.5	**1.5**	0.75	0.9	**0.825**	0.0
30 WPV	1.8	1.8	**1.8**	1.5	1.5	**1.5**	0.65	0.65	**0.65**	0.0
32 WPV	1.8	1.65	**1.725**	1.3	1.35	**1.325**	0.6	0.65	**0.625**	0.0
34 WPV	1.65	1.65	**1.65**	1.3	1.35	**1.325**	0.6	0.65	**0.625**	0.0
36 WPV	1.65	1.5	**1.575**	0.9	1.35	**1.113**	0.3	0.6	**0.75**	0.0
38 WPV	1.5	1.5	**1.5**	0.6	0.95	**0.775**	0.3	0.3	**0.3**	0.0
40 WPV	1.35	1.5	**1.425**	0.3	0.75	**0.525**	0.3	0.3	**0.3**	0.0
Mean	1.99*	2.01*	2*	1.73*	1.57*	1.62*	1.13•	1.23•	1.19•	0.0
SE	0.103	0.103	0.102	0.138	0.085	0.11	0.133	0.128	0.126	
SD	0.472	0.471	0.468	0.631	0.388	0.506	0.609	0.588	0.579	
P value (Chi-test)		1 (100%)			0.999994 (99.99%)			0.999499 (99.95%)		

P value for (A Iran O5) versus the mean of the three doses of the trivalent vaccine average equal 0.89967.

WPV: weeks post vaccination, C: calf, CC: calf control,**: booster dose at 8wpv to a 3 ml dose injected calves, (6.2µg/serotype/ml =9.1µg/serotype/dose 146S), (4.1µg/serotype/ml =6µg/serotype/dose 146S), (2µg/serotype/ml =3µg/serotype/dose 146S),*: the titer over the PT, •: below the PT (PT=1.5).

Fig-(3). FMD serotype (SAT-2/EGY/2012) serum neutralizing antibodies titer in calves vaccinated with trivalent FMD inactivated ISA 206 oil adjuvanted virus vaccine.

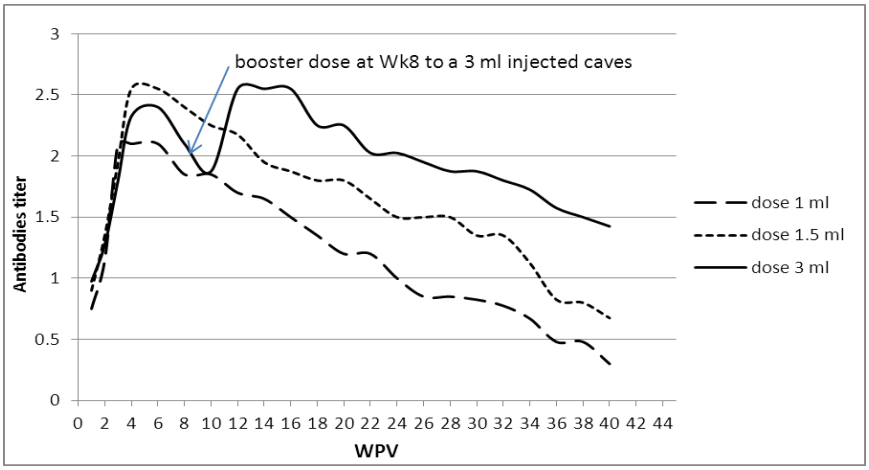

Table – (4). FMD serotype (SAT-2/EGY/2012) serum neutralizing antibodies titer in calves vaccinated with inactivated trivalent FMD ISA-206 oil adjuvanted virus vaccine.

Weeks post vaccination (WPV)	FMD serum neutralizing antibody titer (log_{10}/ml)									
	3ml of the vaccine (6.2µg 146S/ml)			1.5ml (4.1µg 146S/ml)			1ml (2µg 146S/ml)			
	C1	C2	Mean	C3	C4	Mean	C5	C6	Mean	CC
1 WPV	0.9	1.05	**0.975**	0.75	1.05	**0.9**	0.6	0.9	**0.75**	0.0
2 WPV	1.2	1.35	**1.275**	1.2	1.5	**1.35**	0.95	1.35	**1.15**	0.0
3 WPV	1.8	1.8	**1.8**	1.95	1.95	**1.95**	2.1	2.1	**2.1**	0.0
4 WPV	2.4	2.25	**2.325**	2.4	2.7	**2.55**	2.1	2.1	**2.1**	0.0
6 WPV	2.4	2.4	**2.4**	2.7	2.4	**2.55**	1.95	2.25	**2.1**	0.0
8 WPV	2.1**	2.1**	**2.1**	2.4	2.4	**2.4**	1.75	1.95	**1.85**	0.0
10 WPV	1.95	1.8	**1.875**	2.4	2.1	**2.25**	1.75	1.95	**1.85**	0.0
12 WPV	2.4	2.7	**2.55**	2.25	2.1	**2.175**	1.65	1.75	**1.7**	0.0
14 WPV	2.4	2.7	**2.55**	2.1	1.8	**1.95**	1.65	1.65	**1.65**	0.0
16 WPV	2.4	2.7	**2.55**	1.95	1.8	**1.875**	1.35	1.65	**1.5**	0.0
18 WPV	2.1	2.4	**2.25**	1.8	1.8	**1.8**	1.35	1.35	**1.35**	0.0
20 WPV	2.1	2.4	**2.25**	1.8	1.8	**1.8**	1.05	1.35	**1.2**	0.0
22 WPV	1.95	2.1	**2.025**	1.65	1.65	**1.65**	1.05	1.35	**1.2**	0.0
24 WPV	1.95	2.1	**2.025**	1.5	1.5	**1.5**	0.95	1.05	**1**	0.0
26 WPV	1.8	2.1	**1.95**	1.5	1.5	**1.5**	0.75	0.95	**0.85**	0.0
28 WPV	1.8	1.95	**1.875**	1.5	1.5	**1.5**	0.75	0.95	**0.85**	0.0
30 WPV	1.8	1.95	**1.875**	1.35	1.35	**1.35**	0.75	0.9	**0.825**	0.0
32 WPV	1.8	1.8	**1.8**	1.35	1.35	**1.35**	0.65	0.9	**0.775**	0.0
34 WPV	1.65	1.8	**1.725**	0.95	1.3	**1.125**	0.6	0.75	**0.675**	0.0
36 WPV	1.5	1.65	**1.575**	0.7	0.95	**0.825**	0.3	0.65	**0.475**	0.0
38 WPV	1.5	1.5	**1.5**	0.65	0.95	**0.8**	0.3	0.65	**0.475**	0.0
40 WPV	1.35	1.5	**1.425**	0.6	0.75	**0.675**	0.3	0.3	**0.3**	0.0
Mean	1.875*	2.01*	1.94*	1.61*	1.65*	1.628*	1.17•	1.27•	1.2•	
SE	0.09	0.097	0.09	0.137	0.110	0.123	0.129	0.121	0.124	0.0
SD	0.413	0.445	0.424	0.629	0.505	0.56	0.591	0.553	0.568	
P value (Chi-test)	1 (100%)			0.999977 (99.998%)			0.99964 (99.96%)			

P value for (SAT2/EGY/2012) versus the mean of the three doses of the trivalent vaccine average equal 0.9211.

WPV: weeks post vaccination, C: calf, CC: calf control,**: booster dose at 8wpv to a 3ml dose injected calves, (6.2µg/serotype/ml =9.1µg/serotype/dose 146S), (4.1µg/serotype/ml =6µg/serotype/dose 146S), (2µg/serotype/ml =3µg/serotype/dose 146S),*: the titer over the PT, •: below the PT (PT=1.5)

Table-(5). Different concentrations of 146S of FMDV serotypes and mean neutralizing antibody for 40 week post vaccination in cattle.

Types of FMDV	146S conc. (µg/ml)	Mean serum neutralizing antibody/40wk/post vaccination
FMDV type O Pan Asia-2	6.2	2.08
	4.1	1.56
	2	1.25
FMDV type A IranO5	6.2	2
	4.1	1.62
	2	1.19
FMDV type SAT2/EGY/2012	6.2	1.94
	4.1	1.63
	2	1.2

From these results it was clear a dose (6.2µg/ml) showed the immune response from the beginning of the vaccination and elevated to be over the protective titer start from 3WPV-early and rapid protection- also appear with a dose (4.1µg/ml), that agree with (Doel et al., 1994 and Salt et al., 1995) who showed that many experiments with high potent inactivated vaccines (4.1µg-10µg) revealed full protection in cattle by end of 1st week post vaccination upon indirect aerosol challenge from donor pigs. And for (6.2µg/ml) the immune response was over the protective titer persist for a long term (up to 38 WPV and may extend to 40WPV) with P-value=1, that is with very highly potency, that result disagree with (Bayry et al., 1999, Patil et al., 2002, Barnard et al., 2005 and El-Sayed et al., 2012) who showed that the protective titer persist up to 32 weeks post vaccination naturally and up to 36 weeks post vaccination when vaccinated with Montanide ISA-206 which promote long lasting immunity. while in a dose (4.1µg 146S) showed the protective titer up to 30th WPV, which need revaccination after 30th week, but with a dose (2µg/ml or 3µg/dose 146S), the protective titers were remain up to 16th WPV with (P-value is between 0.9895-0.999) that disagree with (Daoud et al., 2013) who explained that the minimal protective antigenic content of 146S should not less than 2.2µg/serotype/dose to give the highest protection rate in guinea pig or in cattle, so in 2µg/ml (3µg/serotype/dose) 146S in that study give protection (not full or high protection) but need revaccination.

Potency testing showed (fig.4-6) the relationship between 146S pay load and log SN/serotype. The estimated R square correlation coefficient for antibodies titer (log SN on linear regression) were 0.802, 0.896 and 0.806 for (O Pan Asia2, A Iran O5 and SAT2/EGY/2012), respectively. The 146S density gradient is the most susceptible, reliable, reproducible and straightforward test so, we use 146S and SNT as a model of estimation of potency of the vaccine for many reasons, PD_{50} has high variability, low repeatability and reproducibility between the PD_{50} tests that due to a small number of animals in each group of animals, both PG_{50} and PPG potency tests employ challenge (considered as a golden standard for potency testing for FMD in cattle) with virulent virus which is a cause of a clinical signs of FMD with considerable pain of animals, the protected animals showed extensive primary lesions in tongue, similar to controlled unvaccinated animals, the costs of animals are high and high risk of the virus escaping may be resulted in outbreaks so, chosen of estimation of 146S and SNT (substitution of PD_{50} and PPG) were used to assess the potency of the vaccine as (Alkan et al., 2008 and Parida, 2009) shown. There are many factors affecting SNT, the cell substrate, PH of the media, the maturity of cells, dose of the virus, the antigenic relationship of the assay virus to the vaccine virus and serum dilutions are encountered before or after with the virus inoculation. Therefore, different laboratories have different log_{10} serum titers. (Pay and Hingley, 1992a and Barnett et al., 2003b) recorded that there is a big difference existed in the correlation of antibody to protection between laboratories, particularly in the case of the O serotype, for that reasons each laboratory testing by serum neutralization should set own its correlation and considered as alternative method.

On conclusion, the using a highly potent vaccine (6.2µg/serotype/ml) that resulted in early rapid immunization and long term effective immunity (up to 38 wpv and more extend) which cover the most area of protection in endemic areas especially in Egypt that by estimating of 146S and SNT and that were an alternative way of protective dose50 (PD_{50}) and protection against the generalization (PPG).

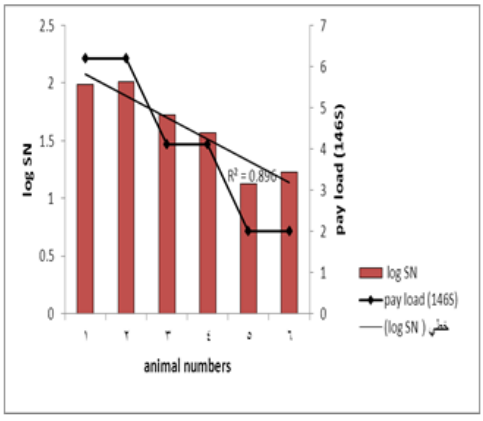

Fig-(5). Potency test data for A Iran O5 serotype.

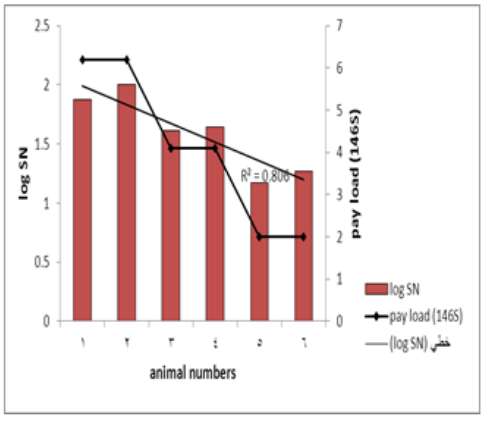

Fig-(6). Potency test data for SAT-2/EGY/2012.

There is a correlation between the protection and 146S pay load and many vaccine manufactures depend on it (fig. 7-9) as increase the content of 146S correlated with the protection.

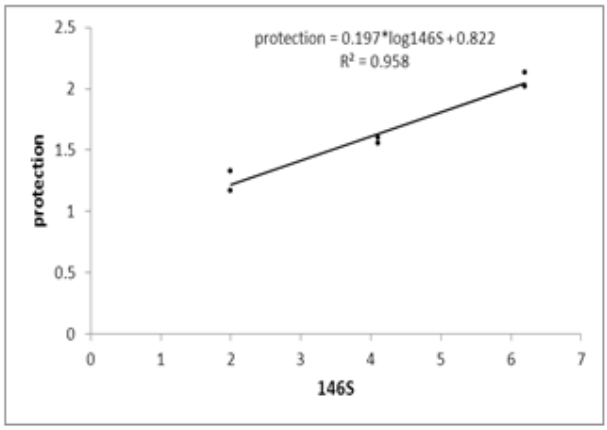

Fig-(7). Relationship between 146S mass and the protection for O Pan Asia-2.

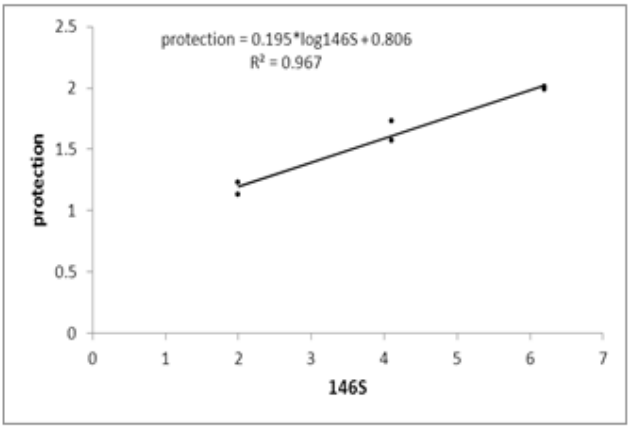

Fig-(8). Relationship between 146S mass and the protection for A Iran O5.

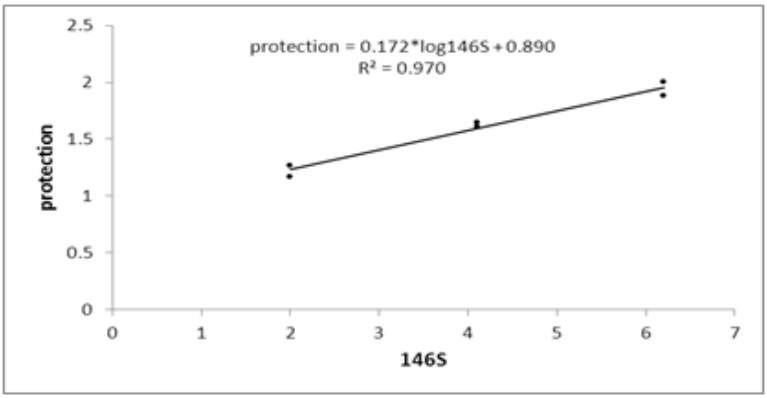

Fig-(9). Relationship between 146S mass and the protection for SAT- 2/EGY/2012

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