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Preparation of FMD bivalent vaccine A&O by estimation of 146s antigen in the cattle dose and its evaluation by using serological technique and challenge test

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SUMMARY

Foot-and-mouth disease virus (FMDV) serotypes (O/1/3/93) and (A /Egy/2009), grown on BHK-21 clone 13 monolayer cell line, inactivated with Binary Ethylenimine (BEI) and adjuvanted with 30% Alhydragel [$AL(OH)_3$] were used for preparation of bivalent gel adjuvant vaccine currently used in Egypt .146s was measured using sucrose density gradient ultracentrifugation and vaccine dose was estimated as 4.2 µg of 146s viral proteins for each vaccine serotypes. Evaluation and testing of FMD vaccine for safety, sterility and potency were carried out. Cattle protective dose 50% (PD_{50}) was determined. The specific developed neutralizing antibody responses against different doses of bivalent vaccine in correlation with the challenge exposure test for serotype (O1/3/93) virus that was done 21 days post vaccination were undertaken. The obtained potency of a vaccine with an overall 50% cattle protective dose (PD_{50}) value was 9.99 (PD_{50}) for serotype (O/ 1/3/93).

INTRODUCTION

Foot-and-mouth disease (FMD) is an extremely contagious viral disease of cloven-hoofed domesticated as well as wild animals and has a great potential for causing severe economic loss. The causal agent, FMD virus (FMDV), is a member of the genus Aphthovirus in the family Picornaviridae and occurs as seven distinct serotypes throughout the world: A, O, C, Asia1 and South African Territories (SAT) 1-3. Vaccination is the most important control and eradication strategy for FMD (Balamurugan et al., 2004, Mason et al., 2003 and Li et al., 2010). In many countries with endemic or with frequent introductions of FMD virus, the control of the disease mainly relies on vaccination of cattle and

other susceptible species. As the economic impact of an FMD outbreak can be large, the quality control of vaccines in most countries is strictly regulated, and in Europe, animal challenge tests are prescribed to show vaccine efficacy. As a result of such a challenge test, animals are either considered protected against clinical signs or not (Goris et al., 2007 and Syed et al., 2008).

In Egypt, where the disease is endemic, prophylactic vaccination is the only means of control. Aluminum hydroxide gel vaccine has been used to control the disease. The Recommendations of the 14th Conference of the Permanent Commission of the Office International des Epizooties (OIE) on Foot and Mouth Disease 1975 and (Pay and Hingley 1992a) form the basis for the testing of foot and mouth disease (FMD) vaccine potency. They require that potency should be measured by a quantitative method, and the minimum acceptance level for any assay method should be lower 95% confidence limit equates with a level of 70% protection in cattle after primary vaccination with a single dose following the observation by Wild and Brown (1968).

It is now well recognized that the major immunogenic component in preparations of foot-and-mouth disease (FMD) virus is the intact virion, the 146s antigen. However, preparations of some FMD strains can contain, in addition to intact virions, quantities of empty particles, the 75S antigen, which is also immunogenic (Pay 1971, Rweyemamu et al., 1979; and 1984, Doel and Chong 1982 and Pay and Hingley 1987). The 12S subunit antigen is of extremely low immunogenicity and plays no real part in the immunogenicity of conventional FMD vaccines. This study designed and aimed to get safe, efficient and good quality FMD bivalent vaccine by application of restricted evaluation potency.

MATERIALS AND METHODS

1- Cell culture:

Baby hamster kidney (BHK-21) clone 13 monolayer cell line was kindly supplied by the Department of FMD, Veterinary Serum and Vaccine Research Institute (VSVRI), Abbassia, Cairo and used for the vaccine preparation and in Serum Neutralization Test (SNT). (Macpherson and Stocher, 1962 and Mowat, 1974)

2-Virus:

Foot-and-Mouth Disease vaccinal strains serotypes (O/ 1/ 3/ 93) and (A/ Egy/ 2009) are maintained at FMD department, (VSVRI), were used for the vaccine production. Each strain (O, A) was passaged once in cattle tongue epithelium and then adapted to BHK-21 clone 13 monolayer cell line. The two virus strains at the 6th passage level were used as seed virus which reseeded to BHK-21 clone 13 monolayer cell line and the cell associated and cell free virus content

from both inoculated monolayer were collected 16–18 hrs post inoculation and kept frozen.

3- Vaccine preparation:

The cell culture supernatant of FMD virus of the 7th passage on BHK monolayer with titer of 10^8 $TCID_{50}$ of both serotypes (O1/3/93/Egypt and A/Egy/2009) were concentrated to1/10 of the original volume using Polyethylene glycol (PEG) 6000 according to Pay and Hingley (1987). The harvested concentrated viruses were treated with chloroform in concentration of 1.5% (Volume/Volume) at (4 - 8°C) for one hour , then clarified by centrifugation at 6000 g for 30min. at (4-8 °C) and stored for further using. Both virus serotypes were inactivated with 1% of 0.1M of Binary ethylenimine (BEI) in 0.2N NaOH and the pH adjusted to 8.0 by sodium bicarbonate. The virus and BEI mixture was mixed well and incubated at 37°C for 24 hours with continuously stirring for inactivation of both virus serotypes. Sodium thiosulphate was added to give a final concentration of 2% to neutralize the action of (BEI).The inactivated FMDV suspension (35% for "O" type and 35% for "A" type), was adjuvanted with 30% aluminum hydroxide gel and stirred on a magnetic stirrer for 2 hrs. to obtained homogenized solution and Saponine was added in concentration 1.5µg/dose as immunostimulant. FMDV concentration in the final vaccine formula was adjusted to equal to 4.2 µg of 146s for each type of the virus per vaccine dose , PH brought to 8.2 with glycol buffer and Sodium thiomersal was used as preservative at a final concentration of 0.0001 (1ml of 10% Sod Thiomersal /10 liter vaccine) according to Moussa et al., (1976). Vaccine of the both serotypes was prepared and equal quantities of each mixed to make a bivalent vaccine. The vaccine was stored at (4-8 °C) until use.

4-Estimation of 146s to determine cattle dose:

The 146S antigen of each viral fluids was estimated before and after virus concentration by using sucrose density gradient ultracentrifugation by determination the absorbance at 254 nm using (ISCO 520 C Density Gradient system) as described by (Doel and Chong, 1982 and Barteling et al., 1990). The concentration of payload antigen of inactivated viral antigen was maintained at 4.2 µg 146S viral particles/dose/serotype.

(The OIE Code sated that the dose should contain not less than 2 µg/dose/ serotype.)

5-Animals:

Three groups of clinically, apparently healthy and FMDV free cattle aged 12–18 months (5calves/each). They were vaccinated with recommended dose (2ml), (0.6ml) and (0.2ml); respectively of bivalent vaccine (antigen payload 4.2 µg 146S viral particles/dose) subcutaneously in the dewlap. Another two unvaccinated healthy FMDV

antibody free cattle aged 12–18 months were kept as unvaccinated controls.

6-Sterility test:

Vaccine was tested for its sterility and purity from any bacterial or fungal contaminants. Vaccines samples were cultured on thioglycolate broth, Sabouraud's, Nutrient agar and phenol dextrose media. If any viable microorganisms were detected, the vaccine was considered unsuitable for field use, according to Code of Federal Regulation of USA (1986)

7 -Safety Test:

Three susceptible cattle were inoculated by single dose of recommended vaccinal dose (2ml) intradermo - lingual in at least (20) sites of the tongue, 4 days later (3x) dose of recommended vaccinal dose was inoculated subcutaneously. After 7 days the vaccine considered safe as no local or general lesions appear and no rise of temperature (Henderson, 1970).

8- Challenge Test:

Three weeks post vaccination the three groups were inoculated by (10^{4}) cattle infective dose 50 (ID50) of the homologous virus serotype (O1/3/93) by intradermo-lingual route and also the two unvaccinated control ones. Animals were observed for 7 days post inoculation of challenge virus. The cattle protective dose 50% (PD50) is calculated by the method of Reed and Muench, (1938).

9-Serological tests:

Blood samples for sera were collected pre and weekly post vaccination and post challenge from all animals and Serum neutralization test (SNT) was performed by the micro technique as described by (Ferreira, 1976 and Pay and Hingley, 1992b). The SN titer of the serum was expressed as the log10 of the inverse dilution which protected 50% of wells and (PD50) were calculated (Reed and Muench, 1938).

RESULTS AND DISCUSSION

The potency of FMD vaccine regularly expressed as the number of 50% of cattle protective dose (PD_{50}) contained in the dose of the vaccine (Berlinzani et al.,1998).Table (1) illustrated SNT titers of cattle vaccinated with different doses of bivalent FMD vaccine (2ml, 0.6ml and 0.2ml) where the mean SNT antibody titers of cattle in the 1st. week for both serotypes and different doses of vaccine were (0.84-0.81, 0.66-0.66 and 0.6-0.6) for serotypes (O&A) respectively while at end of the 2nd week the mean titers were elevated to reach (1.44-1.38 , 1.11-1.14 and 0.9-0.9). At 21 days post vaccination by the third week the mean titers were still elevated (1.89-1.83, 1.56-1.56 and 1.23-1.26) and challenge FMD virus serotype (O/1/3/93) was inoculated so the means titers for (O) strain were dropped at 4th week to (1.68, 1.38 and 1.14) while the means titers for (A) strain

showed persistent elevation to (1.89 , 1.56 and 1.41).

Table (2) showed the changes in body temperature of all animals in the different groups through 8 days post challenge with FMD virus serotype (O/1/3/93) included two positive control ones, as animals number (2 , 3 and 5) of group one , number (6 , 8 and 9) of group two and number (12 , 13 and 15) of group three showed slightly elevation of temperature from the 1st day post challenge and lasted for 3 days ,while animals number (10) of group two and number (11 and 14) of group three showed high elevation of temperature and also the two control animals. These changes in body temperature of post challenge test were correlated with protection, severity of infection and sight of lesions in relation to different doses of vaccine that represented in Table (3). The animals number (13 and15) of group three showed local lesion on the tongue while animals number (10) of group two and numbers (11 and 14) of group three and two control animals showed severe generalized lesions in mouth, fore and hind limbs. The cattle protective dose 50% (PD_{50}) was calculated in Table (3) as 9.99 (PD_{50}) and the results were confirmed by the 146S antigen estimation which showed also in Table (3) as (4.2 µg / virus / dose) all these results and observations are supported by(Pay and Hingley 1992b) who sated that the relationships between serum neutralizing antibody response log_{10} of the inverse dilution and protection from challenge following a single dose primary vaccination and also by Li et al., (2010) who recorded that the antibody titer is one of referenced criteria to evaluate vaccine potency as it is positively linked with protection rate , but it influenced by factors such as vaccine' s antigen content (146S) , animal individual status. Also, other studies of Rweyemamu et al., (1979), Doel and Chong (1982) , Pay and Hingley (1987) , Pay (1971) and Rweyemamu et al., (1984) stated that the major immunogenic component in preparation of FMD virus is the intact Virion , the (146S) antigen in addition to (75S) antigen in some FMD virus strains . From this study, we proposed the estimation of (146S) antigen content of FMD virus as a great efficient method for support vaccine production.

Table: (1): FMDV Potency Evaluation by SNT (up to 28 day post vaccination)

Animal No.	Intervals	Pre-vaccination screen		Day 0		Day 7		Day 14		Day 21 *		Day 28	
	Serotype	O titer	A titer	O titer	A titer	O titer	A titer	O titer	A titer	O titer	A titer	O titer	A titer
1	Vaccine dose 2.00 ml	0.00	0.3	0.00	0.3	0.9	0.6	1.5	1.2	1.8	1.65	1.5	1.8
2		0.00	0.00	0.00	0.00	0.6	0.75	1.2	1.5	1.5	1.8	1.5	1.95
3		0.6	0.3	0.6	0.3	0.9	0.9	1.35	1.35	1.8	1.95	1.8	2.1
4		0.3	0.45	0.3	0.45	1.2	0.9	1.8	1.65	2.4	2.1	1.95	1.8
5		0.00	0.00	0.00	0.00	0.6	0.75	1.35	1.2	1.95	1.65	1.65	1.8
Mean		0.18	0.21	0.18	0.21	0.84	0.81	1.44	1.38	1.89	1.83	1.68	1.89
6	Vaccine dose 0.6 ml	0.00	0.00	0.00	0.00	0.75	0.75	1.2	1.2	1.8	1.65	1.5	1.8
7		0.6	0.6	0.6	0.6	0.9	0.9	1.5	1.35	1..95	1.8	1.65	1.65
8		0.6	0.9	0.6	0.9	0.9	0.9	1.2	1.2	1.5	1.65	1.5	1.8
9		0.00	0.00	0.00	0.00	0.3	0.3	0.75	1.05	1.35	1.5	1.2	1.35
10		0.3	0.00	0.3	0.00	0.45	0.45	0.9	0.9	1.2	1.2	1.05	1.2
Mean		0.3	0.3	0.3	0.3	0.66	0.66	1.11	1.14	1.56	1.56	1.38	1.56
11	Vaccine dose 0.2 ml	0.6	0.6	0.6	0.6	0.6	0.6	0.9	0.9	1.35	1.2	1.2	1.35
12		0.9	0.9	0.9	0.9	1.05	0.75	1.2	1.2	1.35	1.5	1.35	1.65
13		0.6	0.6	0.6	0.6	0.75	1.05	1.05	1.2	1.5	1.5	1.2	1.5
14		0.00	0.00	0.00	0.00	0.3	0.3	0.6	0.6	0.9	1.2	1.05	1.35
15		0.00	0.00	0.00	0.00	0.3	0.3	0.75	0.6	1.05	0.9	0.9	1.2
Mean		0.042	0.42	0.42	0.42	0.6	0.6	0.9	0.9	1.23	1.26	1.14	1.41
16	Control	0.00	*(Day 21 PV) application of Challenge Test by serotype (O1/3/93).										
17		0.00											

- Protective antibody titer (log10) = 1.2.

Table (2): FMD Potency Evaluation Through Temperature (°C) (Post Challenge)

Animal No.	Intervals	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
	Temp	(°C)	(°C)	(°C)	(°C)	(°C)	(°C)	(°C)	(°C)	(°C)
1	Vaccine dose 2.00 ml	38.6	38.3	38.5	38.5	38.2	38.3	38.3	38.2	38.2
2		39.5	38.0	38.5	38.8	37.9	37.9	37.8	37.7	37.6
3		38.9	40.0	38.2	38.6	38.2	38.0	38.1	38.1	38.0
4		39.0	38.9	38.8	39.0	38.4	38.6	38.5	38.4	38.4
5		38.8	38.5	38.6	39.7	38.5	38.2	38.3	38.3	38.2
6	Vaccine dose 0.6 ml	38.7	40.1	38.6	38.9	38.2	38.2	38.2	38.1	38.0
7		39.3	38.9	38.9	38.8	38.6	38.7	38.6	38.5	38.4
8		38.8	39.1	39.1	40.2	38.8	38.3	38.3	38.3	38.5
9		38.8	40.9	39.9	39.6	38.6	38.2	38.3	38.2	38.4
10		39.0	39.5	40.8	40.0	39.6	39.1	39.1	39.0	39.1
11	Vaccine dose 0.2 ml	38.6	40.2	40.4	40.0	38.3	39.3	38.8	38.7	38.7
12		38.7	39.3	38.8	39.1	38.7	40.1	39.2	39.3	39.2
13		38.5	40.3	39.7	39.2	38.0	38.5	38.3	38.4	38.4
14		38.8	39.8	40.5	40.2	38.4	38.7	38.6	38.5	38.4
15		38.9	40.1	38.8	39.0	38.3	38.5	38.5	38.5	38.3
16	control	38.7	40.9	40.1	39.8	38.9	38.6	38.6	38.6	38.5
17		38.9	39.2	40.9	39.9	39.0	38.8	38.8	38.7	38.7

- Normal temperature of Cattle (38.0 – 38.4 °C)

Table (3): Inspection of FMD lesions (Post Challenge)

Animal No.	Vaccine dose	Left Front Leg				Right Front Leg				Left Hind Leg				Right Hind Leg				Tongue	Palatum	Gum	Dental Pad	Nose	CACULATED (PD50) 9.99
		Claw		Byclaw		Claw		Byclaw		Claw		Byclaw		Claw		Byclaw							
		Lat	Med	Lat	Med	Lat	Med	Lat	Med	Lat	Med	Lat	Med	Lat	Med	Lat	Med						
1	2 ml	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
2		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
3		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
4		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
5		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
6	0.6 ml	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
7		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
8		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
9		-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
10		+	+	-	-	+	+	-	-	+	+	-	-	-	-	-	-	+	-	+	-	-	
11	0.2 ml	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	-	+	+	-	
12		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
13		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
14		+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	-	-	-	-	
15		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	
16	control	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	-	+	-	+	
17		+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	-	-	-	-	

The concentration of antigen of inactivated viral antigen was maintained at 4.2 µg 146S viral particles/dose/serotype.

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تحضير لقاح الحمى القلاعية ثنائى العترة (O &A) بحساب كمية ١٤٦s لجرعة الأبقار و تقييمة باستخدام الطرق السيرولوجية و أختبار التحدى.

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**المعمل المركزى للرقابة على المستحضرات الحيوية البيطرية

تم خلال هذا العمل تمرير و تنمية عترتى فيروس الحمى القلاعية النوع (A/Egypt/2009)و (O/1/3/93) على المزارع النسيجية لخلايا كلى اليربوع الذهبى السورى (BHK-21cl 13) وأبطال تأثيريهما باستخدام مادة البنارى أثيل أمين (BEI) و أضافة مادة الهيدراجيل المحفزة بتركيز ٣٠% وذلك لتحضير اللقاح ثنائى العترة وسيلة الوقاية الرئيسية المستخدم حاليا فى مصر. تم قياس الأنتجين (146s) بأستخدام الطرد المركزى الفائق خلال السكروس متدرج الكثافة ومن خلال ذلك قدر محتوى جرعة اللقاح بقيمة ٤.٢ ميكروجرام من البرتين الفيروسى (146s) لكل عترة من اللقاح. تم تقييم و أجراء أختبارات السلامه و النقاوة والفاعلية للقاح بالأضافة لتقدير جرعة حماية الأبقار (PD_{50}). وقد أكدت الدراسة أن تقدير استجابة الأجسام المناعية المتخصصة للجرعات المختلفة من اللقاح و أجراء أختبار التحدى بأستخدام عترة الفيروس (O/1/3/93) فى اليوم ٢١ بعد أجراء التحصين جاءتا متوافقة مع مستوى الحماية ومن خلال ذلك تم حساب قوة اللقاح الذى يوفر الحماية لنسبة ٥٠% من الأبقار (PD_{50}) والتى كانت بقيمة ٩.٩٩ للنوع (O/1/3/93) من عترة الفيروس.