Journal of
Advanced Veterinary Research
Volume 2 (2012) 198-205 **Original Research**

Effect of Different Storage Temperatures on the Efficacy of the Bivalent Foot and Mouth Disease Oil Vaccine

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(Recieved 8 June 2012/ Accepted 6 Juli 2012)

Abstract

The storage stability of locally produced double oil emulsion adjuvant bivalent Foot and mouth disease (FMD) vaccine prepared from type O1/Aga/ EGY/93 strain and A/EGY/1/2006 had been determined depending on its shelf life in different storage temperatures during the registration of this vaccine by the Central Laboratory for Evaluation of Veterinary Biologics, Abbasia, Cairo. Samples of this vaccine were kept at 4°C for period of 27 months; at 25°C for 5 weeks and at 37°C for 3 weeks. The potency of these vaccine samples was evaluated in guinea pigs as laboratory animal's model. The obtained results confirmed that the vaccine keep its potency beyond the normal conservation period at 4°C for two years with 100% protection against challenge with FMDV O1/Aga/EGY/93 and at 25°C for 3 weeks and at 37°C for 1 week, showing 80% protection when storage of the vaccine at 25°C for 4 weeks; at 37°C for 2 weeks. On challenge with A/EGY/1/2006 the vaccine gave 100% protection when storage at 4°C for 21 months; at 25°C for 2 weeks and at 37°C for 1 week. Otherwise it gave 80% protection when storage at 4°C for 24 months; at 25°C for 3 weeks and at 37°C for 2 weeks then became invalid after 27 months at 4°C; after 4 weeks at 25°C and for 3 weeks at 37°C. So it could be concluded that 4°C is the best temperature of choice for storage of the oil inactivated bivalent FMD vaccine.

Keywords: FMD; oil Montanide ISA206; keeping quality; storage; temperature; vaccine

Introduction

Proper storage, transportation and handling of inactivated foot and mouth disease (FMD) vaccines are necessary for successful vaccination campaigns in areas where the disease is endemic. So, the shelf life of theses vaccines must be determined (Butchaiah *et al*., 1985).

Guinea pigs are susceptible animals to FMD and can be protected by aqueous FMD vaccines. The methods of demonstrating the potency of such vaccines using guinea pigs have been described which have a good correlation with the protection afforded to cattle (Black *et al*., 1985).

Foot and mouth disease vaccine adjuvanted with Montanide ISA oil was found to be valid for more than 2 years when the 50% guinea pig protective dose (GPPD50) was calculated (Samira *et al*., 1999).

FMD quadrivalent oil double emulsion (Montanide ISA 206) vaccines were tested in sheep. The oil adjuvant elicited a better immune response at any time than did the aluminum hydroxide gel vaccine (Patil *et al*., 2002). The immune response of vaccinated goats with alhydargel and double oil emulsion Montanide ISA 206 vaccines persisted for 20 and 36 weeks post challenge, respectively (Fathia, 2003). Also, Sonia (2007) and Selim *et al*. (2010) found that such vaccine induced long lasting immunity than that with Alhydragel adjuvant.

The vaccine shelf life indicated by the manufacturer is usually twelve months under the specified conditions of storage. However, the ultimate shelf life of these vaccines remains to be determined (Ferris *et al*., 1984). The shelf life of an inactivate oil adjuvant FMD vaccine at 4°C was tested for a storage period of 15 months, as there was no appreciable vaccine potency loss could be detected during that period by the direct challenge testing of vaccinated cattle and antibody assay(Abaracon *et al*., 1980; Doel, 2003). In addition, Terpestra *et al.* (1994) showed that the potency of two double oil emulsion (DOE) FMD vaccine after a storage period of 1 year at 4°C was equal to that obtained shortly after formulation. Although this potency

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had markedly decreased after 2 years yet a standard dose still induce 50% protection in vaccinated animals. They suggested that DOE vaccine after storage for 2 years provides adequate protection against field infection.

The mean protective serum antibody titers against FMD in calves vaccinated with double oil emulsion (Montanide ISA 206) evaluated by ELISA and SNT was started at the 3rd week post vaccination reached the highest antibody level at the 10th week and continued with the protective level till the 32nd week post vaccination then began to decline under the protective level for both FMD virus types O1/Aga/EGY/93 and A/EGY/1/2006 (Gamil, 2010).

The present work was carried out through the registration steps of the newly produce bivalent FMD oil vaccine aiming to provide useful; accurate and complete information about the vaccine including the proper storage periods at different temperatures.

Materials and methods

Animals:

Guinea pigs:

Two hundred and fifty Albino apparently healthy adult guinea pigs of approximately 500 grams body weight, from lab. Animal house in Veterinary serum and vaccine research Institute, were used for preparation of guinea pigs adapted FMD virus, potency test and stability test of FMD bivalent oil vaccines.

Calves:

Twenty seven apparently healthy native breed calves of six to eight months old of about 250-300 Kg body weight were used. These calves were found to be free from antibodies against FMD virus serotypes O_1/Aga/ EGY/93 and A/EGY/1/2006 as screened by serum neutralization test and ELISA used for safety and potency (study the efficacy of the vaccine via challenge and the duration of antibody level).

Viruses:

Locally isolated FMDV type O_1/Aga/EGY/93 with titer 10^9 $TCID_{50}$ and type A/EGY/1/2006 with titer 10^9 $TCID_{50}$ were supplied by Foot and Mouth Vaccine Research Department (FMDRD), Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. The virus type A was confirmed by world reference laboratory for FMD (WRL) Pirbright London, UK as A/EGY/1/2006. These viruses were used for production of the bivalent FMD vaccines, challenge test.

Cell culture:

Baby Hamster kidney cell line (BHK21) Clone 13 maintained in FMD Department, Abbasia, Cairo using Eagl's medium with 8-10% sterile bovine serum, obtained from Sigma Company, USA, was used for application of serum neutralization test.

Adapation of FMD viruses to guinea pigs:

Foot and mouth disease guinea pig's adapted viruses were obtained following the method described by Carrillo *et al*. (1990) and Núñez *et al*. (2007). 10 male guinea pigs were used for every virus strain where 3 animals were used for each of 3 successive virus passages keeping one animal as control. Guinea pigs were inoculated by intra-dermal injection in the metatarsal pad of the left hind foot with 100µl of a viral suspension obtained after low-speed centrifugation of vesicular fluid and homogenized tissue in phosphate-buffered saline. Animals were euthanized at the 4th day post-infection, and vesicular fluid and epithelia around the vesicles were collected, homogenized, and used for further inoculations.

Preparation of inactivated FMD vaccines:

Foot and mouth disease viruses were propagated in BHK21 cell line in roller bottles and both virus types were inactivated with Binary Ethyleneimine (BEI) according to Bahnemann (1975). The vaccine formulation was carried out according to the method described by Barnett *et al*. (1996), where the oil phase consisted of Montanide ISA 206 mixed with the inactivated viruses as equal parts of an aqueous and oil phase (weight/ weight) and mixed thoroughly. The vaccine was prepared on the base that each dose (2 ml) of vaccine contains not less 10^8 $TCID_{50}$/ dose of each virus type.

Virus titration:

Titration of FMD viruses was carried out using the micro titer technique (SNT) to detect the infectivity titer which expressed as $\log_{10}$ $TCID_{50}$ as described by Reed and Muench (1938).

Antigenicity titration:

Antigenicity titration of FMD viruses used in the preparation of FMD vaccine was carried out by complement fixation test (CFT) using reference hyper-immune serum against FMDV. The CFT was carried out according to the method adopted by Traub and Manso (1944).

Evaluation of the prepared vaccine:

Montanide ISA206 oil was obtained from Seppic Company in france.
Bivalent inactivated montanide ISA206 oil FMD vaccine was prepared and subjected to the following quality control tests:

Sterility test:

It was carried out according to the directions of the Code of Federal Regulation of USA (1986).Testing the freedom of the prepared inactivated FMD vaccine was done by culturing random samples of such vaccines on Tryptose phosphate broth; thioglycolate media, Sabauraud's dextrose agar and mycoplasma medium as reported by OIE (2010).

Safety test:

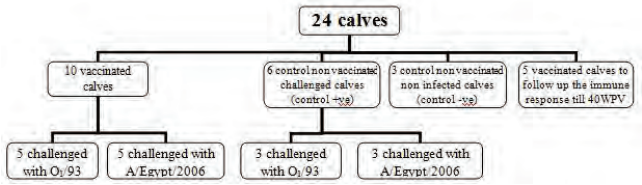
The inactivated FMD viruses were tested for safety in vitro on BHK21 clone 13 cell line according to Terpestra *et al*. (1994) and the safety of the whole prepared vaccines was tested in vivo in 3 susceptible calves according to Henderson (1970) by intradermo-lingual inoculation of 1 ml in 10 sites of the tongue of 3 susceptible calves OIE (2010).

Potency test:

In guinea pigs:

Potency of the prepared vaccine was tested in guinea pigs according to Black *et al*. (1985) and Challa *et al.* (2011) where 30 guinea pigs were divided into 6 groups. The vaccine potency was determined by calculation of the PD50 of formulated vaccines by using guinea pigs according to Reed and Muench (1938).

Experimental design for potency test in calves:



Keeping quality test:

Samples of the prepared DOE bivalent FMD vaccine were stored at 4°C; 25°C and 37°C and their validity was tested on month, week and day intervals through determination of the vaccine potency in Guinea pigs.

Serological assays:

Serum neutralization test (SNT) described by Ferreira (1976) and Enzyme linked immunosrobent assay (ELISA) according to Voller *et al*. (1976) were carried out to determine FMDV induced antibody levels in vaccinated calves.

Results

Results of the present study revealed that FMD virus used in the vaccine preparation was of high infectivity and antigenicity titers (Table 1).

Table 1. Infectivity and antigenicity titers of FMD virus

Serotype of FMDV	Infectivity titer	Complement fixation titer
FMDV/O	10^9 $TCID_{50}$/ ml	64
FMDV/A	10^9 $TCID_{50}$/ ml	32

Calculation of GPPD50 was done according to Reed and Muench (1983), for the bivalent inactivated montanide ISA206 oil adjuvant FMD vaccine it was GPPD50 = 88 GPPD50 as shown in table 2.

The mean antibody titer against FMDV type O1/Aga/EGY/93 (animals no. 1-5) started to increase from 0.32 $\log_{10}$ before vaccination to 0.96 $\log_{10}$ after the first week post vaccination and became as a protective titer at the 2nd WPV (1.53 $\log_{10}$), but the mean antibody titer against FMDV type A/ EGY/1/2006 (animals no. 6-10) started to increase from 0.32 $\log_{10}$ before vaccination to 0.78

log_{10} after the first week post vaccination and became as a protective titer at the 3rd WPV (1.89 log_{10}) using SNT but by using ELISA the mean antibody titer against FMDV type O1/Aga/EGY/93 started to increase from 0.32 log_{10} before vaccination to 1.31 log_{10} after the first week post vaccination and became as a protective titer at the 2nd WPV (1.8 log_{10}) and the mean antibody titer against FMDV type A/ EGY/1/2006 started to increase from 0.32 log_{10} before vaccination to 1.18 log_{10} after the first week post vaccination and became as a protective titer at the 3rd WPV (2.14 log_{10}).

The mean antibody titer for control positive calves before challenge (no. 11-16) remained with neglected non protected antibody titer till starting for challenge (0-0.3 log_{10}). Also the mean antibody titer for control negative cattle (no. 17-19) remains with neglected non protected antibody titer till ending of experiment (0-0.3 log_{10}) (Table 3).

Table 2. GPPD50 for the prepared ISA 206 DOE-FMD vaccines

Vaccine Dilution	No. Of vaccinated Guinea pigs	No. of Protected animals	Cumulative number of protected Animals	Cumulative number of non protected animals	Protection %
Undiluted	5	5	18	0	100%
1/4	5	5	13	0	100%
1/16	5	4	8	1	88%
1/64	5	4	4	2	66%
1/256	5	0	0	7	0%

GPPD50 = 88 GPPD50

Table 3. Results of calves vaccinated with inactivated bivalent FMD oil vaccine and challenged with FMD virus strain O1/Aga/ EGY/93 and A/EGY/1/2006

Calves No.	Treatment	Pre Vaccination		FMD antibody titers (log10/ml)/Week post vaccination estimated by SNT						ELISA						Challenge	
				1WPV*		2WPV		3WPV		1WPV		2WPV		3WPV			
		O	A	O	A	O	A	O	A	O	A	O	A	O	A		
1	Vaccinated with bivalent inactivated ISA 206 oil FMD vaccine	0.3	-	0.9	-	1.5	-	1.8	-	1.2	-	1.7	-	2.1	-	With FMDV O1/93	
2		0.3	-	0.9	-	1.5	-	2.1	-	1.2	-	1.8	-	2.5	-		
3		0.4	-	1.05	-	1.8	-	2.1	-	1.4	-	2	-	2.5	-		
4		0.3	-	0.9	-	1.65	-	1.95	-	1.4	-	1.9	-	2.4	-		
5		0.3	-	1.05	-	1.2	-	1.95	-	1.35	-	1.6	-	2.3	-		
6		-	0.3	-	0.6	-	1.35	-	1.8	-	1.1	-	1.5	-	2	With FMDV A/2006	
7		-	0.3	-	0.6	-	1.35	-	1.95	-	1.1	-	1.6	-	2.3		
8		-	0.4	-	0.9	-	1.6	-	1.95	-	1.2	-	1.8	-	2.2		
9		-	0.3	-	0.9	-	1.5	-	1.8	-	1.2	-	1.8	-	2.2		
10		-	0.3	-	0.9	-	1.2	-	1.95	-	1.3	-	1.6	-	2		
Mean		0.32	0.32	0.96	0.78	1.53	1.4	1.98	1.89	1.31	1.18	1.8	1.66	2.36	2.14		
11	Control positive	0	-	0	-	0	-	0	-	0	-	0	-	0	-	With FMDV O1/93	
12		0.3	-	0.3	-	0.3	-	0.3	-	0.3	-	0.3	-	0.3	-		
13		0	-	0	-	0	-	0	-	0	-	0	-	0	-		
14		-	0.3	-	0.3	-	0.3	-	0.3	-	0.3	-	0.3	-	0.3	With FMDV A/2006	
15		-	0.3	-	0.3	-	0.3	-	0.3	-	0.3	-	0.3	-	0.3		
16		-	0	-	0	-	0	-	0	-	0	-	0	-	0		
17	Control negative	0.3	0	0.3	0	0.3	0	0.3	0	0.3	0	0.3	0	0.3	0	Control -ve	
18		0.3	0	0.3	0	0.3	0	0.3	0	0.3	0	0.3	0	0.3	0		
19		0	0.3	0	0.3	0	0.3	0	0.3	0	0.3	0	0.3	0	0.3		

*WPV: Weeks post vaccination

The results in table 4, demonstrated that the mean SNT antibodies titer against FMDV type A/1/EGY/2006 started to increase from 0.05 $\log_{10}$ before vaccination to reach the peak at the 8th WPV (2.212 $\log_{10}$), then decreased to become non protective by the 40th WPV (1.45 $\log_{10}$) but the mean SNT antibodies titer against FMDV type O1/Aga/EGY/93 started to increase from (0.122 $\log_{10}$) before vaccination to reach the peak at the 8th WPV (2.241 $\log_{10}$), then decreased to become non protective by the 40th WPV (1.476 $\log_{10}$). While the mean ELISA antibodies titer against FMDV type A/1/EGY/2006 started to increase from (0.36 log10) before vaccination to reach the peak at the 8th WPV (2.51 $\log_{10}$), then decreased to become non protective by the 40th WPV (1.52 $\log_{10}$) and the mean ELISA antibodies titer against FMDV type O1/Aga/EGY/93 started to increase from (0.31 $\log_{10}$) before vaccination to reach the peak at the 4th WPV (2.62 log10), then decreased to become non protective by the 40th WPV (1.62 $\log_{10}$).

Table 4. Monitoring the duration of FMD mean antibody titer in vaccinated calves using SNT and ELISA

Weeks post vaccination	Mean SNT titer ($\log_{10}$)		Mean ELISA titer ($\log_{10}$)	
	A	O	A	O
0	0.05	0.122	0.39	0.31
4	2.175	2.139	2.49	2.62
8	2.212	2.241	2.51	2.4
12	2.15	2.125	2.44	2.56
16	1.95	1.99	2.31	2.44
20	1.863	1.881	2.19	2.27
24	1.913	1.911	2.11	2.14
28	1.763	1.761	2.09	2.03
32	1.65	1.779	1.84	1.99
36	1.613	1.635	1.95	1.99
40	1.45	1.476	1.52	1.62

As shown in table 5, the vaccine keep its potency beyond the normal conservation period at 4°C for at least two years and it induced protection against challenge with FMDV O1/Aga/ EGY/93 of 100% for storage at 4°C for 24 month then become invalid but when challenge with FMDV A/1/ EGY/2006 of 100% for storage at 4°C for 21 month then decrease to become 80% at 24 months but still valid and become invalid at 27 months.

Table 5. Protection % of guinea pigs vaccinated with inactivated bivalent FMD/ ISA206 oil vaccine stored at 4ºC after challenged with FMD virus type O1/Aga/ EGY/93 or A/EGY/1/2006

Storage periods	Number of challenged guinea pigs		Number of survived guinea pigs		Protection %	
	O	A	O	A	O	A
0 day	5	5	5	5	100	100
3 months	5	5	5	5	100	100
6 months	5	5	5	5	100	100
9 months	5	5	5	5	100	100
12 months	5	5	5	5	100	100
15 months	5	5	5	5	100	100
18 months	5	5	5	5	100	100
21 months	5	5	5	5	100	100
24 months	5	5	5	4	100	80
27 months	5	5	3	3	60	60

As shown in table 6, the vaccine kept its potency beyond the normal conservation period at 25°C for at least 3 weeks and it induced protection against challenge with FMDV O1/Aga/ EGY/93 of 100% for storage at 25°C for 3 weeks then decrease to become 80% at 4 weeks but still valid and become invalid at 5 weeks but when challenge with FMDV A/1/ EGY/2006 of 100% for storage at 25°C for 2 weeks become invalid at 4 weeks. On the other hand, the vaccine keep its potency beyond the normal conservation period at 37°C for at least 2 weeks and it induced protection against challenge with FMDV O1/Aga/ EGY/93 or with FMDV A/1/ EGY/2006 of 100% for storage at 37°C for 1 weeks then decrease to become 80% at 2 weeks but still valid and become invalid at 3 weeks (Table 7).

Table 6. Protection % of guinea pigs vaccinated with inactivated bivalent FMD/ ISA206 oil vaccine stored at 25ºC after challenged with FMD virus type O1/Aga/ EGY/93 or A/EGY/1/2006

Storage periods	Number of challenged guinea pigs		Number of survived guinea pigs		Protection %	
	O	A	O	A	O	A
0 day	5	5	5	5	100	100
1 weeks	5	5	5	5	100	100
2 weeks	5	5	5	5	100	100
3 weeks	5	5	5	4	100	80
4 weeks	5	5	4	3	80	60
5 weeks	5	5	3	3	60	60

Table 7. Protection % of guinea pigs vaccinated with inactivated bivalent FMD/ ISA206 oil vaccine stored at 37ºC after challenged with FMD virus type O1/Aga/ EGY/93 or A/EGY/1/2006

Storage periods	Number of challenged guinea pigs		Number of survived guinea pigs		Protection %	
	O	A	O	A	O	A
0 day	5	5	5	5	100	100
1 weeks	5	5	5	5	100	100
2 weeks	5	5	5	5	100	100
3 weeks	5	5	5	4	100	80
4 weeks	5	5	4	3	80	60
5 weeks	5	5	3	3	60	60

Discussion

The obtained results shown in table (1) revealed that FMD viruses used in the vaccine preparation had high infectivity and antigenicity titers. It was found that the prepared bivalent oil FMD vaccine was free from aerobic and anaerobic bacteria; fungi and mycoplasma in agreement with the recommendations of the OIE (2010).

Regarding the insurance of complete virus inactivation, it was found that there was no detection of cytopathic effect (CPE) on BHK clone 13 indicating that no viable viral residues in all of tested vaccines. Also no local or general symptoms or lesions developed in cattle and there were no changes in their body temperature. These observations revealed that the tested vaccine is safe as recommended by Henderson (1970) and Terpestra *et al*. (1994).

The achieved results showed that the control non vaccinated animals showed clinical signs of FMD virus infection after challenge with virulent virus at different sites of the tongue with body temperature 41ºC; salivation, appearance of vesicles on the mucous membrane of the mouth, tongue specially at the sites of inoculation and also vesicle between interdigital space. There were no signs or lesions of FMD on the vaccinated animals after challenge. Similar signs were recorded in naturally and experimentally infected animals by McVicar and Sutmoller (1972); Arafa (1980); Deeb *et al*. (1987) and Musser (2004).

The obtained results were shown in tables (3 and 4) recorded that SNT and ELISA titers were parallel with each other indicating the validity of the bivalent oil FMD vaccine on the detected preservation periods at different temperature for 2 years at 4ºC; for 3 weeks at 25ºC and for 2 weeks at 37ºC as shown in table (5, 6 and 7). SNT results against FMDV type O1/Aga/ EGY/93 revealed that the mean protective antibody titers started at the 2nd week post vaccination (1.53 $\log_{10}$), the mean of antibody titer reached the peak of protective level at the 8th week post vaccination (2.241 $\log_{10}$) and the mean of antibody titer continued with protective level till 36th weeks, then declined under the protective level, but SNT results against FMDV type A/EGY/1/2006 results revealed that the mean protective antibody titers started at the 2nd week post vaccination (1.4 $\log_{10}$), the mean of antibody titer reached the peak of protective level at the 8th week post vaccination (2.212 $\log_{10}$) and the mean of antibody titer continued with protective level till 36th weeks, then declined under the protective level. ELISA results against FMDV type O1/Aga/ EGY/93 revealed that the mean protective antibody

titers started at the 2nd week post vaccination (1.8 $\log_{10}$), the mean of antibody titer reached the peak of protective level at the 8th week post vaccination (2.4 $\log_{10}$), but ELISA results against FMDV type A/EGY/1/2006 revealed that the mean protective antibody titers started at the 2nd week post vaccination (1.6 $\log_{10}$), the mean of antibody titer reached the peak of protective level at the 8th week post vaccination (2.51 $\log_{10}$) and the mean of antibody titer continued with protective level till the 36th weeks, then declined under the protective level. These levels of FMD neutralizing antibody indices appear to be higher than the recommended protective titer (1.5 by SNT and 1.8 by ELISA) as shown by Moussa *et al*. (1976); Barteling and Vreeswij (1991); Halima *et al*. (1999) and Abd El-Rahman *et al*. (2007).

The obtained results were shown in tables (5,6 and 7) confirmed that the vaccine keep its potency beyond the normal conservation period at 4°C for at least two years and it induced protection against challenge with FMDV O1/Aga/ EGY/93 of 100% for storage at 25°C for 3 weeks and at 37°C for 1 week, showing 80% protection when storage of the vaccine at 25°C for 4 weeks; at 37°C for 2 weeks. But after 24 month at 4°C, after 3 weeks at 25°C and after 2 weeks at 37°C the vaccine became invalid. On challenge with A/EGY/1/2006 the vaccine gave 100% protection for storage the vaccine at 4°C for 21 months; at 25°C for 2 week and at 37°C for 1 week, with 80% protection for storage of the vaccine at 4°C for 24 months; at 25°C for 3 weeks and at 37°C for 2 weeks then became invalid at 4°C after 27 months; at 25°C after 4 weeks and at 37°C for 3 weeks. So it could be concluded that 4°C is the best temperature for storage of the oil inactivated bivalent FMD vaccine where it still valid for 2 years, but under un recommended temperature as 25°C it still valid for 4 weeks and at 37°C still valid for 2 weeks as shown by Black *et al*. (1985); Samira *et al*. (1999); Abaracon *et al.* (1980); Doel (2003) and Terpestra *et al*. (1994).

References

Abaracon, P., Alonso Fernandez, A., Magallanes, N., Charles, E.G., Durini, L.A., 1980. Protection of cattle following vaccination with oil adjuvant foot and mouth disease vaccine. Boletin de Centro Panamericano de Fiebre Aftosa, 37/38, 41-47.

Abd El-Rahman, A.O., Azab, A.M.H., Agaour, A.M., Fatma, A.A. Moussa, Manal Abo El-Yazeid, 2007. Studies on cellular and humeral immune response in cattle against FMD bivalent vaccine. J.Egyp.Vet.Med.Ass. 67 (2), 265-272.

Arafa, M.H., 1980. Studies on the carrier state of goats exposed to foot and mouth disease virus. MVSc Thesis, Cairo University, Egypt.

Bahnemann, H. G., 1975. Oil-adjuvant vaccine against foot-and-mouth disease. Boletin del Centro Panamericano Fiebre Aftosa 53, 25-30.

Barnett, P., Pullen, L., Williams, L., Doel, T.R., 1996. Assessment of Montanide ISA 25 and ISA 206, two commercially available oil adjuvant. Vaccine 14 (13), 1187-1198.

Barteling, S.J., Vreeswij, K.J., 1991. Development in foot and mouth disease vaccine. Vaccine 9 (2), 75-88.

Black Francis, M.J., Rweyemamu, M.M., Umehara,O., Boge, A., 1985. The relationship between serum antibody titers and protection from foot and mouth disease in pigs after oil emulsion vaccination. Journal of biological standardization 12 (4), 379-389.

Butchaiah, G., Subba Rao, M.V., Madhusudan, P., Rao, B.U., 1985. Storage stability of Foot and Mouth Disease vaccines. Res. Sci. Tech. Off. Int. Epiz. 4 (1), 139-143.

Carrillo, C., Plana, J., Mascarella, R., Bergada, J., Sobrino, F., 1990. Genetic and phenotypic variability during replication of foot-and-mouth disease virus in swine. Virology 179, 890-892.

Code of Federal Regulation of USA 1986. Animal and animal products 9\1986. Published by the office of the federal register national archives and Record administration.

Challa, S., Szczepanek, S.M., Rood , D., Barrette, R.W., Silbart, L.K., 2011. Bacterial Toxin Fusion Proteins Elicit Mucosal Immunity against a Foot-and-Mouth Disease Virus Antigen When Administered Intranasally to Guinea Pigs. Advances in Virology 2011:713769. Epub 2011 Sep 22.

Doel, T.R., 2003. Review of FMD vaccines. Virus Research 91, 81-99.

Deeb,S., Hassan,M.S., Daoud, A., Fadia Fahmy, 1987. Pancreatic change in animals experimentally infected with foot and mouth disease virus. Assuit Veterinary Medical Journal 19 (37), 70–74

Fathia, A.M., 2003. Vaccination of goats with FMD vaccines. MVSc Thesis, Alexandria University, Egypt.

Ferreira, M.E.V., 1976. Prubade microneutralization poraestudies de anticueropos de la fibre aftosa. 13th Centropanamericano Fiebre Aftosa, (21/22), 17-24.

Ferris, N.P., Kitching, R.P., Oxtoby, J.M., Philpot, R.M., Rendle R., 1984. Use of inactivated foot and mouth disease virus antigen in liquid phase blocking ELISA. Journal of Virological Methods 29 (1), 33-41.

Fontaine, G., Dubocland, C., Bornarel, P., 1966. Vaccination against FMD in sheep search for a method of testing immunity. Bull.Off.Int.Epiz. 65, 205-212.

Gamil, M.A., 2010. Studies on the immune response of calves vaccinated inactivated bivalent FMD virus vaccine type O1 and A Egypt 2006. M.V.Sc in Veterinary Science (virology). Benha University, Egypt.

Halima El-Watany; Shawky, M.; Rhshdy, O.H., Samira El-Kilany, 1999. Realtionship between cellular and humeral immune response in animals vaccinated with

FMD vaccine. Zagzig Veterinary Medical Journal 27 (1), 137-143.

Henderson,W.M., 1970. Foot and mouth disease: a definition of the problem and routs on its isolation. British Veterinary Journal 126, 115-120.

McVicar, J.W., Sutmoller, P., 1972. Foot and Mouth disease in sheep and goats: early virus growth in the pharynx and udder. Proc. Ann. Mtg. US Livestock Sanit Association 75, 194-199.

Moussa, A.A.M., Ibrahim, M.H., Hussein, K., 1976. Preliminary study on antibody response of cattle after experimental infection with FMDV. Proc. Of the 13th Arab Vet. Conger., 13-18 February, Cairo, Egypt

Musser, J.M., 2004. A practitioner's primer on foot-and-mouth disease. Journal of the American Veterinary Medical Association 224 (8), 1261-1268.

Núñez, J.I., Molina, N., Baranowski ,E., Domingo, E., Clark, S., Burman, A., Berryman, S., Jackson ,T., Sobrino, F., 2007. Guinea pig-adapted foot-and-mouth disease virus with altered receptor recognition can productively infect a natural host. Journal of Virology 81(16), 8497-8506.

OIE, 2010. Foot and mouth disease, Chapter 2.1.1. In manual of standards for diagnostic tests and vaccine, 4th Ed. 2000, Paris, pp. 77-92.

Patil, P.K., Bayry, J., Ramakrishna, C., Hugar, B., Misra, L.D., Natarajan, C., 2002. Immune response of goats against FMD quadrivalent vaccine: comparison of double oil emulsion and aluminum hydroxide gel vaccine in eliciting immunity. Vaccine 20, 2781-2789.

Reed, L.J., Muench, H., 1938. A simple method for estimating fifty percent (50%) end points. Amer. J. Hyg. 27, 493-497.

Samira El-Kilany, El- Watany, H., Abd El-Rahman, A.O., Fadia Fahmy, 1999. The effect of long term storage at 4°C on the stability of FMD vaccine formulated with different adjuvants. Alexandria Journal of Veterinary Sciences 15 (1), 35-42.

Selim A.M.A., Abouzeid N.Z., Agaour A.M., Sobhy N.M., 2010. Comparative Study for Immune Efficacy of Two Different Adjuvants Bivalent FMD Vaccines in Sheep. Journal of American Science 6 (10), 1292-1298.

Sonia, A.M., 2007. Studies on the preparation of an improved Foot and Mouth Disease oil vaccine. Ph. D. Thesis, Cairo University, Egypt.

Terpestra, C., Dekker, A., Van Maanen, 1994. Shelf life of FMD-double oil emulsion vaccines prepared from stored antigen. European Commision for the control of FMD, Vienna, appendex 15, pp. 80-84.

Traub, E., Manso, I.R., 1944. Uber die herstellung complement bindender merrsch-weinehon sera fur die typen diagnose bie Maul und Klauenseuche. Zbi. Bakt. Lorig. 151, 380-381.

Voller, A., Bidwell, D.E., AnnBartlett., 1976, Enzyme immuno- assay in diagnostic medicine, theory and practice. Bull. World Health Organ 53, 55-65.