



Effect of Carbomer as an Adjuvant for Enhancement of Immune-Response Against FMD Vaccine

Walaa S. Shabana^{1*} and Abeer Abd EL-Sadek²

¹Foot and Mouth Disease Research Department , Veterinary Serum and Vaccine Research Institute (VSVRI), Agricultural Research Center (ARC), Abassia, Cairo, Egypt.

²Department of Infectious Diseases, Faculty of Veterinary Medicine, El-Wadi El-Gadid University, Egypt.

*Corresponding Author, **Walaa S. Shabana**, E-mail: walaa.shabanaomar@gmail.com

ABSTRACT

This work was designed to provide high protective, long-lasting immunity against FMD by enhancing the immunogenicity of the trivalent FMD vaccine using carbomer as adjuvant using G. pigs as an alternative cheapest animal model for quality control testing of the prepared FMD vaccines formulae. Guinea pigs were chosen as experimental models to develop concepts and techniques to study the PD50 of FMD vaccines because of the similarities of clinical symptoms in these animals to those of swine and cattle to saving cost, three different formulae of inactivated trivalent FMD vaccine including serotypes O Pan Asia2, A Iran O5 and SAT2/EGY/2012 were prepared as formula 1- (50% carbomer to 50% antigen); formula 2 (50% Montanide ISA 206 to 50% antigen) and formula 3- (25% Montanide ISA 206 and 25% carbomer with 50 % antigen). All of such formulae were found to be free from foreign contaminants, safe and potent, showing no postvaccinal reactions and high protective levels of specific FMD antibodies in Guinea pigs. Each vaccine formula's immunogenicity was determined by estimation of 50% Guinea pig protective dose (GPPD₅₀) and monitoring of the humeral antibody response of vaccinated G. Pig groups. It was found that Montanide oils 206 with carbomer is the best vaccine formula, followed by Montanide oils 206 and finally carbomer which give early short-lasting immunity.

Keywords: Adjuvants, Carbomer, FMD, Montanide ISA206.

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INTRODUCTION

Foot and mouth disease (FMD) is one of the most highly contagious diseases of cloven-hoofed animals worldwide (**Grubman and Baxt, 2004**). FMD is able to infect G. Pigs, buffalo, goats, pigs and wild cloven-hoofed animals. FMD virus (FMDV) is the real causative agent, represented by seven serotypes (A, C, O, SAT1, SAT2, SAT3 and Asia1) (**Depa et al., 2012**). The main clinical signs in infected animals are fever, vesicular lesions on the tongue, snout, feet and teats, and lameness with high morbidity and low mortality (**Satya, 2009**). The circulating FMDV serotypes in Egypt are serotypes O, A, and SAT2 (**Aidaros, 2002; Abd El-Rahman et al., 2006; Abd El-Aty et al., 2013**). Control of FMD using vaccination depending on the factors influencing the vaccine potency and the induction of a protective antibody response is the

integrity of the structural protein and the intact virion using 146S (**Doel, 2003**).

Guinea pigs were chosen as experimental models to develop concepts and techniques to study the PD50 of FMD vaccines because of the similarities of clinical symptoms in these animals to swine and cattle to save cost (**Richard et al., 1979 and Eman, 2012**). 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 as having a good correlation with cattle protection (**Black et al., 1985**).

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

The quantity of 146S particles in inactivated FMD virus vaccine samples produced in FMD Vaccine Production Center in Thailand could be estimated by the sucrose gradient ultracentrifugation and optical density analysis by using the computer applying system (Shiari *et al.*, 1990). Using 146S assay only or some serological tests which together increase the reliance on estimating potency of specific vaccine batch. They can also be assembled with an interconnected method which is based on the 146S concentration of the final vaccine batch (Alkan *et al.*, 2008). Adjuvants stimulate the immune response and increase the immunity duration and the adjuvant nature can determine the particular type of immune response (Mair *et al.*, 2015)

The capturing of soluble immune mediators such as cytokines and chemokines could result in exemplary intercellular signaling and into more efficient leukocyte recruitment to the site of vaccine delivery. Alternatively, carbomer action could rely on nonimmune cells, whose role in promoting immunity would be revealed in subsequent time (Mair *et al.*, 2015). Carbomer was previously used in horse vaccines (Mumford *et al.*, 1994 and Minke *et al.*, 2007), pigeons and swine vaccines (Vereecken *et al.*, 2000). The adjuvant criteria of polyacrylic acids, designated by the term carbomers, may vary significantly with the number of carboxyl groups present in the final molecule. Polyacrylic acid polymers termed carbomers have been evaluated as adjuvants in animal vaccines with no side effect (Mair *et al.*, 2015; Mumford *et al.*, 1994; Gualandi *et al.*, 1988; Hoogland *et al.*, 2006; Liu *et al.*, 2005 and Tollersrud *et al.*, 2002).

The use of carbomer as an adjuvant can induce robust humoral immunity and T-cell responses to some subunit vaccines (Mumford *et al.*, 1994). Adding carbomer to animal vaccines results in systemic adjuvant activity, including proinflammatory T cell sensitization, fast leukocyte recruitment, proinflammatory cytokine secretion with antigen capture fast by the inflammatory monocytes (Gartlan *et al.*, 2016).

It was shown that Carbomer 934 is actually immunogenic and maybe a relevant alternative to oil in avian species for which safety is a major concern). Aluminum hydroxide was proved to be less immunogenic than carbomer and the last was totally safe by vaccination the young goslings with inducing a good serological response (Jacqueline *et al.*, 2011).

In a trial to improve the rabies vaccine's immunogenicity, water-soluble acrylic acid (carbomer) was used as an adjuvant revealing that it is potent and efficient (Naglaa *et al.*, 2020). It was suggested that to yield potentially high immune response made combination to carbomers with other adjuvant

formulations such as MF59 (Lai *et al.*, 2012 and Dey *et al.*, 2012). It could be concluded that the Montanide oils 206 with carbopol is the best vaccine formula which induced earlier, long-lasting immunity followed by Montanide oils 206 and finally Carbopol which give early, short-lasting immunity when vaccinated 160 calves (El-Sayed and Salma, 2021).

This work was designed to provide high protective, long-lasting immunity against FMD through the enhancement of the immunogenicity of the trivalent FMD vaccine using carbomer as adjuvant using G. pigs as an alternative cheapest animal model for quality control testing of the prepared FMD vaccines formulae.

MATERIALS AND METHODS

1. Guinea pigs

Two hundred thirty-five (235) healthy adult male Albino Guinea pigs approximately 400-500 gm body weight were used to prepare Guinea pig adapted FMD virus to be used for Guinea pig challenge and for determination of the potency of the prepared vaccines formulae. These animals were supplied by Lab Animal Farm (LAF) in Veterinary Serum and Vaccine Research Institute (VSVRI).

2. FMD virus strains

Local FMDV serotypes (A Iran O5, O Pan Asia2 and SAT2/EGY/2012) were inoculated in BHK21 cell culture to prepare virus fluid were supplied by the Department of FMD Vaccine Research (DFMDVR); (VSVRI).

3. Cell culture

Baby Hamster kidney cell line (BHK21) was propagated and maintained using Eagle's Minimum Essential Medium (MEM) supplied with 8-10% newborn calf serum as described by Xuan *et al.*, (2011) and used for SNT, virus propagation and titration for vaccine preparation.

4. Guinea pig adapted virus

Albino Guinea pigs were inoculated by FMD virus strain O pan Asia-2, A Iran O5 and SAT2 / EGY/2012 intra-dermoplanter in the metatarsal pads. After 24-48 hours, the developed lesions were collected aseptically. The lesion extract was re-inoculated in other Guinea pigs five times until the virus became entirely adapted to Guinea pigs, as recommended by Sonia (2007).

5. Virus infectivity and antigenicity

Titration of the used FMD virus serotypes was carried out and the infectivity titer was calculated in log₁₀ TCID₅₀ as described by Reed and Muench (1938). The complement fixation test (CFT) was carried out according to the Health Protection Agency (2009).

6. Virus inactivation

FMDV serotypes (A Iran O5, O Pan Asia2 and SAT2/EGY/2012) at their seventh passage on BHK21 cell line with an infectivity titer of 10^8 TCID₅₀/ml were subjected to inactivation process by a combination of 0.04% formaldehyde and 1 mM binary ethyleneimine (BEI) as the method described by **Barteling and Cassim (2004)** and **Ismail et al., (2013)**. To neutralize the effect of BEI 20% of sodium thiosulfate in a final concentration of 2% and also to neutralize the excess of formalin 20% of sodium bisulfite in a final concentration of 2% were added.

7. Estimation of antigenic content (Total protein and 146S) in FMD virus serotypes

Protein estimation of FMD prepared antigen was performed by Bradford's method. The concentration of 146S particles in the virus preparation was estimated by using sucrose density gradient ultracentrifugation by determining the absorbance at 254 nm using ISCO 520 C Density Gradient system as described by **Doel and Chong (1982)** and **Bartelling et al., (1990)**.

8. Used adjuvants

8.1. Carbomere adjuvant was provided by Lubrizol Co. as a fluffy white powder. It was dissolved in hot water to prepare 0.5% aqueous stock solutions, sterilized by autoclaving at 121°C for 20 min, then stored at 4°C until further use (United States Pharmacopeial Convention, 1990).

8.2. Montanide ISA206 was supplied from Seppic, Paris, France.

9. Preparation of inactivated vaccines

Mixing of the inactivated FMDV serotypes for vaccine preparation was carried out after estimation of virus titer and 146S antigen content from each FMD virus strain.

10. Formulation of the prepared experimental vaccine batches

Three formulae of trivalent inactivated FMD vaccine were prepared according to **Gamil (2010)** and **El-Sayed et al. (2012)** using the mentioned adjuvants as follow:

- Formula (1) prepared with 50% carbomer and 50% antigen
- Formula (2) prepared with 50% Montanide ISA 206 oil and 50% antigen
- Formula (3) prepared with 25% Montanide ISA 206 oil; 25% carbomer with 50 % antigen.

11. Evaluation of the prepared FMD vaccine formulae

11.1. Physical parameters: Physical parameters of the vaccines like viscosity, stability and emulsion type were studied as described by **Stone (1988)**.

11.2. Sterility testing: Sterility assays of all prepared and FMD vaccine formulae were performed by on thioglycolate broth, Sabouraud's agar; Nutrient agar; phenol dextrose media and mycoplasma medium according to (**OIE 2017**).

11.3. Safety testing: The safety of each prepared vaccine formulae was tested by subcutaneous inoculation of double dose in the G. Pigs. (**OIE 2017**)

11.4. Potency test of the prepared FMD trivalent vaccine formulae:

11.4.1. Determination of 50% Guinea pig's protection dose (GPPD₅₀)

It was carried out according to **Black et al., (1985)**, (**Assem, 2010**) and **Challa et al. (2011)** where 100 Guinea pigs were divided into four groups (25 G. pigs/ group) as follow:

- The 1st group was vaccinated with FMD inactivated vaccine adjuvanted with carbomer alone using fourfold dilution (undiluted, 1/4, 1/16, 1/64, 1/256) where 0.5ml of each dilution was inoculated S/C in each of 5 Guinea pigs.
- The 2nd group was vaccinated with FMD inactivated vaccine adjuvanted with ISA206 oil in the same manner in the first group.
- The 3rd group was vaccinated with FMD inactivated vaccine adjuvanted vaccine ISA206 oil and carbomer in the same manner in the 1st and 2nd group.
- The 4th group was kept without vaccination as a test control.

Three weeks later, all Guinea pigs in all groups were challenged with the specific Guinea pig adapted FMD virus (O, A, SAT2); by inoculation intra-dermoplantar. The challenged Guinea pigs were observed for 7 days to detect the infection generalization and the GPPD₅₀ was calculated using the following formula:

The difference of logarithm =

Morbidity next above 50% - 50%

----- X log of the dilution factor
Morbidity next above 50% - Morbidity next below 50%

The Guinea pigs were checked 4-5 days after applying the challenge test for the development of primary and secondary lesions. If the virus generalizes in the guinea pig's body, the vesicles on the un-inoculated feet and the tongue are observed for a positive reaction to the FMDV infection. The observations were recorded and the 50% Protective Dose (PD₅₀) was calculated in all groups and the control groups.

11.4.2. Assessment of the humoral immune response

This test includes 160 G. Pigs divided into 4 groups (50 G. Pigs/ group in the first three groups and 10 G. Pigs in the 4th group) as follow:

- Group (1) was vaccinated with the trivalent carbomer FMD vaccine
- Group (2) was vaccinated with the trivalent oil FMD
- Group (3) was vaccinated with the trivalent oil-carbomer FMD vaccine
- Group (4) was kept without vaccination as a control.

The used vaccine dose of each vaccine formula was 0.5 ml/ G. pig inoculated subcutaneously and serum samples were collected from the all-G. Pigs (vaccinated and non-vaccinated) to follow up the antibody titers against the serotypes of FMDV (A Iran O5, O Pan Asia2 and SAT 2/EGY/2012) through the application of serum neutralization test (SNT) using the microtiter technique as mentioned by **Ferreira (1976)** and indirect ELISA as described by **Voller, et al., (1976)** on week intervals up to 4 weeks then on two weeks interval up to 16 weeks then on four weeks intervals up to 36 weeks post-vaccination.

RESULTS

Table 1: FMD virus parameters

FMD virus types	Evaluated parameters			
	Titer (log10 TCID50/ml)	CFT Value	Total protein (mg/ml)	146S(µg/ml)
O Pan Asia-2	8.5	64	3.8	3
An Iran O5	7.8	32	3.7	2.5
SAT-2/Egy. 2012	7.5	32	3.5	2.5

Table 2: GPPD50 of trivalent FMD with different vaccine formulae challenged with serotype (O):

Vaccine dilution	VG			PG			NPG			CPG			CNPG			Protection %		
	GP1	GP2	GP3	GP1	GP2	GP3	GP1	GP2	GP3	GP1	GP2	GP3	GP1	GP2	GP3	GP1	GP2	GP3
Undiluted	5	5	5	5	5	5	0	0	0	16	18	21	0	0	0	100	100	100
1/4	5	5	5	5	5	5	0	0	0	11	13	16	0	0	0	100	100	100
1/16	5	5	5	4	4	5	1	1	0	6	8	11	1	1	0	85.7	88.8	100
1/64	5	5	5	2	3	4	3	2	1	2	4	6	4	3	1	33.3	57.1	85.7
1/256	5	5	5	0	1	2	5	4	3	0	1	2	9	7	4	0	12.5	33.3

VG: No. of vaccinated guinea pigs, PG: No. of protected guinea pigs, NPG: No. of non-protected guinea pigs, CPG: Cumulative no. of protected guinea pigs, CNPG: Cumulative no. of non-protected guinea pigs, (GPPD50 of GP1) = 40.6, (GPPD50 of GP2) = 78.6 and (GPPD50 of GP3) = 161.7.

Table 3: GPPD50 of trivalent FMD with different vaccine formulae challenged with serotype (A):

Vaccine dilution	VG			PG			NPG			CPG			CNPG			Protection %		
	GP1	GP2	GP3	GP1	GP2	GP3	GP1	GP2	GP3	GP1	GP2	GP3	GP1	GP2	GP3	GP1	GP2	GP3
Undiluted	5	5	5	5	5	5	0	0	0	15	19	21	0	0	0	100	100	100
1/4	5	5	5	5	5	5	0	0	0	10	14	16	0	0	0	100	100	100
1/16	5	5	5	4	4	5	1	1	0	5	9	11	1	1	0	83.3	90	100
1/64	5	5	5	1	4	4	4	1	1	1	5	6	5	2	1	16.6	71.4	85.7
1/256	5	5	5	0	1	2	5	4	3	0	1	2	10	6	4	0	14.3	33.3

VG: No. of vaccinated guinea pigs, PG: No. of protected guinea pigs, NPG: No. of non-protected guinea pigs, CPG: Cumulative no. of protected guinea pigs, CNPG: Cumulative no. of non-protected guinea pigs, (GPPD50 of GP1) = 31.6, (GPPD50 of GP2) = 105.8, and (GPPD50 of GP3) = 161.7.

Table 4: GPPD50 of trivalent FMD with different vaccine formulae challenged with serotype (SAT2)

Vaccine dilution	VG			PG			NPG			CPG			CNPG			Protection %		
	GP1	GP2	GP3	GP1	GP2	GP3	GP1	GP2	GP3	GP1	GP2	GP3	GP1	GP2	GP3	GP1	GP2	GP3
Undiluted	5	5	5	5	5	5	0	0	0	13	18	19	0	0	0	100	100	100
1/4	5	5	5	4	5	5	1	0	0	8	13	14	1	0	0	88.8	100	100
1/16	5	5	5	3	4	4	2	1	1	4	8	9	3	1	1	57.1	88.8	90
1/64	5	5	5	1	3	4	4	2	1	1	4	5	7	3	2	12.5	57.1	71.4
1/256	5	5	5	0	1	1	5	4	4	0	1	1	12	7	6	0	12.5	14.2

VG: No. of vaccinated guinea pigs, PG: No. of protected guinea pigs, NPG: No. of non-protected guinea pigs, CPG: Cumulative no. of protected guinea pigs, CNPG: Cumulative no. of non-protected guinea pigs, (GPPD50 of GP1) = 19.75, (GPPD50 of GP2) = 78.6, and (GPPD50 of GP3) = 105.8.

Table 5: Mean FMD serum neutralizing index in G. Pigs vaccinated with different formulae of trivalent FMD vaccine

WPV*	Mean FMD serum NI in G. Pigs								
	O			A			SAT		
	GP 1	GP 2	GP 3	GP 1	GP 2	GP 3	GP 1	GP 2	GP 3
0	0.6	0.3	0.3	0.15	0.6	0.15	0.45	0.3	0.45
1	1.2	1.1	1.4	1.2	1.2	1.55	1.2	1.1	1.6
2	1.5	1.3	1.6	1.65	1.7	1.9	1.35	1.5	1.8
3	1.8	1.8	1.95	1.8	1.95	2.1	1.6	1.8	1.9
4	2.1	2.2	2.4	2.1	2.2	2.4	1.85	1.9	2.3
6	2.4	2.5	2.7	2.4	2.6	2.7	2.1	2.3	2.8
8	2.7	2.8	2.9	2.7	2.85	3.0	2.4	2.6	3.05
10	2.85	2.9	3.25	2.9	3.0	3.45	2.7	2.8	3.35
12	2.7	3.1	3.25	2.85	3.1	3.25	2.55	3.05	3.25
14	2.5	2.75	2.95	2.7	2.90	3.0	2.4	2.75	3.05
16	2.4	2.7	2.85	2.55	2.70	2.9	2.1	2.55	2.95
18	2.1	2.58	2.7	2.4	2.5	2.75	1.95	2.35	2.8
20	1.8	2.38	2.55	2.25	2.4	2.6	1.8	2.15	2.65
22	1.65	2.24	2.4	2.1	2.35	2.45	1.65	2.05	2.4
24	1.5	2.05	2.3	1.8	2.15	2.4	1.5	1.95	2.25
26	1.15	1.85	2.1	1.2	2.05	2.3	1.15	1.95	2.05
28	0.9	1.77	1.95	1.05	1.85	2.1	1	1.8	1.95
30	0.9	1.70	1.95	0.9	1.7	1.95	0.45	1.8	1.8
32	0.45	1.67	1.8	0.3	1.5	1.95	0.3	1.65	1.7
34	0.3	1.54	1.65	0.3	1.1	1.8	0.3	1.5	1.65
36	0.3	1.12	1.5	0	0.9	1.5	0.3	1.15	1.5
38	0	0.9	1.15	0	0.45	1.05	0	0.9	1.1

*WPV= week post vaccination

PT by SNT= 1.2

Table 6: Mean FMD ELISA antibody titers in G. Pigs vaccinated with different formulae of trivalent FMD vaccine

WPV*	Mean FMD ELISA antibody titer (log10) in G. Pigs								
	O			A			SAT		
	GP 1	GP 2	GP 3	GP 1	GP 2	GP 3	GP 1	GP 2	GP 3
0	0.8	0.5	0.5	0.4	0.85	0.4	0.68	0.53	0.68
1	1.4	1.3	1.6	1.45	1.45	1.8	1.43	1.33	1.83
2	1.7	1.5	1.8	1.9	1.95	2.15	1.58	1.73	2.03
3	2	2	2.15	2.05	2.2	2.35	1.83	2.03	2.13
4	2.3	2.4	2.6	2.35	2.45	2.65	2.08	2.13	2.53
6	2.6	2.7	2.9	2.65	2.85	2.95	2.33	2.53	3.03
8	2.9	3	3.1	2.95	3.1	3.25	2.63	2.83	3.28
10	3.05	3.1	3.45	3.15	3.25	3.7	2.93	3.03	3.58
12	2.9	3.3	3.45	3.1	3.35	3.5	2.78	3.28	3.48
14	2.7	2.95	3.15	2.95	3.15	3.25	2.63	2.98	3.28
16	2.6	2.9	3.05	2.8	2.95	3.15	2.33	2.78	3.18
18	2.3	2.78	2.9	2.65	2.75	3	2.18	2.58	3.03
20	2	2.58	2.75	2.5	2.65	2.85	2.03	2.38	2.88
22	1.85	2.44	2.6	2.35	2.6	2.7	1.88	2.28	2.63
24	1.7	2.25	2.5	2.05	2.4	2.65	1.73	2.18	2.48
26	1.35	2.05	2.3	1.45	2.3	2.55	1.38	2.18	2.28
28	1.1	1.97	2.15	1.3	2.1	2.35	1.23	2.03	2.18
30	1.1	1.9	2.15	1.15	1.95	2.2	0.68	2.03	2.03
32	0.65	1.87	2	0.55	1.75	2.2	0.53	1.88	1.93
34	0.5	1.74	1.85	0.55	1.35	2.05	0.53	1.73	1.88
36	0.5	1.32	1.7	0.25	1.15	1.75	0.53	1.38	1.73
38	0.2	1.1	1.35	0.25	0.7	1.3	0.23	1.13	1.33

*WPV= week post-vaccination

Group (1) vaccinated with the trivalent carbomer FMD vaccine.

Group (2) vaccinated with the trivalent oil FMD

Group (3) vaccinated with the trivalent oil-carbomer FMD vaccine

DISCUSSION

All of the prepared inactivated polyvalent FMD vaccines showed homogenous appearance; free from aerobic and anaerobic bacteria; fungi and mycoplasma were tested on specific media and safe inducing no abnormalities in inoculated G. pigs. There was no noticeable toxicity or prolonged pyrexia was observed in the vaccinated G. Pigs, in agreement with the recommendation of (Stone,1988) and (OIE 2017).

FMD still represents a non-neglectable problem in livestock in many countries, resulting in huge economic losses, especially in developing countries. Regarding Egypt, several outbreaks attack the country due to the infection with either type O, A, SAT2 (Aidaros, 2002). Monovalent

FMD vaccine type O was used for several years where it was the only recorded type in Egypt (Parida Satya, 2009) after that and according to the introduction of type A, a bivalent vaccine was successfully prepared to contain both type O and A (Knowles *et al.*, 2007; Assem, 2010; Gamil, 2010; El-Sayed, 2011 and 2012). More recently, FMDV type SAT2 was recorded in Egypt (Ahmed *et al.*, 2012 and Shawky *et al.*, 2013) and this required the preparation of a trivalent vaccine containing the three present serotypes (A, O, SAT2). The present study was planned as preliminary work to establish such vaccine formulae by determining the best formulae that induce the highest GPPD₅₀.

The obtained results revealed that the virus titers of the FMDV serotypes (O Pan Asia-2, A Iran O5 and SAT/EGY/2012) were 8.5, 7.8 and 7.5

log₁₀ TCID₅₀ /ml with CFT value of 64, 32 and 32 total protein and 146S antigen contents 3.8, 3.7 and 3.5 mg/ml for the three serotypes respectively as tabulated in the table (1).

Determination of GPPD₅₀ induced by the prepared trivalent FMD vaccine formulae revealed that the values of 40.6; 78.6 and 161.7 against serotype O in G. group-1 which vaccinated with FMD carbomer adjuvanted vaccine; group-2 which vaccinated with FMD Montanide ISA 206 oil adjuvanted vaccine and group-3 which vaccinated with FMD Montanide ISA 206 oil carbomer adjuvanted vaccine respectively. It was found that the GPPD₅₀ against serotype A 31.6; 105.8 and 161.7 in group-1; 2 and 3 which vaccinated with FMD carbomer adjuvanted vaccine the; Montanide ISA 206 oil adjuvanted vaccine and Montanide ISA 206 oil carbomer adjuvanted vaccine respectively. The three G. pig groups showed GPPD₅₀ values of 19.75; 78.6 and 105.8 against serotype SAT2, respectively.

These results are tabulated in tables (2,3,4), coming in agreement with **Pay and Hingley (1987)**, who used the same FMD vaccine virus concluding that 2.2 µg of the antigen was required to obtain 1 PD50. However, protection was observed even with lower antigen dose but disagree with **Morgan et al., (1969)** who mentioned that many trials had been conducted to correlate the quantity of 146 S virus particles per vaccine dose and the protection and immunity achieved. The minimum effective dose of purified FMDV A-119 required for eliciting a virus-neutralizing immune response in guinea pigs was about 1.6 µg; this disagreement could be attributed to the different used subtypes of FMDV type A.

Testing of G. pig's serum samples of vaccinated animals with carbomer alone; to confirm the results of GPPD₅₀ using SNT; showed neutralizing antibody titer of 1.2, 1.2 and 1.2 log₁₀/ml against FMDV serotype O Pan Asia-2; A Iran O5 and SAT/EGY/2012 respectively at 1st WPV with peak antibody titers at 10th WPV (2.85, 2.9 and 2.7 respectively and extended till 24th to 25th WPV 1.5, 1.2, 1.5 in the three groups respectively. G. pig's serum samples from animals vaccinated with Montanide ISA206 alone showed antibody titer 1.3, 1.2 and 1.5 against FMDV serotype O Pan Asia-2, A Iran O5 and SAT/EGY/2012, respectively at 2nd or 3rd WPV with peak antibody titer at 12th WPV (3.1, 3.1 and 3.05) respectively and extended till the 32nd to 34th WPV (1.54, 1.5, 1.5) respectively.

On the other side G. pig vaccinated with Montanide ISA206 with carbomer showed antibody titer of 1.4, 1.55 and 1.6 against FMDV serotype O Pan

Asia-2, A Iran O5 and SAT/EGY/2012 respectively at the 1st WPV with peak antibody titer at 10th WPV (3.25, 3.45 and 3.35) and extended till the 36th WPV (1.5, 1.5, 1.5) respectively.

Testing of G. pig's serum samples of vaccinated animals with carbomer alone; to confirm the results of GPPD₅₀ using ELISA; showed antibody titer of 1.7, 1.9 and 1.58 log₁₀/ml against FMDV serotype O Pan Asia-2; A Iran O5 and SAT/EGY/2012 respectively at 2nd WPV with peak antibody titers at 10th WPV (3.05, 3.15 and 2.93 respectively and extended till 24th WPV 1.7, 2.05, 1.73 in the three groups respectively. G. pig's serum samples from animals vaccinated with Montanide ISA206 alone showed antibody titer 1.5, 1.95 and 1.73 against FMDV serotype O Pan Asia-2, A Iran O5 and SAT/EGY/2012 respectively at 2nd WPV with peak antibody titer at 12th WPV (3.3, 3.35 and 3.28) respectively and extended till the 32nd to 34th WPV (1.74, 1.75, 1.73) respectively. On the other side G. pig vaccinated with Montanide ISA206 with carbomer showed antibody titer of 1.6, 1.8 and 1.83 against FMDV serotype O Pan Asia-2, A Iran O5 and SAT/EGY/2012 respectively at the 1st WPV with peak antibody titer at 10th WPV (3.45, 3.7 and 3.58) and extended till the 36th WPV (1.7, 1.75, 1.73) respectively.

Such titers appear to be higher than the recommended protective antibody titer indicating that the prepared trivalent FMD vaccine able to induce acceptable immune level in vaccinated G. pigs where the obtained antibody titer against the three serotypes was found to be higher than the recommended protective antibody titer (PT=1.2) coming in a parallel manner with that of GPPD₅₀. These finding coming in agreement with those of **Motamedi et al., (2007)** who found that after the vaccines were inoculated subcutaneously on the back foot in two groups of guinea pigs, just two of ten animals showed antiserum titration above the protective titration (PT=1.2), and the other eight animals were below the PT.

CONCLUSION

Finally, it could be concluded that G. pigs can be used as an animal model for FMD vaccine evaluation instead of cattle to low the required cost and efforts. Also, it could be concluded that the FMD vaccine adjuvanted with carbomer alone give early immunity than the oil vaccine alone but the combination between carbomer and Montanide ISA206 oil induces early immunity with high antibody levels extends longer than that induced by other adjuvants and further studies are required in cattle to confirm the results.

Declaration of Competing interest

On behalf of all authors, I hereby declare that no conflict of interest may interfere with the publication of the manuscript.

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