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Comparative Immunological Studies on the Combined FMD and RVF Vaccine Using Different Oils

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Abstract

Background: Foot and mouth disease (FMD) and Rift valley fever (RVF) are consider very important diseases in Egypt and the main way for their control is the perfect vaccination, using a vaccine that could improve early and long lasting immunity through selection of the best adjuvants. Also, the formulation of combined vaccine is aimed to save cost; effort and minimize the stress on the animal which may lead to undesirable effect on the animal immune response.

Objective: Formulation of combined vaccine of RVF and FMD using different oil adjuvants select the best formula which induces long lasting immunity.

Method: Formulation of different vaccine formulae from RVF and trivalent FMD in single and combined forms using Montanide ISA206, 201 and POLYVAC™ 50 (Mukta oil industries). Vaccination of groups of sheep to follow up their cell mediated immunity using lymphocytic proliferation assay and interleukine-6 and the humeral immune response using SNT and ELISA. Application of potency test by challenge groups of sheep vaccinated with combined FMD and RVF with different adjuvants with FMDV serotypes O, A, SAT2 and evaluation of the ED_{50} on mice to detect the potency test against RVF.

Results: Sheep vaccinated with combined FMD and RVF adjuvanted with Montanide ISA201 exhibited high cellular and humeral immunity than that induced by vaccine adjuvanted either with Montanide ISA 206 or POLYVAC™ 50 showing high protection reached to 100% when challenged by the 3 serotypes of FMDV.

Conclusion: There is no difference in the immunity between vaccination of sheep with FMD and RVF alone or using combined FMD and RVF vaccine. The duration of immunity from Montanide oils 201 in combined RVF and FMD vaccine is longer and start earlier then followed by ISA 206 then POLYVAC™ 50.

Keywords: *Combined FMD and RVF Vaccine; Montanide ISA206; Montanide ISA201; POLYVAC™ 50*

Introduction

Foot and mouth disease (FMD) is a highly infectious disease of cloven footed animals including cattle, sheep, goats, pigs and also wild animals. Foot and mouth disease virus (FMDV) is the main etiologic agent of the disease which causes an acute disease characterized by fever, lameness and vesicular lesions on the feet, tongue, snout and teats, with high morbidity and low mortality [1]. The etiological agent, FMDV belongs to genus: Aphthovirus, family: Picornaviridae. The virus exists in the form of seven serologically and genetically distin-

guishable types, namely, O, A, C, Asia1, SAT1, SAT2, and SAT3, also a large number of subtypes have evolved within each serotype [2]. In Egypt, FMD serotypes SAT2, A and O were since 1950 [3-7]. Control of FMD in animals was considered to be the corner stone to eliminate the disease in endemic areas through effective vaccination of susceptible animals [1] beside controlling the in and out motion of the live susceptible animals.

Rift valley fever (RVF) is an acute viral disease that can cause severe disease in domestic animals as buffalo, camels, cattle, goats and sheep and human. Disease in these species is characterized by fever, severe illness, abortions, and a high morbidity and mortality rate. The etiologic cause is RVF virus (RVFV) belongs to genus: Phlebovirus, family: Bunyaviridae [8,9]. The most significant economic losses of RVF are due to death in young animals and abortion among infected livestock [10]. In Egypt, RVF outbreaks had occurred in 1993, 1999, and most recently in 2003. In most cases these outbreaks were believed to have begun as epizootics among sheep, goats, cattle, and camels, which serve as amplifying hosts of the virus. The outbreaks of RVF in Upper Egypt during 1977 were preceded by epizootics that occurred to the south of Egypt in Sudan, Kenya, and Uganda and were thought to result from the movement of herd animals into Egypt from the south [11,12].

The most common risk factor in Egypt between FMD and RVF is the movement of herd animals from another neighbor endemic area.

Vaccine oil adjuvant is very important factor affecting the immune response and stimulate specific component either humeral or cell mediated immunity [13]. Since the inactivated vaccine has a disadvantages of short duration of immunity, oil adjuvant vaccines have been shown to be more effective in conferring a longer duration of immunity than the aqueous adjuvant based FMD vaccines [14,15]. An oily vaccine was prepared by using Montanide oil as adjuvant to FMD, combined FMD with RVF and RVF vaccines giving high titer of antibodies and long duration of antibodies [16]. Furthermore, Montanide oil adjuvant for inactivated RVF vaccine induced early immune response in sheep rather than the alum gel [17]. As well as, it was sufficient to protect the animal all over the year with only one vaccine dose rather than the alum gel which needed 2nd booster dose to maintain the protection all over the year. Duration of immunity from Montanide oils (201, 206, 61 and 50) FMD vaccines is a long-lived immunity which ranged between 32 and 38 weeks post vaccination, but the Montanide ISA 201 FMD vaccine is superior to the others in the rapid cellular immune response of the vaccinated animals which showed its highest level within 2 weeks [18].

A combined vaccine is that one consists of 2 or more separate immunogens physically combined into a single product [19]. Several benefits of a combined vaccine must be fulfilled as aiming to prevent multiple diseases caused by different organisms [20] and also reducing the number of injections and therefore the degree of pain and side effect of repeated vaccination. Also, the result of this combination must be linked with the humeral immune response against the targeted organisms. The combined vaccine against FMD and RVF has been studied before and revealed an efficient humeral immune response against FMDV and RVFV higher than that induced by the single one [21].

Vaccination of pregnant ewes at 2 - 3 months before parturition with FMD/RVF combined inactivated oil vaccine provide them with high antibody titers that could be transferred to their lambs through the colostrum and enable the newly born lambs to withstand any FMD and RVF infections for at least 3 - 4 months of age and depending on the previous results. The newly born lambs should be vaccinated at this age [22].

The protective antibody titer of FMD using SNT is 1.5 $\log_{10}$ and 1.8 $\log_{10}$ by ELISA [23] but the protective antibody titer of RVF using SNT is 1.5 $\log_{10}$ [9].

This study comparatively evaluates the potency of combined FMD and RVF vaccine blend with international oil adjuvant and that of indigenously prepared oil adjuvant vaccine in addition to study the relation of antigenic payload in terms to serum antibodies in vacci-

nated animals.

Materials and Methods

Ethical approval

The experiment was carried out according to the protocol of Institutional Animal Ethics Committee and the authors had a permission of the animal owners at the private farms.

Animals

Sheep

Eighty native breed sheep were found to be serologically negative for the presence of antibodies against FMDV type A, O or SAT2 and RVFV as proved by SNT and ELISA. These animals were used for evaluation of the potency of the prepared vaccine formulae and classified as demonstrated in figure 1.

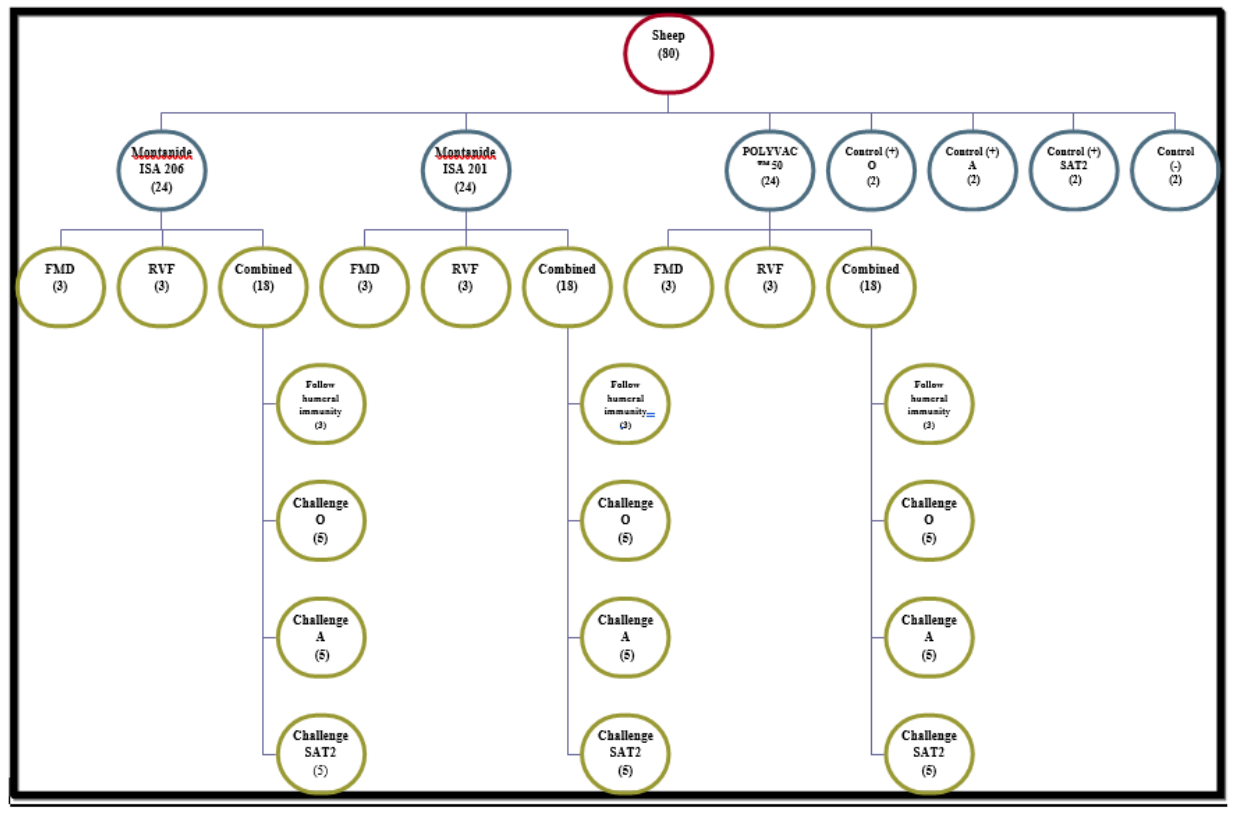

Figure 1: *Scheme of sheep.*

Suckling baby mice

One hundred Suckling Albino Swiss baby mice, 2 - 4 days old, (Charles River Strain, USA) were used for the safety testing of the prepared vaccine formulae.

Weaned mice

Three Hundred and fifty Swiss Albino weaned mice; 21 - 28 days old were used for titration of the virus and testing the potency of the prepared vaccine for RVF by evaluating the ED_{50}. All mice were supplied by the Laboratory Animal Breeding Unit, Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo, Egypt.

Cell culture

Baby Hamster kidney cell line (BHK21) Clone 13 was obtained from VSVRI using Eagl's medium with 8 - 10% bovine serum as described by Xuan., *et al*. [24] and used for application of SNT, virus titration and vaccine preparation.

Viruses

FMD virus strains

Local FMD virus type O pan Asia, A Iran O5 and SAT2/EGY/2012 propagated in BHK21 cell line monolayer cultures were used for preparation of virus infected fluids and supplied by Department FMD Research, VSVRI. The titer of the three types was 10^9 $\log_{10}$ $TCID_{50}$/ml as calculated by Reed and Muench [25] with Complement Fixation value 1/64 detected according to Health Protection Agency [26].

RVF virus (ZH 501)

RVF virus was supplied by RVF Research Department, VSVRI with a titter of $10^{8.5}$ $TCID_{50}$/ml following the techniques recommended by El-Nimr [27].

Oil adjuvants

- Montanide ISA 201 oil was obtained from Seppic Company, France.
- Montanide ISA 206 oil was obtained from Seppic Company, France.
- PolyVAC™50 oil was obtained from Mukta industries company, E-mail: mail@muktaindustries.com, India.

Virus purification

Aseptically, the harvested culture media from FMD and RVF virus infected BHK21 cell cultures were centrifuged in a cooling centrifuge at 3000 rpm for 20 minutes to remove cell debris.

Virus concentration

The tissue culture viral fluids of the three serotypes of FMDV (O pan Asia, A Iran O5 and SAT2/EGY/2012) and RVFV were centrifuged at 7000 revolution/min for 30 minutes and then concentrated by Poly Ethylene Glycol (PEG)-6000 to reach to 1/10 of its original volume [21].

Virus inactivation

1% M Binary ethylenamine (BEI) in 0.2N NaOH was added to the virus suspension to give final concentration of 0.001 M of BEI. The virus and BEI mixtures were mixed well and the pH was adjusted to 8.0 by sodium bicarbonate. The virus was placed on a magnetic stirrer in the incubator at 37°C for 18h for FMD and RVF viruses inactivation. After 18 hrs. of incubation sodium thiosulphate was added in a final concentration of 2% to neutralize the BEI action according to Ismail., *et al* [28].

Vaccine formulations

Nine vaccine formulae were prepared as follow:

- **Formula-1:** Consisted of trivalent FMD vaccine adjuvant with Montanide ISA 206.
- **Formula-2:** Consisted of RVF vaccine adjuvant with Montanide ISA 206.

- **Formula-3:** Consisted of combined trivalent FMD and RVF vaccine adjuvant with Montanide oil ISA 206.
- **Formula-4:** Consisted of trivalent FMD vaccine adjuvant with Montanide oil ISA 201.
- **Formula-5:** Consisted of RVF vaccine adjuvant with Montanide oil ISA 201.
- **Formula-6:** Consisted of combined trivalent FMD and RVF vaccine adjuvant with Montanide oil ISA 201
- **Formula-7:** Consisted of trivalent FMD vaccine adjuvant with POLYVAC™ 50 oil.
- **Formula-8:** Consisted of RVF vaccine adjuvant with POLYVAC™ 50 oil.
- **Formula-9:** Consisted of combined trivalent FMD and RVF vaccine adjuvant with POLYVAC™ 50 oil.

All the vaccine formulae were prepared by mixing equal volumes of inactivated FMD virus strains and inactivated RVF virus as aqueous phase. That aqueous antigen mixture was added in equal weight (w/w) to oil phase and mixed thoroughly [21].

Quality control testing of the prepared vaccine formulae

Sterility test

The prepared vaccines were tested for their freedom of aerobic and anaerobic bacteria, fungal and mycoplasma contaminants where vaccine samples were cultured on thioglycolate broth, Sabouraud's, Nutrient agar; phenol dextrose media and mycoplasma medium according to OIE [9] and Code of Federal Regulation of USA [29].

Safety test

Three to five days old Swiss Albino suckling mice were used for the safety test of inactivated FMD and RVF viruses according to Randall., *et al* [30].

Potency test

Evaluation of the cellular immune response

*Lymphocyte blastogenesis using XTT assay

Blood samples were collected from all sheep groups on the 1^{st}; 3^{rd}; 7^{th}; 14^{th}; 21^{st} and 28^{th} days post vaccination to be subjected to lymphocyte blastogenesis using XTT assay according to Slater., *et al.* [31] and EL-Naggar [32] through separation of lymphocytes as described by Lucy [33] and Lee [34] and determination of viable cell number according to Mayer., *et al* [35].

*Estimation of interleukin

Estimation of the level of interleukin in the sera of vaccinated and control sheep including IL-6 levels was carried out using sheep IL-6 ELISA Kit Catalog No. EKE51028 supplied by Biomatik Company, Wilmington, Delaware, USA.

Evaluation of sheep humeral immune response to the prepared vaccines in sheep

Serum samples collected from sheep before and after vaccination (4 times on week intervals then every month up to 40 weeks) were subjected to estimation of antibody titers against the three serotypes of FMDV (O pan Asia, A Iran O5 and SAT2/ EGY/2012) and RVFV by SNT using the micro titer technique described by Ferreira [36] and indirect ELISA according to Voller., *et al* [37]. The used dose of all vaccine formula was 1 ml inoculated subcutaneously.

Determination of challenge protection percentage in sheep against FMDV serotypes

Fifteen sheep from each vaccine formula of combined FMD and RVF and control positive sheep were inoculated with the challenged FMDV either serotype A or O or SAT2 with titer 10^4 ID_{50} on the base of the tongue [9]. All challenged animals were kept under daily observation and clinically examined for 1 week post infection, where lesions on the gum and oral mucosa as well as inter digital space were recorded. Control negative sheep were included in this experiment.

Determination of mice ED_{50} for RVF

Fivefold dilutions of each RVF vaccine were prepared in suitable media starting from 1:1 to 1:625. Five groups of weaned mice (21 - 28 days old) were used for each dilution and each mouse was inoculated with two doses of 0.2 ml of the vaccine I/P, one week a part. Seven days after the second inoculation, all animals were challenged via I/P route with 0.1 ml of RVF virus containing 10^3 $MIPLD_{50}$/ml in addition to other two groups of mice, one inoculated with challenge virus as positive control and one kept as non-vaccinated non challenged negative control. All groups of mice were kept under observation for 21 days and deaths were recorded daily. The ED50/ml was calculated according to the method of Reed and Muench [25]. Deaths occurring during the 1st day were considered as nonspecific.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) in the SPSS-12 statistical software package for P.C.S. Multiple comparisons of means were made using Duncan's multiple range tests at $P < 0.05\%$.

Results and Discussion

Usually, the attention of vaccine researchers is directed toward the vaccine evolution aiming to provide a maximum immunogenicity to livestock, especially against the most dangerous diseases like FMD and RVF. Through the present work, nine formulae of inactivated oil trivalent FMD; RVF and combined FMD and RVF vaccines were successfully prepared where of them were found to be free from foreign contaminants; safe for vaccinated sheep and suckling mice and potent inducing adequate protection for vaccinated animals coming in agreement with the earlier report [29].

Regarding the cellular immune response of sheep to FMD and/or RVF vaccine, evaluation of the cellular immunity included estimation of the lymphocyte blastogenesis in addition to IL-6 levels was done. The efficient induction of early protection against contact infections by FMDV and/or RVF relies on the rapid assimilation of appropriate innate immune defense, probably leading to the enhanced induction of specific immune responses [38]. Table 1 showed that the cellular immune response of sheep to the inactivated FMD ISA 206 oil vaccine (formula-1) revealed increasing mean delta optical density of lymphocyte blastogenesis assay at day 1, 3, 7, 14, 21 and 28 DPV from 0.32 at the day 1 to reach its maximum value (0.95) at the 14th DPV then declined after at the 21st DPV (0.81), but in sheep vaccinated with inactivated RVF 206 oil vaccine (formula-2), showed an increase in the mean value from (0.35) at the day 1 to reach its maximum value (0.94) at the 14th DPV then declined after at the 21st DPV (0.79) while the inactivated combined FMD and RVF inactivated vaccine with Montanide 206 oil (formula-3) revealed increasing from 0.42 at the day 1 to reach its maximum value (0.97) at the 14th DPV then declined after at the 21st DPV (0.90).

Used adjuvant	Used Vaccine formula	Delta optical density of lymphocyte blastogenesis					
		1st DPV*	3rd DPV	7th DPV	14th DPV	21st DPV	28th DPV
Montanide Oil ISA 206	Vacc. (1)	0.32	0.45	0.67	0.95	0.81	0.76
	Vacc. (2)	0.35	0.45	0.64	0.94	0.79	0.72
	Vacc. (3)	0.42	0.54	0.77	0.97	0.90	0.87
Montanide Oil ISA 201	Vacc. (4)	0.41	0.82	1.19	0.92	0.91	0.79
	Vacc. (5)	0.40	0.79	1.15	0.95	0.91	0.81
	Vacc. (6)	0.43	0.88	1.21	1.05	0.99	0.89
POLYVAC™ 50	Vacc. (7)	0.11	0.21	0.48	0.54	0.61	0.50
	Vacc. (8)	0.12	0.19	0.46	0.54	0.59	0.48
	Vacc. (9)	0.15	0.25	0.51	0.58	0.67	0.52
Control		0.10	0.12	0.13	0.12	0.13	0.10

Table 1: *Mean delta optical density of lymphocyte blastogenesis assay in sheep vaccinated with the prepared vaccine formulae.*

The cellular immune response of sheep to the inactivated FMD ISA 201 oil vaccine (formula-4) showed mean delta optical density of lymphocyte blastogenesis assay increasing from 0.41 at the day 1 to reach its maximum value (1.19) at the 7th DPV then declined after at the 14th DPV (0.92), but in sheep vaccinated with inactivated RVF 201 oil vaccine (formula-5), showed an increase in its mean value from (0.40) at the day 1 to reach its maximum value (1.15) at the 7th DPV then declined after at the 14th DPV (0.95). The inactivated combined FMD and RVF Montanide 201 oil vaccine (formula-6) revealed a mean delta optical density of lymphocyte blastogenesis assay increased from (0.43) at the day 1 to reach its maximum value (1.21) at the 7th DPV then declined after at the 14th DPV (1.05).

Using the inactivated FMD POLYVAC™ 50 vaccine (formula-7) the mean delta optical density of lymphocyte blastogenesis assay showed an increase in the mean value from (0.11) at the day 1 to reach its maximum value (0.61) at the 21st DPV in vaccinated sheep and then declined after at the 28th DPV (0.50), but in sheep vaccinated with inactivated RVF POLYVAC™ 50 vaccine (formula-8), mean delta optical density of lymphocyte blastogenesis assay showed an increasing value from (0.12) at the day 1 to reach its maximum value (0.59) at the 21st DPV then declined after at the 28th DPV (0.48). The inactivated combined FMD and RVF inactivated POLYVAC™ 50 oil vaccine (formula-9), mean delta optical density of lymphocyte blastogenesis assay showed an increase in the mean value from (0.15) at the day 1 to reach its maximum value (0.67) at the 21st DPV in vaccinated sheep and then declined after at the 28th DPV (0.52).

Table 2 showed that the cellular immune response of sheep to the inactivated FMD ISA 206 oil vaccine revealed increasing mean delta optical density of Interleukin-6 at day 1, 3, 7, 14, 21 and 28 DPV from (0.89) at the day 1 to reach its maximum value (3.88) at the 14th DPV then declined after at the 21st DPV (3.62), but in sheep vaccinated with inactivated RVF 206 oil vaccine, showed an increase in the mean value from (0.90) at the day 1 to reach its maximum value (3.86) at the 14th DPV then declined after at the 21st DPV (3.60) while the inactivated combined FMD and RVF inactivated vaccine with Montanide 206 oil revealed increased from (0.99) at the day 1 to reach its maximum value (3.94) at the 14th DPV then declined after at the 21st DPV (3.71).

Used adjuvant	Used Vaccine formula	IL-6 (ng/ml) at DPV*					
		1st DPV*	3rd DPV	7th DPV	14th DPV	21st DPV	28th DPV
Montanide Oil ISA 206	Vacc. (1)	0.89	1.44	2.11	3.88	3.62	3.17
	Vacc. (2)	0.90	1.48	2.11	3.86	3.60	3.19
	Vacc. (3)	0.99	1.54	2.25	3.94	3.71	3.25
Montanide Oil ISA 201	Vacc. (4)	1.43	2.51	4.78	3.97	3.81	3.43
	Vacc. (5)	1.40	2.50	4.74	3.93	3.79	3.40
	Vacc. (6)	1.48	2.54	4.72	4.1	3.87	3.49
POLYVAC™ 50	Vacc. (7)	0.40	1.01	1.77	2.54	2.95	2.55
	Vacc. (8)	0.42	1.05	1.73	2.50	2.89	2.50
	Vacc. (9)	0.72	1.21	1.84	2.87	3.47	2.94
Control		0.40	0.35	0.32	0.3	0.39	0.4

Table 2: *Interleukin-6 immune response expressed as mean delta optical density of sheep vaccinated with the prepared vaccine formulae.*

**DPV: day post vaccination.*

Vacc. (1) Trivalent FMD adjuvanted with Montanide ISA206

Vacc. (2) RVF adjuvanted with Montanide ISA206.

Vacc. (3) Combined FMD and RVF adjuvanted with Montanide ISA206

Vacc. (4) Trivalent FMD adjuvanted with Montanide ISA201

Vacc. (5) RVF adjuvanted with Montanide ISA201

Vacc. (6) Combined FMD and RVF adjuvanted with Montanide ISA201.

Vacc. (7) Trivalent FMD adjuvanted with POLYVAC™ 50

Vacc. (8) RVF adjuvanted with POLYVAC™ 50.

Vacc. (9) Combined FMD and RVF adjuvanted with POLYVAC™ 50.

The interleukin-6 immune response expressed as mean delta optical density increasing from (1.43) at the day 1 to reach its maximum value (4.78) at the 7 DPV then declined after at the 14th DPV (3.97), but in sheep vaccinated with inactivated RVF 201 oil vaccine, showed an increase in its mean value from (1.40) at the day 1 to reach its maximum value (4.74) at the 7th DPV then declined after at the 14 DPV (3.93). The inactivated combined FMD and RVF Montanide 201 oil vaccine revealed a mean delta optical density of interleukin-6 assay increased from (1.48) at the day 1 to reach its maximum value (4.72) at the 7th DPV then declined after at the 14th DPV (4.1).

Using the inactivated FMD POLYVAC™ 50 vaccine the mean delta optical density of interleukin-6 assay showed an increase in the mean value from (0.40) at the day 1 to reach its maximum value (2.95) at the 21st DPV in vaccinated sheep and then declined after at the 28th DPV (2.55), but in sheep vaccinated with inactivated RVF POLYVAC™ 50 vaccine, showed an increasing value from (0.42) at the day 1 to reach its maximum value (2.89) at the 21st DPV then declined after at the 28th DPV (2.50). The inactivated combined FMD and RVF inactivated POLYVAC™ 50 vaccine, showed an increase in the mean value from (0.72) at the day 1 to reach its maximum value (3.47) at the 21st DPV in vaccinated sheep and then declined after at the 28th DPV (2.94).

From the above results and the statically analysis of cellular immunity it revealed that the Montanide oils 201 in combined RVF and FMD vaccine induced earlier immunity followed by ISA 206 then POLYVAC™ 50

These results agree with Mossad., *et al.* [39] who mentioned that the Delta optical density of lymphocyte blastogenesis assay and interleukin6 and 12 at day 0, 3, 7, 14, 21 and 28 days post vaccination (DPV) showed that a significant difference between vaccinated and control groups started at 3rd DPV and increased gradually till 21st DPV using trivalent FMD Montanide inactivated vaccine.

Evaluation of the humeral FMD type-O antibody titer in vaccinated sheep with different prepared oil adjuvant vaccine formulae using SNT and ELISA data (Table 3 and 4) showed differences in the onset, intensity and duration of the FMD serotype O antibodies. Concerning the onset of protective antibody titer, it is clear that inactivated FMD ISA 206 oil vaccine induced titers of (1.68 by SNT and 1.8$\log_{10}$ by ELISA) in the 2nd week post vaccination and inactivated FMD ISA 201 oil vaccine induced titers of (1.52 by SNT and 1.84 $\log_{10}$ by ELISA) in the 1st week post vaccination while inactivated FMD POLYVAC™ 50 vaccine showed later immune response in the 3rd WPV (1.61 by SNT, 1.82 $\log_{10}$ by ELISA). On the other side the inactivated combined FMD/RVF ISA 206 oil vaccine induced protective type O antibody titer (1.69 by SNT and 1.8 $\log_{10}$ by ELISA) in the 2nd week post vaccination and inactivated combined FMD/RVF ISA 201 oil vaccine induced antibody titers (1.55 by SNT and 1.92 $\log_{10}$ by ELISA) in the 1st week post vaccination while inactivated combined FMD/RVF POLYVAC™ 50 vaccine showed later immune response in the 3rd WPV (1.65 by SNT and 1.92 $\log_{10}$ by ELISA).

Used adjuvant	Used Vaccine formula	FMD type-O serum neutralizing antibody titers /WPV*																
		0WPV*	1W	2W	3W	4W	6W	8W	10W	12W	14W	16W	20W	24W	28W	32W	36W	40W
Montanide Oil ISA 206	Vacc. (1)	0.45	1.45	1.68	1.71	2.24	2.51	2.82	3.11	2.84	2.72	2.61	2.32	2.12	1.84	1.72	1.31	1.25
	Vacc. (3)	0.3	1.35	1.69	1.74	2.13	2.55	2.7	3.3	2.97	2.85	2.67	2.46	2.25	1.95	1.83	1.45	1.35
Montanide Oil ISA 201	Vacc. (4)	0.32	1.52	1.61	1.79	2.24	2.68	3.35	3.32	3.05	2.87	2.79	2.57	2.40	2.09	1.7	1.59	1.24
	Vacc. (6)	0.3	1.55	1.73	1.86	2.34	2.72	3.31	3.28	3.1	2.93	2.83	2.62	2.46	2.19	1.81	1.65	1.37
POLYVAC™ 50	Vacc. (7)	0.29	1.23	1.40	1.61	2.05	2.38	2.48	2.42	2.81	2.68	2.38	2.04	1.74	1.61	1.57	1.5	0.71
	Vacc. (9)	0.27	1.13	1.45	1.65	1.95	2.42	2.55	2.58	2.85	2.76	2.46	2.12	1.84	1.75	1.62	1.55	0.75
Control		0.3	0.3	0.4	0.3	0.4	0.4	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3

Table 3: *Mean FMD type-O serum neutralizing antibody titers expressed in $\log_{10}$ in different vaccinated sheep groups.*

Used adjuvant	Used Vaccine formula	FMD type-O ELISA antibody titer /WPV*																
		0WPV*	1W	2W	3W	4W	6W	8W	10W	12W	14W	16W	20W	24W	28W	32W	36W	40W
Montanide Oil ISA 206	Vacc. (1)	0.45	1.41	1.8	1.92	2.14	2.68	2.84	3.16	3.14	3.06	2.85	2.61	2.46	2.04	1.96	1.72	1.42
	Vacc. (3)	0.6	1.52	1.8	2	2.28	2.87	2.96	3.33	3.23	3.11	2.93	2.72	2.51	2.17	2	1.79	1.61
Montanide Oil ISA 201	Vacc. (4)	0.9	1.84	1.89	2.15	2.46	2.87	3.46	3.36	3.31	3.17	3.01	2.87	2.64	2.36	2.07	1.92	1.65
	Vacc. (6)	0.6	1.92	1.92	2.21	2.57	2.98	3.57	3.48	3.35	3.2	3.08	2.9	2.72	2.47	2.18	1.98	1.68
POLYVAC™ 50	Vacc. (7)	0.43	1.25	1.54	1.82	2.12	2.51	2.71	2.8	2.93	2.84	2.55	2.3	2.01	1.87	1.8	1.19	0.95
	Vacc. (9)	0.42	1.35	1.64	1.92	2.21	2.63	2.81	2.83	3.01	2.99	2.69	2.45	2.12	1.93	1.84	1.28	1.01
Control		0.45	0.45	0.7	0.6	0.7	0.7	0.6	0.6	0.6	0.6	0.45	0.45	0.45	0.45	0.3	0.3	0.3

Table 4: *Mean FMD type-O ELISA titer in different vaccinated sheep groups.*

It is clear that peak of the protective antibody titers induced by the inactivated FMD ISA 206 oil vaccine (3.11 by SNT and 3.14 $\log_{10}$ by ELISA) appear in the 10th week post vaccination and by the inactivated FMD ISA 201 oil vaccine (3.35 as SNT and 3.46 $\log_{10}$ as ELISA) in the 8th week post vaccination (WPV) while the inactivated FMD POLYVAC™ 50 vaccine induced the peak of antibody titers in the 12th WPV (2.81 by SNT, 2.93 $\log_{10}$ by ELISA). On the other side the inactivated combined FMD/RVF ISA 206 oil vaccine induced peak protective antibody titers (3.3 by SNT and 3.33 $\log_{10}$ by ELISA) in the 10th week post vaccination and (3.31 as SNT and 3.57 $\log_{10}$ as ELISA) appeared in the 8th week post vaccination (WPV) by the inactivated combined FMD/RVF ISA 201 oil vaccine while the inactivated combined FMD/RVF POLYVAC™ 50 vaccine showed antibody titers (2.85 as SNT, 3.01$\log_{10}$ by ELISA) in the 12th WPV.

Regarding the duration of the protective type-O antibody titers, it is clear that inactivated FMD ISA 206 oil vaccine showed protective titers of (1.72 by SNT and 1.96 $\log_{10}$ by ELISA) up to the 32th week post vaccination and those induced by the inactivated FMD ISA 201 oil vaccine (1.59 as SNT and 1.92 $\log_{10}$ as ELISA) up to the 36th week post vaccination (WPV) while inactivated FMD POLYVAC™ 50 vaccine showed later protective antibody titers in the 36th WPV (1.5 by SNT and 1.8 $\log_{10}$ by ELISA) in the 32th WPV. It was noticed that the protective type O antibody titers induced by inactivated combined FMD/RVF ISA 206 oil vaccine (1.83 by SNT and 2 $\log_{10}$ by ELISA) up to the 32th week post vaccination and 36th week by the inactivated combined FMD/RVF ISA 201 oil vaccine (1.65 as SNT and 1.98 $\log_{10}$ as ELISA) while inactivated combined FMD/RVF POLYVAC™ 50 vaccine showed later protection titers in the 36th WPV (1.55 by SNT) and (1.84 $\log_{10}$ as ELISA) the 32th WPV.

These results came parallel to those described by Dong., *et al.* [40] who mentioned that the ELISA antibodies against FMDV type O were compared as induced by Montanide oils 201and 206 showing that the antibody titer induced by oil 201-vaccine were higher than those induced by the oil 206-vaccine on 3dpv, 7dpv, 14dpv, 21dpv and 28dpv. This means that the immune stimulating effect of 201oil is better than that of 206-vaccine. Also the obtained antibody titer of FMD is considered high protective as determined by SNT and ELISA as recommended by OIE [23] as 1.5 $\log_{10}$ by SNT and 1.8 $\log_{10}$ by ELISA.

FMDV serotype (A) antibody titers induced in vaccinated sheep the different prepared vaccine formulae are determined by SNT and ELISA (Table 5 and 6) showing differences in the onset, intensity and duration. These tables demonstrate that inactivated FMD ISA 206 oil vaccine induced protective titer (1.63 by SNT) in the 2nd week post vaccination and (1.92 $\log_{10}$ by ELISA) in the 3rd week post vaccination

and (1.59 by SNT) appeared in the 1st week post vaccination and (1.81 log_{10} by ELISA) in the 2nd WPV by the inactivated FMD ISA 201 oil while inactivated FMD POLYVAC™ 50 vaccine showed later protection in the 3rd WPV (1.59 by SNT and 1.82 log_{10} by ELISA). On the other side, protective titers (1.59 by SNT) in the 2nd WPV and (2.13 log_{10} by ELISA) in the 3rd WPV; (1.52 by SNT) appear in the 1st WPV and (1.89 log_{10} by ELISA) in the 2nd WPV and (1.52 as SNT, 1.97 log_{10} as ELISA) in the 3rd WPV were obtained by inactivated combined FMD/RVF ISA 206 oil vaccine; FMD/RVF ISA 201 oil and FMD/RVF POLYVAC™ 50 respectively.

Used adjuvant	Used Vaccine formula	FMD type-A serum neutralizing antibody titers /WPV*																
		0WPV*	1W	2W	3W	4W	6W	8W	10W	12W	14W	16W	20W	24W	28W	32W	36W	40W
Montanide Oil ISA 206	Vacc. (1)	0.15	1.07	1.63	1.79	1.87	2.37	2.54	3.05	2.84	2.94	2.68	2.29	2.04	1.91	1.81	1.49	1.34
	Vacc. (3)	0.27	1.02	1.59	1.77	1.92	2.2	2.65	3	2.91	2.89	2.62	2.38	2.12	1.95	1.7	1.43	1.4
Montanide Oil ISA 201	Vacc. (4)	0.21	1.59	1.67	1.74	2.24	2.54	3.19	3.06	3	2.84	2.63	2.34	2.12	2	1.76	1.62	1.39
	Vacc. (6)	0.3	1.52	1.71	1.87	2.3	2.68	3.24	3.18	3.1	2.9	2.71	2.42	2.21	2.09	1.83	1.75	1.47
POLYVAC™ 50	Vacc. (7)	0.20	1.05	1.29	1.59	1.79	2.05	2.34	2.49	2.59	2.67	2.38	2.17	1.82	1.67	1.59	1.51	0.82
	Vacc. (9)	0.27	1.02	1.33	1.52	1.85	2.14	2.43	2.54	2.65	2.76	2.45	2.21	1.91	1.75	1.65	1.55	0.62
Control		0.3	0.3	0.4	0.3	0.4	0.4	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3

Table 5: *Mean FMD type-A serum neutralizing antibody titers expressed in log_{10} in different vaccinated sheep groups.*

Used adjuvant	Used Vaccine formula	FMD type-A ELISA antibody titer /WPV*																
		0WPV*	1W	2W	3W	4W	6W	8W	10W	12W	14W	16W	20W	24W	28W	32W	36W	40W
Montanide Oil ISA 206	Vacc. (1)	0.45	1.21	1.56	1.92	2.24	2.48	2.81	3.17	3.11	3.09	2.73	2.51	2.38	2.24	1.96	1.62	1.44
	Vacc. (3)	0.55	1.3	1.75	2.13	2.35	2.57	2.93	3.25	3.20	3.15	2.96	2.74	2.52	2.32	2.15	1.62	1.52
Montanide Oil ISA 201	Vacc. (4)	0.7	1.54	1.81	2.23	2.40	2.77	3.57	3.30	3.24	3.07	2.97	2.57	2.46	2.36	2.17	1.90	1.65
	Vacc. (6)	0.6	1.69	1.89	2.47	2.65	2.97	3.68	3.42	3.41	3.24	3.14	2.82	2.63	2.47	2.32	1.99	1.69
POLYVAC™ 50	Vacc. (7)	0.43	1.20	1.54	1.82	2.12	2.41	2.61	2.7	2.92	3.07	2.45	2.27	2.17	2.01	1.98	1.87	0.95
	Vacc. (9)	0.39	1.34	1.72	1.97	2.25	2.21	2.75	2.85	2.99	3.15	2.79	2.59	2.42	2.24	2.02	1.84	0.86
Control		0.45	0.45	0.7	0.6	0.7	0.7	0.6	0.6	0.6	0.6	0.45	0.45	0.45	0.45	0.3	0.3	0.3

Table 6: *Mean FMD type-A ELISA antibody titer in different vaccinated sheep groups.*

The recorded peak of FMD type-A antibody titers were (3.05 by SNT and 3.17 log_{10} by ELISA in the 10th WPV; (3.19 by SNT and 3.57 log_{10} by ELISA) in the 8th WPV and (2.67 by SNT and 3.07 log_{10} by ELISA) in the 14th WPV induced by the inactivated FMD ISA 206 oil; FMD ISA 201 oil and FMD POLYVAC™ 50 vaccine respectively. FMD type-A antibody titers (3 as SNT and 3.25 log_{10} as ELISA) appear in the 10th WPV; (3.24 as SNT and 3.68 as ELISA) appeared in the 8th WPV and (2.76 by SNT and 3.15 log_{10} by ELISA) in the 14th WPV were obtained by the inactivated combined FMD/RVF ISA 206 oil; FMD/RVF ISA 201 oil and FMD/RVF POLYVAC™ 50 respectively.

Among the type A duration of immunity, it is clear that inactivated FMD ISA 206 oil; FMD ISA 201 oil and POLYVAC™ 50 vaccines provided protective antibody titers (1.81 by SNT and 1.96 log_{10} by ELISA) up to the 32th WPV; (1.62 by SNT and 1.90 log_{10} by ELISA) up to the 36th WPV and (1.51 as SNT and 1.87 log_{10} as ELISA) up to the 36th WPV respectively. On the other side combined FMD/RVF ISA 206 oil;

FMD/RVF ISA 201 oil and FMD/RVF POLYVAC™ 50 showed protective titer (1.7 by SNT and 2.15 log_{10} by ELISA) up to the 32th WPV; (1.75 by SNT and 1.99log_{10} by ELISA) up to the 36th WPV and (1.55 by SNT and 1.84 log_{10} by ELISA) up to 36th WPV respectively.

Demonstration of FMD type SAT2 antibody titers induced in vaccinated sheep with the prepared different oil vaccine formulae by SNT and ELISA (Table 7 and 8) showed differences in the onset, intensity and duration. Concerning the onset of protective type SAT2 antibody titers as (1.67 as SNT and 1.92 log_{10} as ELISA) appeared in the 2nd WPV; (1.5 as SNT and 1.8 log_{10} as ELISA) in the 1st WPV and (1.50 as SNT, 1.82 log_{10} as ELISA) in the 3rd WPV were obtained by inactivated FMD ISA 206 oil; FMD ISA 201 oil and FMD POLYVAC™ 50 vaccines respectively. Combined FMD/RVF ISA 206 oil; FMD/RVF ISA 201 oil and FMD/RVF POLYVAC™ 50 showed protective titer (1.71 by SNT and 1.96 log_{10} by ELISA) in the 2nd WPV; (1.58 by SNT and 1.85 log_{10} by ELISA in the 1st WPV and (1.55 as SNT, 1.91log_{10} as ELISA) in the 3rd WPV respectively.

Used adjuvant	Used Vaccine formula	FMD type-SAT2 serum neutralizing antibody titers /WPV*																
		0WPV*	1W	2W	3W	4W	6W	8W	10W	12W	14W	16W	20W	24W	28W	32W	36W	40W
Montanide Oil ISA 206	Vacc. (1)	0.48	1.27	1.67	1.86	2.12	2.28	2.59	3	2.83	2.79	2.56	2.39	2.18	1.81	1.79	1.54	1.32
	Vacc. (3)	0.51	1.32	1.71	1.92	2.19	2.37	2.76	3.09	2.97	2.82	2.61	2.47	2.26	1.95	1.86	1.68	1.47
Montanide Oil ISA 201	Vacc. (4)	0.21	1.5	1.71	2.24	2.61	2.83	3.24	3.09	3	2.91	2.64	2.37	2.24	1.97	1.76	1.64	1.52
	Vacc. (6)	0.3	1.58	1.87	2.32	2.71	2.92	3.33	3.18	3.15	3	2.71	2.49	2.31	2.02	1.89	1.73	1.57
POLYVAC™ 50	Vacc. (7)	0.27	1.12	1.29	1.50	1.75	2.06	2.37	2.42	2.68	2.50	2.49	2.27	2.08	1.76	1.59	1.5	1.22
	Vacc. (9)	0.15	1.23	1.35	1.55	1.84	2.14	2.42	2.42	2.71	2.55	2.51	2.33	2.14	1.81	1.65	1.52	0.75
Control		0.3	0.3	0.4	0.3	0.4	0.4	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3

Table 7: *Mean FMD type-SAT2 serum neutralizing antibody titers expressed in log_{10} in different vaccinated sheep groups.*

Used adjuvant	Used Vaccine formula	FMD type-SAT2 ELISA antibody titer /WPV*																
		0WPV*	1W	2W	3W	4W	6W	8W	10W	12W	14W	16W	20W	24W	28W	32W	36W	40W
Montanide Oil ISA 206	Vacc. (1)	0.76	1.47	1.92	2.10	2.40	2.54	2.89	3.27	3.13	2.97	2.76	2.54	2.44	2.11	1.97	1.84	1.62
	Vacc. (3)	1.08	1.57	1.96	2.17	2.49	2.62	3.01	3.34	3.22	3.07	2.86	2.62	2.51	2.23	2.04	1.97	1.72
Montanide Oil ISA 201	Vacc. (4)	0.51	1.8	2.01	2.50	2.81	3.13	3.39	3.29	3.3	3.21	2.94	2.67	2.54	2.17	2.06	1.92	1.82
	Vacc. (6)	0.6	1.85	2.2	2.55	2.93	3.25	3.55	3.38	3.36	3.24	3.08	2.7	2.61	2.27	2.18	1.99	1.88
POLYVAC™ 50	Vacc. (7)	0.42	1.35	1.65	1.82	2.11	2.33	2.61	2.83	2.92	2.89	2.69	2.46	2.22	1.99	1.84	1.8	1.21
	Vacc. (9)	0.4	1.52	1.77	1.91	2.26	2.46	2.79	2.99	3.01	2.96	2.73	2.57	2.33	2.10	1.92	1.84	1.24
Control		0.45	0.45	0.7	0.6	0.7	0.7	0.6	0.6	0.6	0.6	0.45	0.45	0.45	0.45	0.3	0.3	0.3

Table 8: *Mean FMD type-SAT2 ELISA antibody titer in different vaccinated sheep groups.*

The recorded peak of FMD type SAT2 antibody titers were (3.00 by SNT and 3.27 log_{10} by ELISA) in the 10th WPV; (3.2 by SNT) in the 8th WPV and (3.39 log_{10} by ELISA) in the 10th WPV and (2.68 as SNT, 2.92 log_{10} as ELISA) in the 12nd WPV induced by the inactivated FMD ISA 206 oil; FMD ISA 201 oil and FMD POLYVAC™ 50 vaccines respectively. Peak titers of FMD type SAT2 antibodies obtained by inactivated FMD ISA 206 oil; FMD ISA 201 oil and FMD POLYVAC™ 50 vaccines were (3.09 by SNT and 3.34 log_{10} by ELISA) in the 10th WPV; (3.33 by SNT and 3.55 log_{10} by ELISA) in the 8th WPV and (2.71 by SNT) in the 14th WPV and (3.01 log_{10} by ELISA) in the 12th WPV respectively.

Regarding the duration of FMD type SAT2 it was clear that inactivated FMD ISA 206 oil; FMD ISA 201 oil and FMD POLYVAC™ 50 vaccines provided specific immune titers of (1.54 by SNT and 1.84 log_{10} by ELISA) up to the 36th WPV; (1.52 by SNT and 1.82 log_{10} by ELISA) up to the 40th WPV and (1.50 by SNT and 1.80 log_{10} by ELISA) up to 36th WPV respectively. The inactivated combined FMD/RVF ISA 206 oil; FMD/RVF ISA 201 oil and FMD/RVF POLYVAC™ 50 vaccines provided specific protective immune titers of (1.68 by SNT and 1.97 log_{10} by ELISA) up to the 36th WPV; (1.57 by SNT and 1.88 log_{10} by ELISA) up to the 40th WPV and (1.52 by SNT and 1.84 log_{10} by ELISA) up to the 36th WPV respectively.

Our results come parallel to the result approved by EL-Sayed., *et al.* [18] who indicated that vaccines emulsified using Montanide ISA 201 adjuvant elicited a protective humoral immune response from the 2nd WPV for ISA 201 oil by SNT and ELISA titers of (1.62 ± 0.047^{a} and 1.8 ± 0.049^{a}); (1.59 ± 0.076^{a} and 1.836 ± 0.077^{a}) and (1.71 ± 0.06^{b} and 1.96 ± 0.074^{b}) by SNT and ELISA for serotypes O, A, SAT2, respectively., and ISA 206 showed antibody titer by SNT and ELISA of (1.5 ± 0.082^{a} and 1.84 ± 0.084^{a}); (1.56 ± 0.037^{a} and 1.818 ± 0.052^{a}) and ($1.5 \pm 0.106^{a,b}$ and $1.81 \pm 0.104^{a,b}$) for FMD virus serotypes O, A and SAT2, respectively. Also agreed with Pratik., *et al.* [41] who study the effect of the oil adjuvant Polyvac 50 (POLYVAC™ 50) and found that it is as efficient as Montanide ISA 50 V2, this means that antibody start later than Montanide ISA 201 or 206 and the duration may exceed them or equal.

Humeral antibody titer against RVF in vaccinated sheep with the different prepared oil vaccine formulae measured by SNT and ELISA and tabulated in table 9 and 10 showed differences in the onset, intensity and duration. Concerning the onset of protective immune response in vaccinated sheep, it is clear that titers of (1.51 by SNT and 0.235 by ELISA); (1.5 by SNT and 0.248 by ELISA) and (1.52 by SNT, 0.235 by ELISA) were recorded on the 2nd; 1st and 3rd WPV by the inactivated RVF ISA 206 oil; RVF ISA 201 oil and RVF POLYVAC™ 50vaccines respectively. Inactivated combined FMD/RVF ISA 206 oil; FMD/RVF ISA 201 oil and FMD/RVF POLYVAC™ 50 vaccines induced RVF antibody titers of (1.57 by SNT and 0.237 by ELISA) in the 2nd WPV; (1.59 by SNT and 0.253 by ELISA) in the 1st WPV and (1.58 by SNT and 0.242 by ELISA) in the 3rd WPV. These vaccine formulae recorded their peak of induced RVF antibody titers (2.81 by SNT and 0.325 by ELISA) in the 10th WPV; (3.33 by SNT and 0.335 by ELISA) in the 8th WPV and later (2.68 by SNT and 0.300 by ELISA) in the 12th WPV respectively. But in inactivated combined FMD/RVF ISA 206 oil; FMD/RVF ISA 201 oil and FMD/RVF POLYVAC™ 50 vaccines induced antibody titers of (2.87 by SNT and 0.334 by ELISA) in the 10th WPV; (3.58 by SNT and 0.340 by ELISA) in the 8th WPV and later (2.70 by SNT and 0.310 by ELISA) in the 12th WPV respectively.

It was found that the inactivated RVF ISA 206 oil; RVF ISA 201 oil and RVF POLYVAC™ 50 vaccines induced RVF antibody titers of (1.52 by SNT and 0.230 by ELISA) up to the 36th WPV; (1.51 by SNT and 0.235 by ELISA) up to the 40th WPV and (1.70 by SNT and 0.235 by ELISA) up to the 36th WPV respectively. The duration of protective RVF immune levels in vaccinated sheep was at 36 WPV as (1.58 by

Used Adjuvant	Used Vaccine formula	RVF serum neutralizing antibody titers /WPV*																
		0WPV*	1W	2W	3W	4W	6W	8W	10W	12W	14W	16W	20W	24W	28W	32W	36W	40W
Montanide Oil ISA 206	Vacc. (2)	0.45	1.44	1.51	1.70	1.82	2.00	2.53	2.81	2.74	2.60	2.50	2.39	2.26	2.12	1.7	1.52	1.1
	Vacc. (3)	0.52	1.33	1.57	1.77	1.85	2.2	2.58	2.87	2.76	2.61	2.55	2.41	2.29	2.16	1.8	1.58	1.2
Montanide Oil ISA 201	Vacc. (5)	0.53	1.5	1.63	1.92	2.2	3.00	3.33	3.29	3.21	2.79	2.55	2.48	2.34	2.28	2.18	1.83	1.51
	Vacc. (6)	0.7	1.59	1.67	1.96	2.4	3.2	3.58	3.35	3.27	2.92	2.84	2.51	2.38	2.24	2.20	1.88	1.55
POLYVAC™ 50	Vacc. (8)	0.56	0.6	1.0	1.52	1.74	2.15	2.33	2.64	2.68	2.58	2.48	2.36	2.18	2.09	1.9	1.7	1.42
	Vacc. (9)	0.5	0.9	1.2	1.58	1.79	2.19	2.36	2.67	2.70	2.60	2.51	2.38	2.22	2.13	2.09	1.8	1.44
Control		0.47	0.41	0.46	0.38	0.41	0.43	0.37	0.39	0.46	0.40	0.43	0.46	0.39	0.46	0.41	0.40	0.39

Table 9: *Mean RVF serum neutralizing antibody titers expressed in log_{10} in different vaccinated sheep groups.*

Used Adjuvant	Used Vaccine formula	RVF- ELISA optical density /WPV*																
		0WPV*	1W	2W	3W	4W	6W	8W	10W	12W	14W	16W	20W	24W	28W	32W	36W	40W
Montanide Oil ISA 206	Vacc. (2)	0.013	0.154	0.235	0.040	0.249	0.267	0.317	0.325	0.316	0.290	0.280	0.269	0.266	0.253	0.240	0.230	0.209
	Vacc. (3)	0.019	0.163	0.237	0.247	0.256	0.270	0.323	0.334	0.320	0.297	0.285	0.281	0.271	0.260	0.257	0.239	0.213
Montanide Oil ISA 201	Vacc. (5)	0.019	0.248	0.260	0.275	0.279	0.290	0.335	0.330	0.322	0.310	0.279	0.275	0.272	0.260	0.251	0.247	0.235
	Vacc. (6)	0.017	0.253	0.271	0.280	0.286	0.296	0.340	0.336	0.326	0.300	0.286	0.279	0.275	0.269	0.259	0.244	0.239
POLY-VAC™ 50	Vacc. (8)	0.017	0.168	0.189	0.235	0.240	0.265	0.272	0.287	0.300	0.273	0.270	0.265	0.257	0.249	0.241	0.235	0.222
	Vacc. (9)	0.021	0.177	0.196	0.242	0.247	0.271	0.286	0.297	0.310	0.281	0.274	0.270	0.261	0.258	0.250	0.239	0.227
Control		0.013	0.013	0.010	0.016	0.016	0.019	0.016	0.014	0.014	0.015	0.014	0.016	0.013	0.016	0.019	0.016	0.019

Table 10: *Mean RVF-ELISA optical density in different vaccinated sheep groups.*

**WPV: Week Post Vaccination;*

Vacc. (1): Trivalent FMD adjuvanted with Montanide ISA206;

Vacc. (2): RVF adjuvanted with Montanide ISA206;

Vacc. (3): Combined FMD and RVF adjuvanted with Montanide ISA206;

Vacc. (4): Trivalent FMD adjuvanted with Montanide ISA201;

Vacc. (5): RVF adjuvanted with Montanide ISA201;

Vacc. (6): Combined FMD and RVF adjuvanted with Montanide ISA201;

Vacc. (7): Trivalent FMD adjuvanted with POLYVAC™ 50;

Vacc. (8): RVF adjuvanted with POLYVAC™ 50;

Vacc. (9): Combined FMD and RVF adjuvanted with POLYVAC™ 50.

SNT and 0.239 by ELISA); and 40th WPV as (1.55 as SNT and 0.239 as ELISA) and 36 WPV as (1.8 by SNT and 0.239 by ELISA) induced by inactivated combined FMD/RVF ISA 206 oil; FMD/RVF ISA 201 oil and FMD/RVF POLYVAC™ 50 respectively.

From the above results and the statically analysis of humeral immunity it revealed that the Montanide oils 201 in combined RVF and FMD vaccine induced early and long lasting immunity followed by ISA 206 then POLYVAC™ 50

These results agreed with what reported by El-Bagoury., *et al.* [42] who prepared inactivated tissue culture adapted Rift Valley Fever (RVF) virus vaccines using peanut oil; code liver oil, Montanide ISA 206 oil and aluminum hydroxide gel as adjuvants. Such vaccines were found to be sterile and safe inducing no systemic or local clinical signs in vaccinated lambs. Comparative evaluation of four experimentally prepared vaccines in sheep after a single dose indicated that the oily prepared vaccines greatly stimulated the humoral immune response as estimated by SNT and ELISA compared with aluminum hydroxide gel vaccine. Protective serum RVF antibody titers induced by peanut oil and Montanide ISA 206 oil adjuvanted RVF virus vaccine started at 2nd WPV while those induced by code liver oil and Aluminum hydroxide gel vaccine started at 3rd WPV. These protective titers persisted till the 44th WPV for Montanide ISA 206 oil, 32nd WPV for peanut oil and code liver oil and 24th WPV for Aluminum hydroxide gel vaccines then declined under the protective level. They concluded that the use of Montanide ISA 206 was preferable as where it induced the superior immunological response followed by peanut oil then code liver oil and the aluminum hydroxide gel.

The clinical signs described in challenged vaccinated sheep after 4 WPV (Table 11) included either the lesions on tongue epithelial or on buccal mucosa and feet. The lesions vary from erosion, vesicles and ulceration. These signs appear to be characteristic for FMD as stated by Orsel., *et al.* [43]; El-Sayed., *et al.* [44]; Juleff., *et al.* [45] and OIE [23]. In the 4 sheep vaccinated with combined RVF and FMD adjuvanted with Montanide ISA 206 vaccine, one of the four challenged sheeps with serotype (SAT2) showed generalized infection (means 80% protection against serotype SAT2), but all rest of challenged sheep either with FMD serotype O and A give 100% protection. But all the 12 sheep vaccinated with combined RVF and FMD adjuvanted with Montanide ISA 201 vaccine gave protection 100% with FMD serotype O, A and SAT2 after challenge.

When using combined RVF and FMD adjuvanted with POLYVAC™ 50 vaccine one of each group from the 12 vaccinated sheep show generalized infection with either FMD serotype O, A and SAT2 after challenge (means 80% protection against FMD serotype O, A and SAT2).

Also the results of estimating the ED_{50} for different adjuvants for RVF and or combined RVF and FMD were illustrated in table 12, it showed that the all forms of adjuvants either with RVF and or combined RVF and FMD were valid within the permissible limit not more than 0.02/ml according to Randal., *et al.* [30] and Gihan., *et al* [46].

Animal No.	Used vaccine formula	Determined FMD lesions							Challenged virus	Protection %
		Oral lesion			Fore limb		Hind limb			
		Tongue	Gum	Lip	L	R	L	R		
1	Combined RVF and FMD vaccine inactivated and adjuvanted with Montanide ISA 206 (Vacc. 3)	+	-	-	-	-	-	-	FMDV (O)	100%
2		-	-	+	-	-	-	-		
3		-	-	-	-	-	-	-		
4		-	-	-	-	-	-	-		
5		-	+	-	-	-	-	-		
6		-	-	-	-	-	-	-	FMDV (A)	100%
7		-	-	-	-	-	-	-		
8		-	-	-	-	-	-	-		
9		-	-	-	-	-	-	-		
10		-	-	-	-	-	-	-		
11		-	-	+	-	-	-	-	FMDV (SAT2)	80%
12		-	-	-	-	-	-	-		
13		+	-	-	-	-	-	+		
14		-	-	-	-	-	-	-		
15		-	+	-	-	-	-	-		
16	Combined RVF and FMD vaccine inactivated and adjuvanted with Montanide ISA 201 (Vacc. 6)	-	-	-	-	-	-	-	FMDV (O)	100%
17		-	-	-	-	-	-	-		
18		-	+	-	-	-	-	-		
19		+	-	-	-	-	-			
20		-	-	-	-	-	-	-		
21		-	-	-	-	-	-	-	FMDV (A)	100%
22		-	-	-	-	-	-	-		
23		-	-	-	-	-	-	-		
24		-	-	+	-	-	-	-		
25		-	-	-	-	-	-	-		
26		-	-	-	-	-	-	-	FMDV (SAT2)	100%
27		-	-	-	-	-	-	-		
28		-	-	-	-	-	-	-		
29		-	-	-	-	-	-	-		
30		-	-	-	-	-	-	-		

31	Combined RVF and FMD vaccine inactivated and adjuvanted with POLYVAC™ 50 (Vacc. 9)	-	-	-	-	-	-	-	FMDV (O)	80%
32		+	-	-	-	+	-	-		
33		-	-	-	-	-	-	-		
34		-	-	-	-	-	-	-		
35		-	-	-	-	-	-	-		
36		-	-	-	-	-	-	-	FMDV (A)	80%
37		-	+	-	-	-	-	-		
38		-	-	-	-	-	-	-		
39		-	-	-	-	-	+	-		
40		-	-	-	-	-	-	-		
41		-	-	-	-	-	-	-	FMDV (SAT2)	80%
42		-	-	-	-	-	-	-		
43		-	-	-	-	-	-	-		
44		+	-	-	-	+	-	-		
45		-	-	-	-	-	-	-		
46	Control positive (O)	+	+	+	+	+	+	+	FMDV (O)	0%
47		+	+	+	+	+	+	+		
48	Control positive (A)	-	+	+	+	+	+	+	FMDV (A)	0%
49		+	-	+	+	+	+	+		
50	Control positive (SAT2)	+	+	+	+	+	+	+	FMDV (SAT2)	0%
51		+	+	+	-	+	+	+		
52	Control negative	-	-	-	-	-	-	-	No challenge	
53		-	-	-	-	-	-	-		

Table 11: *Protection percentage in sheep vaccinated with combined FMD/RVF and challenged against the three types of FMD virus.*

ED_{50}/ml of RVF vaccine formulae					
Formula (2)	Formula (3)	Formula (5)	Formula (6)	Formula (8)	Formula (9)
0.0014	0.0012	0.0010	0.0009	0.0018	0.0017

Table 12: *ED_{50}/ML for different types of the prepared RVF vaccine formulae.*

Vaccine formula (2) RVF with Montanide ISA206.

Vaccine formula (3): Combined FMD and RVF with Montanide ISA206.

Vaccine formula (5): RVF with Montanide ISA201.

Vaccine formula (6): Combined FMD and RVF with Montanide ISA201.

Vaccine formula (8): RVF with POLYVAC™ 50.

Vaccine formula (9): combined FMD and RVF with POLYVAC™ 50.

**The Permissible limit is not more than 0.02/ml.*

Conclusion

It was clear that there is no difference in the levels of immunity between vaccination of sheep with FMD and RVF alone or using combined FMD and RVF vaccine but from the economical view it is cheaper to prepare combined vaccine and also to save effort of the veterinarians and the stress factor the animal during vaccination.

Montanide oils 201 in combined RVF and FMD vaccine induced earlier and longer last immunity followed by ISA 206 then POLYVAC™ 50.

Bibliography

1. Depa PM., *et al.* "Update on epidemiology and control of foot and mouth disease - A menace to international trade and global animal enterprise". *Veterinary World* 5.11 (2012): 694-704.
2. Longjam N., *et al.* "A brief review on diagnosis of foot and-mouth disease of livestock: Conventional to molecular tools". *Veterinary Medicine International* (2011): 905768.
3. Aidaros HA. "Regional status and approaches to control and eradication of FMD in the Middle East and North Africa". *Revue Scientifique Et Technique (International Office of Epizootics)* 21.3 (2002): 451-458.
4. Farag MA., *et al.* "ELISA as a rapid method for detecting the correlation between the field isolates of foot and mouth disease and the current used vaccine strain in Egypt". *Veterinary Medical Journal* 53.4 (2005): 949-955.
5. Abed El- Rahman AO., *et al.* "Isolation and identification of serotype O of foot and mouth disease virus from imported Bulls and its correlation to the current used vaccine strain O1/3/1993". Proceedings 3rd International Conference Veterinary Research Division. NRC, Cairo, Egypt (2006): 91-100.
6. Satya P. "Vaccination against foot-and-mouth disease virus: Strategies and effectiveness". *Expert Review of Vaccines* 8.3 (2009): 347-365.
7. Shawky M., *et al.* "Isolation and molecular characterization of foot and mouth disease SAT2 virus during outbreak 2012 in Egypt". *Journal of Animal and Veterinary Advances* 3.2 (2013): 60-68.
8. Mirabela R., *et al.* "An assembly model of rift valley fever virus". *Frontiers in Microbiology* 3 (2012): 254.
9. OIE. OIE Rift Valley Fever Factsheet (2013).
10. WHO. "Rift Valley Fever". Fact Sheet (2012): 207.
11. Summerpa SK., *et al.* "Sever rift valley fever may present with a characteristic clinical syndrome". *The American Journal of Tropical Medicine and Hygiene* 82.3 (2010): 371-375.
12. Hanafi AH., *et al.* "Virus isolation sand high population density implicate culex antennatus (Becker) (Diptera: Culicidae) as a vector of rift valley fever virus during an outbreak in the Nile Delta of Egypt". *Acta Tropica* 119 (2011): 119-124.
13. Lombard M., *et al.* "A brief of vaccines and vaccination". *Revue Scientifique Et Technique* 26.1 (2007): 29-48.
14. Patil PK., *et al.* "Immune responses of sheep to quadrivalent double emulsion foot-and-mouth disease vaccines: Rate of development of immunity and variations among other ruminants". *Journal of Clinical Microbiology* 40 (2002): 43674371.

15. Lubroth J., *et al.* "Veterinary vaccines and their use in developing countries". *Revue Scientifique et Technique International Office of Epizootics* 26.1 (2007): 179-201.

16. Sonia AM. "Comparative studies on immune response of FMD and RVF combined vaccine containing OIMS 1313 oil adjuvant". M. V. Sc. Thesis, Microb. Faculty of Veterinary Medicine, Alexandria University, Egypt (2003).

17. Ragaa AE "Studies on production of oil inactivated rift valley fever vaccine using Montanide (IMS)". M. Vet. Sic Thesis, (Infectious Disease). Faculty of Veterinary Medicine. El Monufia University (2007).

18. EL-Sayed EM., *et al.* "Comparative study on the immunopotentiator effect of ISA 201, ISA 61, ISA 50, ISA 206 used in trivalent foot and mouth disease vaccine". *Veterinary World* 8.10 (2015): 1189-1198.

19. Postema AS., *et al.* "Challenges in the development, licensure, and use of combination vaccines". *Clinical Infectious Diseases* 33 (2001): S261-S266.

20. Falk LA., *et al.* "Manufacturing issues with combining different antigens: A regulatory perspective". *Clinical Infectious Diseases* 33 (2001): S351-S355.

21. Shabana W. "Preparation of combined oil vaccine against foot and mouth disease and rift valley fever in sheep". PhD. Faculty of Veterinary Medicine, Cairo University (2014).

22. Gamal WM., *et al.* "Tracing the antibody mediated acquired immunity by foot and mouth disease and rift valley fever combined vaccine in pregnant ewes and their lambs". *Veterinary World* 7.11 (2014): 922-928.

23. OIE World Organisation for Animal Health. Manual of diagnostic tests and vaccines for terrestrial animals, OIE, Paris (2017).

24. Xuan H., *et al.* "Establishment of persistent infection with foot and mouth disease virus in BHK-21 cells". *Virology Journal* 8 (2011): 169.

25. Reed LJ and Muench H. "A simple method for estimating fifty percent (50%) end points". *The American Journal of Tropical Medicine and Hygiene* 27 (1938): 493-497.

26. Health Protection Agency. Complement fixation tests. by: Standards unit, Department for Evaluations, standards and Training 1.3 (2009): 23.

27. El Nimr MM. "Studies on the inactivated vaccine against rift valley fever. Ph. D. Thesis (Microbiology). Faculty of Veterinary Medicine". Assuit University, Egypt (1980).

28. Ismail AH., *et al.* "Optimization of the inactivation process of FMD virus serotype SAT-2 by binary ethyleneimine (BEI)". *Journal of Veterinary Advances* 3.3 (2013): 117-124.

29. Code of Federal Regulation of USA. Animal and Animal Products 9\2019. Office of the Federal Register National Archives and Record Administration (2019).

30. Randall R., *et al.* "Immunization against Rift Valley fever virus. Studies on the immunogenicity of lyophilized formalin inactivated vaccine". *Journal of Immunology* 92 (1964): 293-299.

31. Slater TF., *et al.* "Studies on succinate-tetrazolium reductase system: III. Points of coupling of four different tetrazolium salts". *Biochimica et Biophysica Acta* 77 (1963): 383-393.

32. EL-Naggar H. "Preparation of inactivated lyophilized NDV vaccine, M.V.Sc in Veterinary Science (Virology). Cairo University (2012).

33. Lucy FL. "Chicken Lymphocyte stimulation by mitogenes. A microassay with whole blood cultures". *Avian Disease Journal* 22 (1977): 296-307.

34. Lee LF. "Proliferative response of chicken B and T lymphocyte to mitogen". *Veterinary Medicine Journal* 15 (1984): 44-52.

35. Mayer S., *et al.* "Anomalous reactivity of sera containing cold lymphocytotoxins with chronic leukemic lymphocytes". *Tissue Antigens* 4 (1974): 266-270.

36. Ferreira MEV. "Micro titer neutralization test for the study of FMD antibodies". *Bol. Cent. Pan. Am. Fiebre Aftosa* 21 (1976): 22-23.

37. Voller A., *et al.* "Microplate enzyme immunoassay for the immune diagnosis of virus infection". *The American Society for Microbiology* (1976): 506-512.

38. Barnett PV., *et al.* "Further studies on the early protective responses of pigs following immunization with high potency foot and mouth disease vaccine". *Vaccine Journal* 19 (2002): 3197-208.

39. Mossad W Gamal El-Din., *et al.* "Humeral and cellular immune response of Egyptian trivalent foot and mouth disease oil vaccine in sheep". *Research Opinions in Animal and Veterinary Sciences* 4.4 (2014): 178-185.

40. Dong Li., *et al.* "The comparison of the efficacy of swine FMD vaccine emulsified with oil adjuvant of ISA 201 VG or ISA 206 VG". *Journal of Biosciences and Medicines* 1 (2013): 22-25.

41. Pratik S Pawar., *et al.* "Comparative performance of Foot and Mouth disease vaccine prepared using different adjuvants and payloads". *Veterinarian Journal* (2014).

42. El-Bagoury GF., *et al.* "Comparative evaluation of prepared inactivated Rift Valley Fever virus vaccine with different adjuvants". *Benha Veterinary Medical Journal* 25.1 (2013): 106-114.

43. Orsel K., *et al.* "Foot and mouth disease virus transmission during the incubation period of the disease in piglets, lambs, calves, and dairy cows". *Preventive Veterinary Medicine* 88.2 (2009): 158-163.

44. El-Sayed E., *et al.* "Studies on the duration of immunity induced in cattle after natural FMD infection and post vaccination with bivalent oil vaccine". *Veterinary World* 5.10 (2012): 603-608.

45. Juleff ND., *et al.* "The importance of FMDV localization in lymphoid tissue". *Veterinary Immunology and Immuno- Pathology* 148 (2012): 145-148.

46. Gihan KM., *et al.* "Studies on the keeping quality of binary inactivated Rift Valley fever Vaccine". *Assiut Veterinary Medical Journal* 39.77 (1998): 169-179.

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