IMMUNOLOGICAL STUDIES OF COMBINED FOOT AND MOUTH DISEASE AND RIFT VALLEY FEVER OIL VACCINE

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Abstract

Foot-and-mouth disease (FMD) is an economically important disease of cloven-hoofed animals. In Egypt, FMD assumes as enzootic form and attacks susceptible animals causing high losses in milk and meat production. Rift valley fever virus causes serious and fatal disease in animals and man. It produces high abortion rate among pregnant ewes and cows, causes heavy mortalities in young lambs and calves. In the present study the Montanide ISA 50 oil adjuvanted combined FMD/RVF vaccine was tested in sheep and compares it with single vaccines either FMD or RFV alone.The mean of antibody titers continued with the protective level till the 32 to 36th week post vaccination, In single vaccination either FMD or RFV alone while till to 40th WPV in combined FMD/RVF vaccine.In the final we can conclude that the use of Montanide ISA 50 as an oil adjuvant in prepared vaccines improve the immune response against FMD and RVF, giving high titer of antibodies against both diseases, and long duration of immunity in combined FMD/RVF vaccines.

Keywords : Foot and mouth diseasevaccine,Rift valley fever vaccine, Combined vaccine, Montanide ISA 50.

1. INTRODUCTION

Foot and mouth disease is one of the most troubles worlds wide viral disease of animals specially cloven footed of both wild and domestic animals **(1)**.The causative agent is a single stranded positive- sense RNA virus that belongs to the genus Aphthovirus in the family Picornaviridae. There are seven immunologicaly distinct serotype of FMD virus, namely, O, A, C, Asia1, Sat1, Sat2 and Sat3 **(2).** In Egypt, the disease is enzootic and outbreaks have been reported since 1950 **(3)**. FMD serotype O was the most prevalent since 1960 and onwards (**4-6**).Since 1950,1953 and 1956 serotype A didn't recorded in Egypt **(4),** serotype A FMD virus introduced to Egypt through live animals importation, and the sever clinical signs occurred among cattle and buffaloes **(7). Recently** FMD serotype SAT 2 outbreaks in Egypt were reported in eight (8) out of 27 governorates concentrated mainly in the Delta area and very few along the Nile in the southern parts of the country (**8-9).**The control of FMD in animals was considered to be important to effectively contain the disease in endemic areas, so that vaccination of animals is effective in limiting the spread of FMD **(10).**Most foot-and-mouth disease vaccines are made of BEI (binary Ethyleneinmine) inactivated virus that is adjuvanted with either aluminum hydroxide-saponin (AS) or oil adjuvant. Oil adjuvants are generally preferred over AS vaccines because among other advantages, they produce longer lasting immunity **(11).**

Rift Valley Fever (RVF) is an acute, sub acute or mild arthropod born viral disease of many species of animals as well as human being. The disease characterized by high mortality rates among calves and lambs as well as abortion of pregnant ewes and cows (***13***). RVF causes serious and fatal disease in animals and human being characterized by a short incubation period, fever, leucopenia and necrotic changes in the liver (***14)***. RVFV is a negative-strand

RNA virus belongs to the family Bunyaviridae, genus Phlebovirus **(15&16).** RVFV has traditionally caused recurrent outbreaks affecting humans and ruminants predominantly in Sub-Saharan Africa, but spread to Egypt in 1977 and to the Arabian Peninsula in 2000***(17).***after the appearance of the disease, identification and isolation of virus occurred and the Egyptian authorities succeeded in preparing a safe potent inactivated vaccine (***18***). Other studies were conducted by (***19)*** to improve the vaccine and to raise its efficiency.

Due to the danger of both FMD and RVF diseases, systemic vaccination and quarantine measures are usually applied specially in enzootic areas as effective control measures. Combined vaccines are used for many human and animal diseases. However we have very few examples of combinations comprising anti-FMD valiancy which would allow easy immunization without additional handling .

Many authors recommended the use of combined vaccines against some infectious diseases in cattle and sheep that revealed good immunity as single vaccine (***20***).

The increase number of vaccines which are administered to the animal at different age and time make it necessary to study the immune response of animals vaccinated with two vaccines at the same time as a combined vaccine and compare it with the single vaccine to save efforts and times at launching vaccination campaign for more than one disease.

2. Material and Methods

2.1.AnimalsTwenty one adult susceptible sheep local breed of about 35-50 kg body weight, clinically healthy and free from antibodies against FMD and RVF viruses before the experimental work using serum neutralization test according to ***(21) .***

2.2.FMD viruses

The viruses usedin this study were locally isolated FMDV strains O_1/3/93 , A/1/ Egypt 2006 and SAT2/2012 of cattle origin . The viruses were typed at Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo and confirmed by Pirbright, World Reference Laboratories, United Kingdom.The antigens are stored at -70^{o}C and used for preparation of vaccines and serologically tested for determination of antibodies against FMD virus types.

2.3.RVF virus (ZH 501)

The original virus was that isolated from a human patient in Zagazig, Sharquia province during 1977 outbreak and supplied by NAMRU-3 after being identified to be RVF virus. It was twice passaged I/C into suckling mice and has a final titer of 2×10^7 $MICLD_{50}$/ml. It was considered as the seed virus and preserved at -70^{o}C.

3. Titration of FMD and RVF antigensused in vaccines preparation

3.1.Titration of FMD virus in tissue culture plates to detect the infectivity titer which expressed as $\log_{10}$ $TCID_{50}$ as described by ***(22)*** .

3.2. titration of RVF virus in tissue culture tubes as recommended by ***(17&18)***for detection of infectivity titer which expressed as $\log_{10}$ $TCID_{50}$ as described by ***(22)***

Complement fixation test used for detection of antigenicity of both FMD and RVF viruses used in vaccines preparation.

4.Virus inactivation and safety testing

FMD virus strains O_1 /Aga/ 93 , A/1/Egypt /2006 and SAT 2/2012 were inactivated by 0.1% M Binary ethylene amine (BEA; Sigma) as previously described ***(23).***

Montanoid Oil : ISA 50 Montanoid Oil was obtained from Seppic, Paris, France.

5.Preparation of the oil adjuvant vaccines

The inactivated and clarified virus harvest was concentrated with 8% (w : v) polyethylene glycol

(PEG-6000) The inactivated oil adjuvenated FMD Vaccines were formulated according to **(22).**The ratio of the aqueous antigen to the oil adjuvant was 50:50 . The emulsions were produced by recycling the aqueous antigen-oil mixture several times. Sterility and safety of the prepared vaccines were done according to **(23).**

6.Preparation of combined oil adjuvant vaccine (FMD and RVF viruses)

The combined vaccine prepared from the previous inactivated FMD and RVF viruses is prepared as follows:

Mixing (4) parts of inactivated FMD virus with (1) part of inactivated RVF virus.That aqueous antigen mixture added in equal volumes (v/v) to (oil phase emulsion pH adjusted to 7.3-7.4 and mixed thoroughly.

7. Experimental Design

Three groups(five sheep for each) werevaccinated with the tested vaccines.First sheep group was injected with FMD vaccine, second group vaccinated with combined (FMD&RVF) vaccine, third group vaccinated with RVF vaccine and fourth group was kept as negative control non vaccinated two sheep for each group . Serum samples were collected weekly post vaccination for one month then every weeks post-vaccination till the protective antibody level declined to non protective level.The immune response was evaluated through the estimation of humoral immune level using SNT.

8.Serum neutralization test (SNT)

It was performed using the technique as described by **(20) .**

9.Enzyme linked immunosrobent assay (ELISA)

It was carried out according to the method described by ***(24)*** **.**

2. RESULTS

The obtained results are shown in tables **(1-2&3**) . It revealed that serum antibody protective titer against FMDV evaluated by mean of SNT started at 3rd week post vaccination with FMD vaccine with the titer of(1.68-1.65&1.63) log_{10} fortypes O –A &SAT2 respectively ,while in combined vaccine the protective level at 2nd WPV with the titer (1.6 - 1.59 &1.74) log_{10} and (1.89,1.83&1.74) at 3rd WPV fortypes (O - A &SAT2)respectively.

The higher antibody level following vaccination was at the 10th week with the titer of (2.7 - 3.0 log_{10}) forFMD O,A &SAT 2 and combined respectively , The mean of antibody titers continued with the protective level till the 36th WPV for (O, A) and 32th WPV for type SAT2 ' while in combined vaccine protective level continued till 40WPV for type (O, A) and till 36th WPV for type SAT2 after that the immunity duration started to decline under the protective level by SNT**.**

From tables (4) we noticed that serum antibody protective titer against RVF evaluated by mean of SNT started at 2nd week post vaccination with FMD vaccine with the titer of (1. 8 &2.07) log_{10} forsheep vaccinated with RVF vaccine and combined vaccine respectively. The higher antibody level following vaccination was at the 10th week with the titer of (3.1-3.28) log_{10} forsheep vaccinated with RVF vaccine and combined vaccine respectively. The mean of antibody titers continued with the protective level till the 36th week post vaccination in sheep vaccinated with RVF vaccine and till 40th WPV in combined vaccine then started to decline under the protective level.

Table (1) Frequency of serum antibody titers against type (O) , in sheep vaccinated with trivalent FMD vaccine and combined FMD &RVF vaccines by using SNT expressed log_{10}

Weeks post vaccination	*Antibody titre against FMDV type O_1 in FMD vaccine*					*Mean*	*Antibody titre against FMDV type O_1 in combined FMD &RVF vaccine*					*Mean*
	1	**2**	**3**	**4**	**5**		**1**	**2**	**3**	**4**	**5**	
O	**0**	**0.3**	**0.45**	**0**	**0.15**	**0.18**	**0.0**	**0.3**	**0.45**	0.0	**0.15**	**0.18**
1	**0.9**	**0.9**	**0.6**	**0.45**	**0.6**	**0.7**	**0.3**	**0.6**	**0.9**	**0.3**	**0.9**	**0.6**
2	**1.05**	**1.2**	**1.2**	**1.05**	**1.2**	**1.14**	**1.5**	**1.5**	**1.65**	**1.8**	**1.65**	**1.6**
3	**1.55**	**1.65**	**1.65**	**1.8**	**1.8**	**1.69**	**1.8**	**1.65**	**1.8**	**2.1**	**2.1**	**1.89**
4	**1.65**	**1.8**	**1.8**	**2.1**	**1.95**	**1.86**	**1.95**	**2.1**	**2.25**	**2.4**	**2.7**	**2.28**
6	**2.25**	**2.4**	**2.25**	**2.4**	**2.4**	**2.34**	**2.55**	**2.7**	**2.7**	**2.7**	**2.85**	**2.7**
8	**2.25**	**2,7**	**2.4**	**2.7**	**2.7**	**2.55**	**2.55**	**3.0**	**2.85**	**3.0**	**3.0**	**2.9**
10	**2.55**	**2.4**	**2.85**	**2.85**	**2.85**	**2.7**	**2.85**	**2.85**	**3.0**	**3.15**	**3.3**	**3.0**
12	**2.55**	**2.4**	**2.7**	**2.85**	**2.85**	**2.67**	**2.85**	**2.85**	**3.15**	**3.3**	**3.3**	**3.09**
14	**2.55**	**2.4**	**2.55**	**2.85**	**3.15**	**2.7**	**2.7**	**2.7**	**3.0**	**3.3**	**3.45**	**3.03**
16	**2.4**	**2.25**	**2.55**	**2.7**	**3.0**	**2.85**	**2.7**	**2.7**	**3.0**	**3.3**	**3.45**	**3.03**
18	**2.4**	**2.25**	**2.4**	**2.7**	**3.0**	**2.55**	**2.7**	**2.7**	**3.0**	**3.15**	**3.45**	**3.0**
20	**2.4**	**2.1**	**2.4**	**2.7**	**2.7**	**2.46**	**2.4**	**2.55**	**2.85**	**3.0**	**3.15**	**2.8**
22	**2.25**	**2.1**	**2.1**	**2.7**	**2.7**	**2.37**	**2.4**	**2.55**	**2.85**	**3.0**	**3.0**	**2.76**
24	**1.95**	**1.95**	**1.8**	**2.4**	**2.55**	**2.13**	**2.25**	**2.4**	**2.55**	**2.7**	**2.7**	**2.5**
28	1.95	1.8	1.65	2.1	2.4	**1.98**	**2.25**	**2.1**	**2.25**	**2.7**	**2.55**	**2.37**
32	1.8	1.65	1.5	1.95	2.4	**1.8**	**2.1**	**1.8**	**2.15**	**2.4**	**2.4**	**2.2**
36	1.5	1.5	1.35	1.65	2.1	**1.62**	**1.95**	**1.8**	**1.95**	**1.95**	**2.4**	2.0
40	1.2	1.35	1.2	1.35	1.65	**1.35**	**1.65**	**1.5**	**1.5**	**1.65**	**2.1**	**1.68**
44	0.9	0.9	0.75	1.05	0.96	**1.02**	1.5	1.2	1.2	1.5	1.8	1.44

***Antibody titers expressed as log_{10} $TCID_{50}$**

Table (2) Frequency of serum antibody titers against type (A) , in sheep vaccinated with trivalent FMD vaccine and combined FMD &RVF vaccines by using SNT expressed log_{10}

Weeks post vaccination	*Antibody titre against FMDV type A in FMD vaccine*					*Mean*	*Antibody titre against FMDV type A in combined FMD &RVF vaccine*					*Mean*
	1	2	3	4	5		1	2	3	4	5	
O	0.3	0.3	0.45	0.15	0.15	0.3	0.3	0.3	0.45	0.15	0.15	0.27
1	0.8	0.8	0.5	0.35	0.5	0.8	0.3	0.6	0.9	0.3	0.9	0.6
2	1.05	1.2	1.2	1.2	1.2	1.05	1.5	1.5	1.65	1.65	1.65	1.59
3	1.65	1.65	1.65	1.65	1.65	1.65	1.8	1.8	1.8	1.95	1.8	1.83
4	1.8	1.65	1.65	1.8	1.65	1.8	2.1	1.95	1.95	2.1	1.95	2.0
6	2.1	1.8	2.1	2.1	1.8	2.1	2.4	2.4	2.4	2.7	2.4	2.5
8	2.25	2.1	2.4	2.4	2.4	2.25	2.55	2.55	2.7	2.7	2.7	2.64
10	2.7	2.55	2.7	2.85	2.7	2.7	3.0	3.0	3.15	3.0	3.15	3.0
12	2.55	2.4	2.7	2.85	2.7	2.55	3.0	3.0	3.3	3.3	3.15	3.15
14	2.4	2.4	2.55	2.7	2.55	2.4	2.7	2.7	3.0	3.0	3.0	2.88
16	2.4	2,4	2.55	2.55	2.55	2.4	2.7	2.7	3.0	3.0	3.0	2.88
18	2.4	2.25	2.4	2.4	2.4	2.4	2.7	2.55	2.7	2.85	2.7	2.7
20	1.95	2.25	2.4	2.4	2.4	1.95	2.4	2.25	2.55	2.7	2.55	2.5
22	1.95	2.1	2.1	2.25	2.25	1.95	2.1	2.25	2.4	2.4	2.4	2.3
24	1.8	1.8	2.1	2.25	2.25	1.8	1.95	2.1	2.4	2.25	2.25	2.2
28	1.5	1.5	1.8	1.95	2.1	1.5	1.8	1.8	2.1	2.25	2.25	2.0
32	1.5	1.5	1.5	1.95	1.8	1.5	1.65	1.8	1.8	1.95	2.1	1.86
36	1.2	1.35	1.2	1.8	1.65	1.2	1. 5	1.5	1.65	1.8	1.95	1.68
40	0.9	1.2	0.9	1.5	1.35	0.9	1.5	1.2	1.5	1.5	1.8	1.5
44	0.6	0.9	0.75	2.1	1.05	0.6	1.2	0.9	1.2	1.2	1.5	1.2

***Antibody titers expressed as log_{10} $TCID_{50}$**

Table (3) Frequency of serum antibody titers against type (SAT 2), in sheep vaccinated with trivalent FMD vaccine and combined FMD &RVF vaccines by using SNT expressed log_{10}

Weeks post vaccination	*Antibody titre against FMDV type A in FMD vaccine*					*Mean*	*Antibody titre against FMDV type A in combined FMD &RVF vaccine*					*Mean*
	1	2	3	4	5		1	2	3	4	5	
O	0.3	0.3	0.45	0.15	0.15	0.27	0.3	0.3	0.45	0.15	0.15	0.3
1	0.6	0.6	0.6	0.9	0.9	0.72	0.6	0.6	0.6	0.9	0.9	0.72
2	1.2	1.2	1.2	1.5	1.8	1.38	1.5	1.65	1.65	1.8	2.1	1.74
3	1.65	1.5	1.5	1.7	1.8	1.63	1.8	1.8	2.1	1.95	2.1	1.95
4	1.8	1.65	1.65	1.8	2.1	1.8	2.1	2.1	2.25	2.1	2.4	2.2
6	2.1	1.8	2.1	2.1	2.4	2.1	2.4	2.25	2.55	2.4	2.7	2.5
8	2.25	2.1	2.4	2.4	2.7	2.37	2.55	2.7	2.85	2.85	3.0	2.8
10	2.7	2.4	2.7	2.85	2.85	2.7	3.0	2.85	3.15	3.15	3.3	3.1
12	2.55	2.55	2.7	2.7	2.85	2.67	3.0	3.0	3.0	3.0	3.0	3.0
14	2.4	2.55	2.55	2.55	2.7	2.55	2.85	3.0	3.0	3.0	3.0	2.97
16	2.4	2.25	2.55	2.55	2.7	2.49	2.7	2.85	3.0	2.85	2.85	2.85
18	2.25	2.55	2.4	2.55	2.55	2.46	2.7	2.85	2.85	2.7	2.85	2.8
20	1.95	2.25	2.4	2.4	2.55	2.31	2.25	2.7	2.85	2.4	2.7	2.6
22	1.95	2.1	2.1	2.25	2.55	2.19	2.25	2.4	2.55	2.25	2.55	2.4
24	1.8	1.8	2.1	2.25	2.4	2.07	2.1	2.25	2.4	2.25	2.4	2.3
28	1.8	1.65	1.8	1.8	2.1	1.89	2.1	2.1	2.1	2.1	2.25	2.1
32	1.65	1.5	1.65	1.95	1.8	1.71	1.8	1.95	1.95	1.95	2.25	1.9
36	1.5	1.2	1.35	1.65	1.65	1.47	1.5	1.65	1.65	1.8	1.8	1.68
40	0.9	0.75	0.9	1.2	1.5	0.9	0.9	1.2	1.35	1.5	1.5	1.3
44	0.6	0.6	0.6	0.9	1.05	0.75	0.6	0.9	0.9	1.05	1.2	0.96

Table (4) Frequency of serum antibody titers against RVF in sheep vaccinated with RVF and combined FMD &RVF vaccines by using SNT expressed log_{10}

Weeks post vaccination	*Antibody titre against RVF of sheep vaccinated with RVF*					*Mean*	*Antibody titre against RVF of sheep vaccinated with combined FMD &RVF vaccine*					*Mean*
	1	2	3	4	5		1	2	3	4	5	
O	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.3	0.0	0.15	0.15
1	0.6	0.6	0.9	0.6	0.6	0.7	0.3	0.6	0.9	0.3	0.9	0.6
2	1.2	1.8	2.1	1.95	1.8	1.8	1.8	1.8	2.4	2.4	1.95	2.07
3	1.8	2.4	2.4	2.7	2.4	2.34	2.4	2.4	2.7	2.7	2.4	2.52
4	2.4	2.7	2.85	2.85	2.7	2.7	2.7	2.7	3.15	3.0	2.85	2.88
6	2.7	3.0	3.0	2.85	2.85	2.88	3.15	3.15	3.3	3.3	3.15	3.18
8	2.7	3.0	3.0	3.0	3.0	2.94	3.1	2.7	3.3	3.3	3.3	3.38
10	3.0	3.0	3.3	3.15	3.0	3.1	3.1	3.3	3.6	3.15	3.1	3.28
12	2.85	2.85	2.85	3.0	3.0	2.9	3.0	2.85	3.3	3.0	2.85	3.02
14	2.7	2.7	2.85	2.85	2.85	2.79	3.0	2.7	3.15	3.0	2.55	2.9
16	2.55	2.4	2.7	2.55	2.4	2.52	2.7	2.4	2.85	2.7	2.25	2.76
18	2.4	2.1	2.4	2.1	2.4	2.28	2.7	2.4	2.7	2.7	1.95	2.49
20	2.1	1.8	2.4	1.95	2.1	2.1	2.4	2.1	2.7	2.7	1.8	2.4
22	2.1	1.8	2.25	1.95	1.95	2.0	2.25	2.4	2.7	2.4	1.8	2.28
24	1.8	1.65	2.25	1.8	1.8	1.86	2.4	2.1	2.4	2.1	1.5	2.1
28	1.5	1.5	2.1	1.8	1.8	1.7	1.8	1.8	2.1	1.8	1.5	1.8
32	1.5	1.5	1.8	1.8	1.5	1.6	1.8	1.8	2.1	1.8	1.5	1.8
36	1.35	1.35	1.5	1.35	1.2	1.35	1.5	1.65	1.65	1.8	1.8	1.7
40	1.2	0.9	1.35	1.05	0.9	1.08	0.9	1.2	1.35	1.5	1.5	1.3
44	0.6	0.6	0.9	0.6	0.75	0.69	0.9	1.05	1.05	0.9	1.2	1.0

Figure no. 1: *Immune status of sheep vaccinated with trivalent FMD vaccine against type(O) tested by ELISA and SNT.*

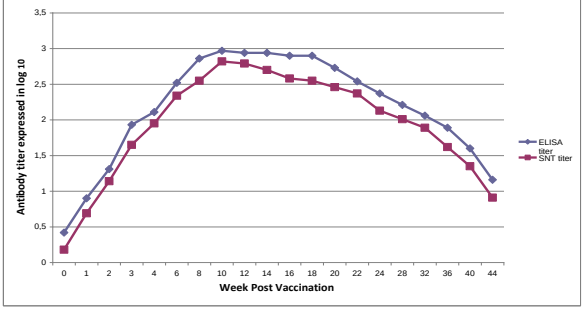


Figure no . 2 : *Immune status of sheep vaccinated with trivalent FMD vaccine against type (A) tested by ELISA and SNT.*

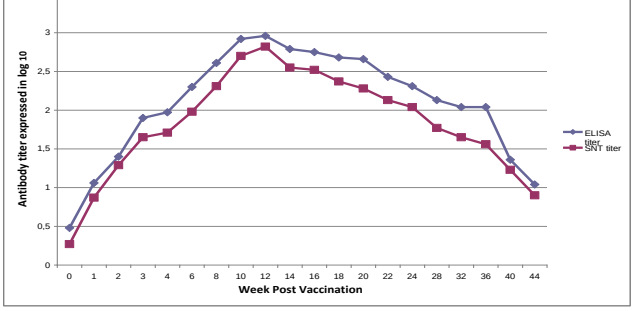


Figure no . 3 : *Immune status of sheep vaccinated with trivalent FMD vaccine against type (SAT2) tested by ELISA and SNT.*

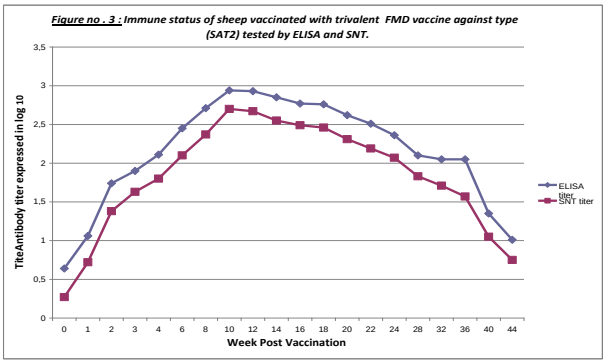


Figure no. 4 : *Immune status of sheep vaccinated oil adjuvant RVF vaccine tested by ELISA and SNT .*

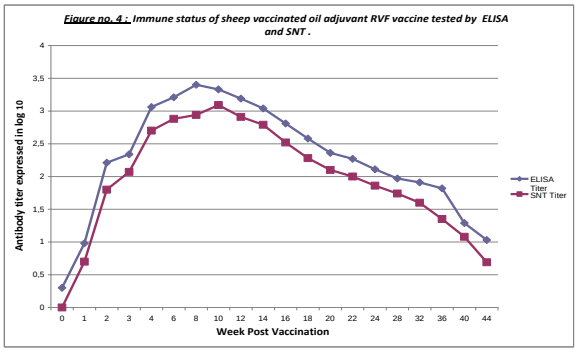


Figure no. 8 : *Humeral immune status of sheep vaccinated with combined(FMD and RVF) vaccine against (RVF) tested by ELISA and SNT .*

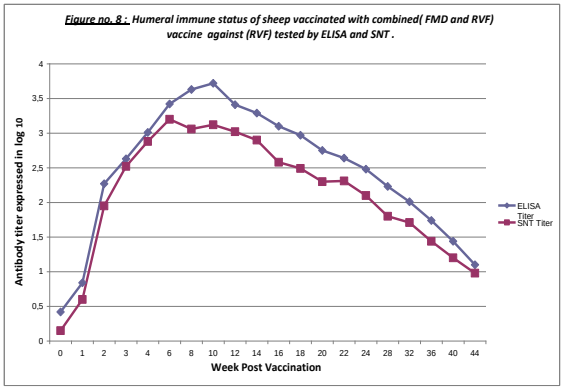


Figure no. 7 : *Immune response of sheep vaccinated with combined(FMD and RVF) vaccine against FMD type (SAT2) tested by ELISA and SNT .*

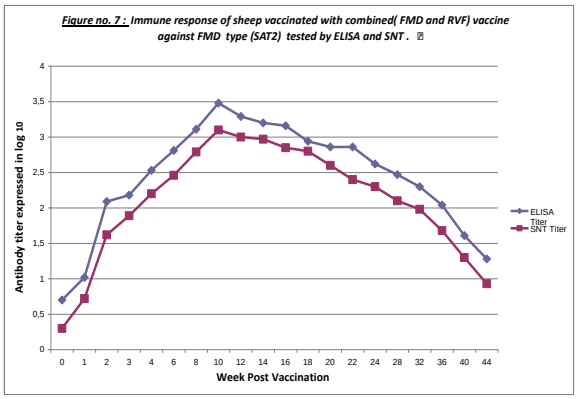


Figure no. 6 : *Immune status of sheep vaccinated with combined (FMD and RVF) vaccine against FMD type (A) tested by ELISA and SNT .*

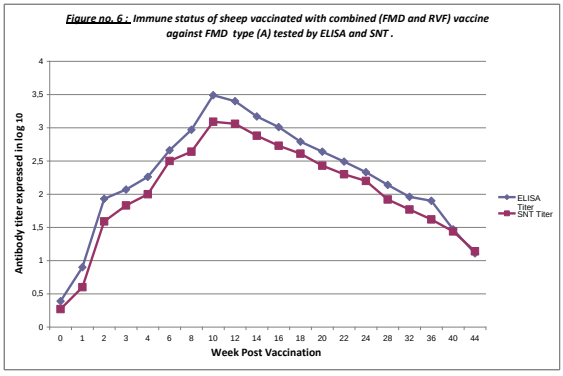
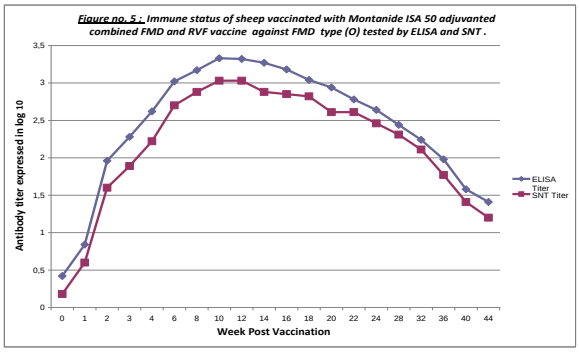


Figure no. 5 : *Immune status of sheep vaccinated with Montanide ISA 50 adjuvanted combined FMD and RVF vaccine against FMD type (O) tested by ELISA and SNT .*



4. DISCUSSION

Foot and mouth disease is considered the major infectious disease affecting cattle, buffaloes, sheep and other cloven footed animals. It characterized by fever, vesicular eruptions of the mouth mucosa, teats and the coronary bands of the hooves. The disease in sheep tends to be mild, transient or even inapparent infection, while in lambs it may be peracute and cause sudden death. It is enzootic in Africa, Asia, Europe, Philippines and South America (***25***). The control of FMD in sheep was considered to be important to effectively contain the disease in endemic areas, (***10***).

Rift valley fever virus causes serious and fatal disease in animals and man. It produces high abortion rate among pregnant ewes and cows, causes heavy mortalities in young lambs and calves (***26***).

The progress in vaccine production is directed towards the selection of the proper adjuvant that can elaborate high and long lasting immunity. Adjuvants can influence the immune response and prolong the immune response and stimulate specific components of the immune response either humoral or cell mediated immunity (***27***).

Three experimental of FMD, RVF and combined FMD/RVF vaccines batches . The viruses inactivated by binary ethyleimimine and adjuvanted with Montanide ISA50 oil adjuvant.

Regarding the results of innocuity, sterility and potency of different inactivated prepared vaccines, the results obtained in there is no detection of cytopathic effect (CPE) on BHK cells after three passages of the inactivated viruses used in vaccine preparation, indicating that there is no viable viral residues after the inactivation process. Also, FMD lesions did not appear on susceptible sheep when inoculated at different sites of the tongue.

in tables **(2-3&4**) findings agreed with **(28)** who found that the peak titer with oil FMD vaccines was not reached before 60-80 days post vaccination. Our results also agreed with ***(29)*** who recorded that the immunogenicity of FMD vaccines can be considerably increased by the use of proper adjuvants, in FMD vaccines prepared for cattle and sheep.

Our results also agreed with ***(29)*** who recorded that the immunogenicity of FMD vaccines can be considerably increased by the use of proper adjuvants, in FMD vaccines prepared for cattle and sheep. Also our results were supported by ***(30)*** who used the serum neutralizing antibody assay for determining the potency of FMD vaccines The finding indicated that protective capacity of the prepared vaccine.

The obtained results were in agreement with ***(31)*** who found that oil FMD vaccines gave high and long duration immunity, while disagreed with ***Samir (32 &33)*** in that the protective titer of antibody continued with protective level till the 38th week post vaccination, then decreased than that results obtained by him in which the protective level started at 3rd week post vaccination .

These results were supported by ***(34)*** who compared between using of different Montanide ISA oil adjuvants and different Montanide IMS oil adjuvants in emergency FMD vaccine for Guinea pigs. Regarding the study of humoral immune response of sheep vaccinated with combined FMD and RVF vaccine, the obtained results of SNT.

The obtained results in **tables (4)** agreed with those of ***(35&36)*** where they mentioned that the use of oil adjuvant in FMD vaccine involved a more efficient antigen stimulus and more sustained antibody response, ***(37)*** who found that mixed vaccination with anthrax and FMD were as good as FMD vaccination on its own, ***(38)*** mentioned that the antibodies developed from vaccination of cattle by FMD virus, rabies and *Brucella*

Intradermol

abortus were as high as of the individual vaccine of each,

Our results were agreed with ***(39)*** who prepared RVF/FMD combined vaccine which protect animals well against challenge with the virulent viruses, ***(40&41)*** who studied the vaccination of sheep with combined RVF and sheep pox vaccine which protect animals well against challenge with the virulent viruses, Assessment of neutralizing antibodies of RVF after vaccination was considered by ***(42)*** as a way for evaluating the protective capacity of prepared vaccines. ***(43)*** suggested that the protective titer was 1.7 $\log_{10}$, while ***(44&45)*** mentioned that serum neutralizing protecting titer considered not less than 1.0 $\log_{10}$. These results agreed with ***(46) .***

The protective antibody level of sheep vaccinated with Montanide ISA 50 as an oil adjuvant (FMD and RVF) vaccine results revealed that the prepared vaccine improve the immune response against FMD and RVF, giving high titer of antibodies against both diseases and indicated that the combined FMD/RVF vaccine conferred long duration of immunity than conferred by single vaccine against these diseases when used alone.

Finally we can concluded that using of combined FMD/RVF vaccines give long duration of immunity and must be used to save efforts and times at launching vaccination .

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دراسات مناعية على اللقاح المركب الزيتى لمرضى الحمى القلاعية وحمى الوادى المتصدع

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الملخص العربى

نظرا لتعدد اللقاحات التى يتم التحصين بها فى أوقات و أعمار مختلفة للحيوانات فكان من الضروربدراسة تأثير التحصين بلقاحين مختلفين فى نفس الوقت ، لذا أجريت هذه الدراسة لإنتاج لقاحمركب لمرضى الحمى القلاعية ثلاثى العترة وحمى الوادى المتصدع كمحاولة لتحسين مناعة الحيوانات للقاح وذلك باستخدام مانتونيد أى.أس.أيه ٥٠ . بدأ مستوى الحماية المناعية للقاح الثلاثى للعترات الموجودة فى مصر(O - A &SAT2) بأستخدام مانتونيد أى.أس.أيه ٥٠ كعامل محفز فى الاسبوع الثالث للتحصين بلقاح الحمى القلاعية (١,٦٨-١,٦٥-١,٦٣) لو١٠ سجل اعلى مستوى مناعى فى الاسبوع العاشر من التحصين (٧,٢) لو١٠واستمر مستوى الحماية المناعية حتى الأسبوع ٣٢ للعترة سات ٢ وحتى الاسبوع ٣٦ من التحصين بالنسبة لعترتى اللقاح (O – A) .بينما حقق اللقاح المركب مستوى مناعي أفضل حيث إستمر حتى الأسبوع ٣٦للعترة سات ٢ من التحصين والأسبوع ٤٠ بالنسبة لعترتى اللقاح (O – A). كما أظهرت النتائج أن الأجسام المناعية وصلت لمستوى الحماية عند الأسبوع الثانى من التحصين وكانت(١,٨٧- ٢,.٧) لو١٠ بالنسبة للقاح حمى الوادى المتصدع الاحادى والمركب مع الحمى القلاعية على التوالى واستمر المستوى المناعى حتى الأسبوع ٣٦ للقاح حمى الوادى المتصدع وحتى الاسبوع ٤٠ بالنسبة للقاح المركبمن النتائج السابقة نستخلص ان اللقاح المركب حقق مستوى مناعى أفضل من اللقاح الأحادى.لذا نوصى بانتاج لقاح مركب للحمى القلاعية وحمى الوادى المتصدع لما حققه من مستوى مناعى أفضل معالوضع فى الإعتبار توفير الوقت والجهد فى عمليات التحصين .