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Efficacy of Inactivated Rift Valley Fever Vaccine Adjuvanted with Poly Lactic-Co-Glycolic Acid

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ABSTRACT

Rift Valley fever virus (RVFV) is a serious emerging pathogen affecting human and livestock in sub-Saharan Africa, Egypt, Yemen, and Saudi Arabia, Since the first description of an outbreak in Kenya 1931. Inactivated tissue culture adapted Rift valley fever (RVF) virus vaccines were prepared using poly (lactic-co-glycolic acid) PLGA in different ratio and aluminum hydroxide gel as adjuvants. The prepared vaccines were sterile and safe induced no systemic or local clinical signs in sheep. The comparative evaluation of prepared vaccines in vaccinated sheep after a single dose showed that PLGA prepared vaccines stimulated the cellular and humeral immune response as compared with aluminium hydroxide gel vaccine. Application of such vaccines will add value to improve the locally produced RVF vaccine, as poly (lactic-co-glycolic acid) 50:50 induced early immuno-response, poly (lactic-co-glycolic acid) 85:15 induce prolonged immuno-response and the mixture of poly (lactic-co-glycolic acid) 50:50, 85:15 in a ratio (1:1) induce early and prolonged immuno-response.

Keywords: *RVF, vaccine, SNT, ELISA, PLGA*

INTRODUCTION

Rift Valley fever virus, a Phlebovirus from the family *Bunyaviridae*, is potentially transmitted by different species of insect vectors that have a wide global distribution (Gubler, 2002). Infected animals develop necrotic hepatitis, hemorrhage, and abortion, with death rates up to 100% among newborn animals, and the disease is associated with symptoms ranging from uncomplicated acute febrile illness to retinitis, hepatitis, renal failure, severe hemorrhagic disease, and death (Bird et al., 2009).

The most effective method of RVF control is vaccination of susceptible animals. In Egypt, many trials for preparation of either live attenuated or inactivated vaccines were carried out beginning early with the first outbreak and extended until now to reach the safest and potent vaccine from the locally isolated strains (Abou-Elfadl, 2007). The most cost-effective way of controlling infectious diseases is through vaccination, even so it is difficult to deliver at least two to three doses of conventional vaccines for primary immunization to achieve protection. In this regard, aluminum hydroxide is most frequently used as an adjuvant in veterinary medicine (Clements and Griffiths, 2002). Also, PLGA is biodegradable in water by the hydrolysis of its ester linkages. The methyl-side groups in PLA make it more hydrophobic than PGA, therefore lactide rich PLGA copolymers are less hydrophilic, which absorb less water, and subsequently are slower in its degradation (Hirenkumar and Steven, 2011). Biodegradable polymer microspheres in recent years have received much attention for controlled release of antigens according to Gupta and Siber (1995), because it reduces the number of doses needed for primary immunization to as few as a single dose. It targets an antigen to an antigen-presenting cell after parenteral inoculations. It can modulate immune responses toward an antigen. Polymers can be easily coupled with

an immuno-modulator, antigen, and ligand easier physically or chemically. One of the advantages of the polymeric adjuvant that there are efficient delivery as well as protection against the degradation of antigens in vivo. The antigen may be directed to various cells in the immune system based on the microspheres size, the molecular weight of polymer, and the ratio of lactic to glycolic acid in the polymer, allowing the slow release of the antigen (Gupta et al., 1998). The microspheres composed of PLGA encapsulated antigens based on the size of a microsphere, molecular weight of the polymer, and the ratio of glycolic acid to lactic in the polymer. A variety of vaccine antigens have been encapsulated in microspheres which is composed of poly (lactic/glycolic) acid (PLGA). For the aforementioned reasons, the present study investigated the use of poly (lactic-co-glycolic acid) as an adjuvant with RVF virus inactivated vaccine to induce high prolonged potent immunity in vaccinated sheep.

MATERIAL AND METHODS

Virus strain

RVF virus (ZH501) Zagazig Human 501 strain was propagated in BHK-21 cell-line of a titer $10^{7.5}$ TCID 50/mL supplied by RVF Department, Veterinary Serum and Vaccine Research Institute (VSVRI). It was used for the preparation of inactivated vaccine, ELISA, and SNT.

Cell lines

Baby hamster kidney (BHK-21) cell line was used for propagation, titration of the virus, and testing the safety of prepared inactivated virus suspension **(Mackpherson and Stocker, 1962).**

Animals

Swiss mice, 3-4 weeks old. These mice were used in toxicity test to determine safe concentration of PLGA adjuvants.

Sheep and experimental design

Twenty-five healthy RAHAMANY breed sheep, 3-4-month-old, were used for evaluation of their immune response to the prepared vaccines. All of these animals were screened using SNT and proved to be free from RVF antibodies, the sheep were divided into 5 groups as follows: -

Group 1(G1): five animals, each vaccinated subcutaneously (S/C) with 1 mL of inactivated RVF-vaccine adjuvanted with poly (lactic-co-glycolic acid) 50:50, Group 2(G2): five animals, each vaccinated subcutaneously (S/C) with 1 mL of inactivated RVF-vaccine adjuvanted with poly (lactic-co-glycolic acid) 85:15, Group 3(G3): five animals, each vaccinated subcutaneously (S/C) with 1 mL of inactivated RVF-vaccine adjuvanted with a mixture of poly (lactic-co-glycolic acid) 50:50 and 85:15 in percent 1:1, Group 4(G4): five animals, each vaccinated subcutaneously (S/C) with 1 mL of inactivated aluminum hydroxide gel RVF-vaccine, and Group 5 (G5): five animals were kept as non-vaccinated (negative control).

Samples collection

All sera were collected from groups 1, 2, 3, 4, and 5 on the day of vaccination (zero-day), then weekly till the 4th-week post-vaccination and monthly till protective antibody level declined. The sera were inactivated at 56°C for 30 minutes and stored at -20°C before being examined by indirect enzyme-linked immunosorbent assay (ELISA) and the Serum Neutralization Test (SNT).

Blood samples were collected from vaccinated and non-vaccinated sheep with anticoagulant (Heparin 20-40 IU/ml) for the evaluation of cell-mediated immune response using lymphocyte blastogenesis assay and for the phagocytic activity test according to **Chang et al. (1996)** method.

Adjuvants

Poly (lactic-co-glycolic acid) was obtained from Seppic, Paris, France. Ratios was prepared according to **Sales-Junior et al. (2005).** Aluminum hydroxide gel was obtained from (Alliance Bio Company, USA).

Cell-mediated immune response

It was performed by measuring the lymphocyte blastogenesis using XTT tetrazolium salt assay, and phagocytic activity evaluation was done based to Scudiero et al. (1988) method.

Serum neutralization test (SNT)

It was achieved using the microtechnique as described formerly by Ramon (1925), to detect the specific neutralizing antibodies against the RVFV in the serum samples of vaccinated sheep.

Enzyme-linked immunosorbent assay (ELISA)

An indirect IgG ELISA assay was conducted to measure the antibody titers in sheep serum (Randall et al., 1964; OIE, 2016).

Statistical analysis

All experiments were conducted in triplicates, the obtained data were analyzed and graphically represented using the statistical package for social science using SPSS-21 software (2014) for obtained means and standard error. The data were analyzed using two-way ANOVA to determine the statistical significance of differences among groups and times. Duncan's test was used for making a multiple comparisons among the groups for testing the inter-grouping homogeneity.

RESULTS

Inactivation of virus

The tissue culture-adapted RVF virus (ZH501) was inactivated by using 1% of 0.1 M BEI at a final concentration of 0.001 M **(Eman, 1995).** It was observed that the infectivity of the virus was completely diminished after 24 h from treatment.

Sterility test

Vaccines were sterile as they were free from any fungal and bacterial contaminants.

Safety test of inactivated RVFV in baby mice

Inactivated vaccines were safe. No mortality occurred indicates optimum inactivation process.

Evaluation of cellular immune response by Lymphocyte blastogenesis

Cell proliferation was early high in G.1 expressed in optical density, but more strong in G.2 and (Table 1 and Fig. 1).

Phagocytic activity test

The phagocytic activity was expressed by phagocytic % as well as phagocytic index in different groups as shown in Tables (2) and (3), Figs. (2) and (3). Results revealed that there was early high macrophage activity in G.1 but stronger in G.2, G.3 and a nearly slight elevation in G.4

Humoral immune response in vaccinated sheep with inactivated RVF vaccines

Mean values of neutralizing titers of sheep

Table (4) and Fig. (4) showed that the mean neutralizing titer in G.1 reached above the protective level (40) at the 2nd-week post-vaccination and increased gradually till it reached the peak at the 3rd-month post-vaccination and still within the protective level till 9 months. The mean neutralizing titer in G.2 showed that was nearly about the protective at the 3rd-week post-vaccination and increased gradually till it reached the peak at the 5th-month post-vaccination and still within the protective level till 12 months, and the same was observed for G.3. It also showed that the mean neutralizing titers in sheep vaccinated with inactivated RVF vaccine (Aluminum hydroxide gel) in G.4 reached the protective level at the 2nd-week post-vaccination and increased gradually till it reached the peak at the 2nd-month post-vaccination then the level decreased and declined to a non-protective level at the end of 9th-month post-vaccination.

ELISA optical density in sheep vaccinated with different forms of inactivated RVF vaccines

Table (5) and Fig. (5), it was clear that the optical density started to appear at a positive level (cut off 0.175) in G.1 at the 2nd-week post-vaccination and was increased gradually till it reached the peak at the 3rd month post-vaccination and still in positive level till 9 months. The mean optical density in G.2 showed that was nearly about the positive level at the 3rd-week post-vaccination and was increased gradually till it reached the peak at the 5th-month post-vaccination and still within the positive level till 12 months. The same result was observed with G.3.

The optical density of sheep vaccinated with aluminum hydroxide gel inactivated RVF-vaccine G.4 started to show at the positive level at the 2nd-week post-vaccination, that reached the peak at the 2nd-month post-vaccination, and disappeared at the end of 9th-month post-vaccination. The previous data showed that there is a correlation between the results of SNT and ELISA tests which agrees with Eman (1995) and Hassan et al. (2001).

Table (1). Results of lymphocyte blastogenesis assay obtained in different sheep groups after vaccination with RVF prepared vaccines.

Animal	**Mean optical densities of cell proliferation assay**						
Groups	**Days post vaccination**						
	1	**3**	**5**	**7**	**10**	**15**	**21**
Gp. 1	f 0.140 BC 0.016	e 0.292 B 0.009	c0.498A 0.019	a0.721 A 0.014	b0.558 C 0.014	d0.365B 0.003	f0.161 B 0.11
Gp. 2	d 0.323 A 0.009	c 0.476 A 0.003	c 0.479 A 0.005	b 0.51 B 0.005	a0.735 B 0.006	c 0.490A 0.005	e0.208 A 0.002
Gp. 3	e0.157 B 0.003	d0.201 C 0.004	c0.352 B 0.006	b0.471 C 0.002	a0.765 A 0.002	b0.471A 0.001	f0.105 C 0.001
Gp. 4	a0.11 C 0.012	ab0.09 D 0.021	c0.065 C 0.025	a0.11 D 0.012	a0.12 D 0.010	a0.11C 0.012	ab0.08 D 0.006
Gp.5	de0.048 D 0.004	cd0.051 E 0.002	bc0.058 C 0.002	b0.064 E 0.002	a0.08 E 0.003	de0.044D 0.003	e0.04 E 0.002

Means ±SE in the same column and carrying different superscript are significant different at ($p<0.05$)
LSD at $p<0.05= 0.11$
A,B,C, mean ± SE in the same column (representing difference between groups)
a,b,c, mean ± SE in the same row (representing difference along experimental time)
G1: RVF vaccine with poly (lactic-co-glycolic acid) 50:50 adjuvant.
G2: RVF vaccine with poly (lactic-co-glycolic acid) 85:15 adjuvant.
G3: RVF vaccine with mixture of poly (lactic-co-glycolic acid) 50:50and 85:15 in ratio (1:1)
G4: RVF vaccine with alum. Hydroxide gel
G5: group of control non-vaccinated sheep

Table (2). Results of phagocytic percentage obtained in different sheep groups after vaccination with RVF prepared vaccines. Means + SE in the same column and carrying different superscript are significant different at ($p<0.05$).

Animal	**Phagocytic percentage (%)**						
Groups	**Days post vaccination**						
	1	**3**	**5**	**7**	**10**	**15**	**21**
Gp. 1	0 C 0	50 B 2.887	60 B 2.887	70 A 0.577	66.7 C 2.065	50 B 4.410	0 A 0
Gp. 2	50 A 2.887	66.7 A 2.137	70.8A 0.569	75 A 1.528	89.47 A 0.312	75 A 1.528	0 A 0
Gp. 3	25 B 1.000	66.7 A 2.139	68.3 A 0.208	75 A 3.606	80 B 2.887	60 B 2.082	0 A 0
Gp. 4	24 B 1.528	32 C 1.528	37 C 1.000	31 B 1.155	31 D 2.887	25 C. 1.155	0 A 0
Gp.5	21 B 1.732	18 D 1.155	19 D 1.732	20 C 2.309	17 E 2.309	19 C 2.333	0 A 0

LSD at $p<0.05= 6.82$
A,B,C mean ± SE in the same column (representing difference between groups)
G1: RVF vaccine with poly (lactic-co-glycolic acid) 50:50 adjuvant.
G2: RVF vaccine with poly (lactic-co-glycolic acid) 85:15 adjuvant.
G3: RVF vaccine with mix of poly (lactic-co-glycolic acid) 50:50and 85:15 (1:1) adjuvant.
G4: RVF vaccine with alum. Hydroxide gel.
G5: group of control non-vaccinated sheep.

Table (3). Results of phagocytic indices obtained in different sheep groups after single vaccination with RVF prepared vaccines.

Animal	**Phagocytic index**						
Groups	**Days post vaccination**						
	1	**3**	**5**	**7**	**10**	**15**	**21**
Gp. 1	d 0 D 0	b0.5 A 0.031	ab0.55 A 0.021	a0.6 A 0.015	b0.5 C 0.021	c0.33 C 0.021	d0 A 0
Gp. 2	e 0 D 0	d0.33 B 0.017	c0.45 AB 0.051	b0.58 A 0.010	a0.79 A 0.012	b0.63 A 0.038	e0 A 0
Gp. 3	d0.5 A 0.010	d0.5 A 0.017	c0.55 A 0.015	b0.6 A 0.015	a0.8 A 0.012	e0.4 BC 0.023	f0 A 0
Gp. 4	e0.2 B 0.012	d0.28 B 0.012	c0.35 B 0.038	bc0.41 B 0.023	a0.58 B 0.021	b0.45 B 0.015	f0 A 0
Gp.5	a0.13 C 0.036	a0.11 C 0.031	a0.11 C 0.021	a0.1 C 0.021	a0.12 D 0.006	a0.11 D 0.012	0 A 0

Means± SE in the same column and carrying different superscript are significant different at ($p<0.05$)
LSD at $p<0.05= 0.11$
A,B,C, mean ± SE in the same column (representing difference between groups)
a,b,c, mean ± SE in the same row (representing difference along experimental time)
G1: RVF vaccine with poly (lactic-co-glycolic acid) 50:50 adjuvant.
G2: RVF vaccine with poly (lactic-co-glycolic acid) 85:15 adjuvant.
G3: RVF vaccine with mix of poly (lactic-co-glycolic acid) 50:50and 85:15 (1:1) adjuvant.
G4: RVF vaccine with alum. Hydroxide gel.
G5: group of control non- vaccinated sheep.

Table (4). Mean values of neutralizing titers of sheep vaccinated with different forms of inactivated RVF vaccines

Animal group	Mean neutralizing titers at different period post vaccination															
	Before	Weeks post vaccination.				Months post vaccination										
		1st w.	2nd w.	3rd w.	4th w.	2nd m.	3rd m.	4th m.	5th m.	6th m.	7th m.	8th m.	9th m.	10th m.	11 th m.	12 th m.	13th m.
G 1	0	fgh 16 B ±0	efgh 42.67 A ±10.67	cde 64 A ±0	de 74.67 B ±28.22	c 128 AB ±0	a 256 A ±0	b 204.8 A ±42.67	c 128 B ±0	cd 102.4 A ±21.33	cde 64 B ±0	efg 51.2 B ±10.67	efgh 42.6 AB ±10.67	efgh 32 BC ±0	fgh 16 B ±0	gh 8 B ±0	h 2 B ±0
G 2	0	f 8 C ±0	ef 32 E ±0	ve 53.33 E ±10.67	cde 64 B ±0	cde 74.67 BC ±28.22	bcd 85.33 B ±21.33	b 128 B ±0	a 256 A ±0	b 204.8 A ±0	b 128 A ±0	bc 102.4 A ±21.33	bcd 85.3 A ±21.33	cde 76.8 A ±28.22	cde 64 A ±0	de 51.2 A ±10.67	ef 32 A ±0
G 3	0	e 32 A ±0	de 51.2 A ±10.67	de 53.3 A ±10.67	de 64 B ±0	cde 85.3 B ±21.33	bc 128 B ±0	b 170.6 B ±42.67	a 256 A ±0	bc 128 A ±0	bc 128 A ±0	cd 102.4 A ±21.33	cde 76.8 A ±28.22	64 AB ±0	cde 51.2 A ±21.33	de 42.6 A ±10.67	e 32 A ±0
G 4	0	e 4 D ±0	cd 42.6 A ±10.67	c 64 A ±0	b 128 A ±0	a 170.6 A ±42.67	b 128 B ±0	c 64 C ±0	c 64 C ±0	cd 51.3 B ±10.67	cd 46.6 C ±10.67	cde 42.8 BC ±0	cde 40.3 AB ±0	de 32 C ±0	e 4 B ±0	e 2 B ±0	e 0 B ±0
G5	0	d 0 E ±0	d 0 B ±0	d 2 B ±0	c 2 C ±0	b 4 C ±0.67	b 4 C ±0	c 2 D ±0	a 8 D ±0	b 4 C ±0	c 4 D ±0.67	d 2 C ±0	d 2 C ±0	d 4 C ±0	d 2 B ±0	d 0 B ±0	d 0 B ±0

Means + SE in the same column and raw carrying different superscript are significant different at ($P < 0.05$)
LSD at $p<0.05$ =48
A,B,C mean ± SE in the same column (representing difference between groups)
a,b,c mean ± SE in the same row (representing difference along experimental time)
G1: RVF vaccine with poly (lactic-co-glycolic acid) 50:50 adjuvant.
G2: RVF vaccine with poly (lactic-co-glycolic acid) 85:15 adjuvant.
G3: RVF vaccine with mix of poly (lactic-co-glycolic acid) 50:50and 85:15 (1:1) adjuvant.
G4: RVF vaccine with alum. Hydroxide gel.
G5: group of control non- vaccinated sheep.

Table (5). ELISA optical density in sheep vaccinated with different forms of inactivated RVF vaccines.

Animal group	Mean values of ELISA optical density indices at different period post vaccination															
	Before	Weeks post vaccination.				Months post vaccination										
		1st w.	2nd w.	3rd w.	4th w.	2nd m.	3rd m.	4th m.	5th m.	6th m.	7th m.	8th m.	9th m.	10th m.	11 th m.	12 th m.	13th m.
G 1	0.042	fg 0.096 A ±0.002	c 0.176 B ±0.004	cd 0.178 C ±0.004	c 0.185 C ±0.003	b 0.268 B ±0.006	a 0.309 A ±0.006	c 0.201 C ±0.004	bc 0.192 C ±0.003	cd 0.188 C ±0.004	de 0.186 C ±0.004	e 0.18 B ±0.004	ef 0.178C ±0.004	g 0.159 C ±0.005	h 0.124 C ±0.02	gh 0.098 C ±0.002	h 0.038 C ±0.002
G 2	0.053	gh 0.066 C ±0.004	g 0.145 C ±0.003	fg 0.178 B ±0.004	g 0.19 A ±0.003	f 0.208 B ±0.006	e 0.224 B ±0.006	b 0.261 B ±0.006	a 0.329 A ±0.005	b 0.267 B ±0.006	f 0.209 B ±0.008	g 0.198 B ±0.005	f 0.185 B ±0.004	f 0.182 A ±0.004	g 0.179 A ±0.003	h 0.175 B ±0.003	gh 0.169 A ±0.002
G 3	0.049	d 0.094B ±0.002	c 0.179 B ±0.004	bc 0.182 B ±0.004	b 0.188 B ±0.004	b 0.198 C ±0.005	ab 0.201 A ±0.005	b 0.208 A ±0.005	a 0.352 A ±0.006	b 0.287 A ±0.006	bc 0.229 A ±0.006	c 0.207 A ±0.005	d 0.185 A ±0.004	e 0.179 B ±0.004	e 0.178 B ±0.004	de 0.176 A ±0.004	f 0.098 B ±0.02
G 4	0.041	gh 0.047 C ±0.002	d 0.175 B ±0.004	de 0.182C ±0.004	d 0.187 C ±0.004	a 0.214 A ±0.006	b 0.208 C ±0.007	de 0.189 D ±0.007	d 0.189 D ±0.007	d 0.187 D ±0.008	e 0.178 D ±0.008	f 0.121 C ±0.004	g 0.098 D ±0.004	h 0.043 D ±0.005	gh 0.041 D ±0.005	h 0.038 D ±0.005	h 0.021 D ±0.004
G5	0.001	g 0.009 D ±0.002	gh0.0017E ±0.002	f 0.0033 E ±0.002	ef 0.004 E v0.003	e 0.0049 E v0.004	a 0.0057 E ±0.004	b 0.0065 E ±0.003	ab 0.0048 E ±0.003	b 0.0047 E ±0.004	b 0.0047 E ±0.004	a 0.0056 E ±0.005	f 0.003 E ±0.002	fg 0.0021 E ±0.002	f 0.0019 E ±0.002	f 0.0013 E±0.002	0 E ±0

Means + SE in the same column and raw carrying different superscript are significant different at ($P < 0.05$)
LSD at $p<0.05=0.02$
A,B,C mean ± SE in the same column (representing difference between groups)
a,b,c mean ± SE in the same row (representing difference along experimental time)
G1: RVF vaccine with poly (lactic-co-glycolic acid) 50:50 adjuvant.
G2: RVF vaccine with poly (lactic-co-glycolic acid) 85:15 adjuvant.
G3: RVF vaccine with mix of poly (lactic-co-glycolic acid) 50:50and 85:15 (1:1) adjuvant.
G4: RVF vaccine with alum. Hydroxide gel.
G5: group of control non- vaccinated sheep. (Cut off value =0.175)

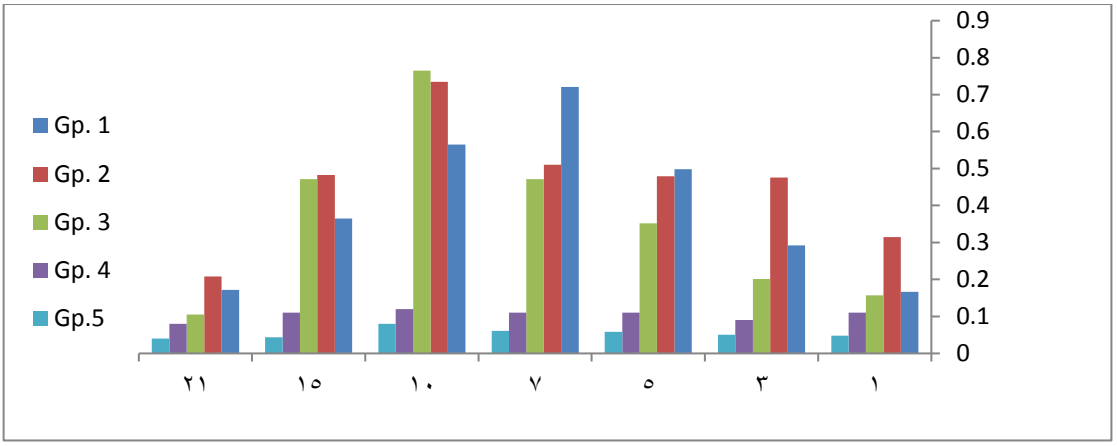

Figure (1). Results of lymphocyte blastogenesis assay obtained in different sheep groups after vaccination with RVF prepared vaccines.

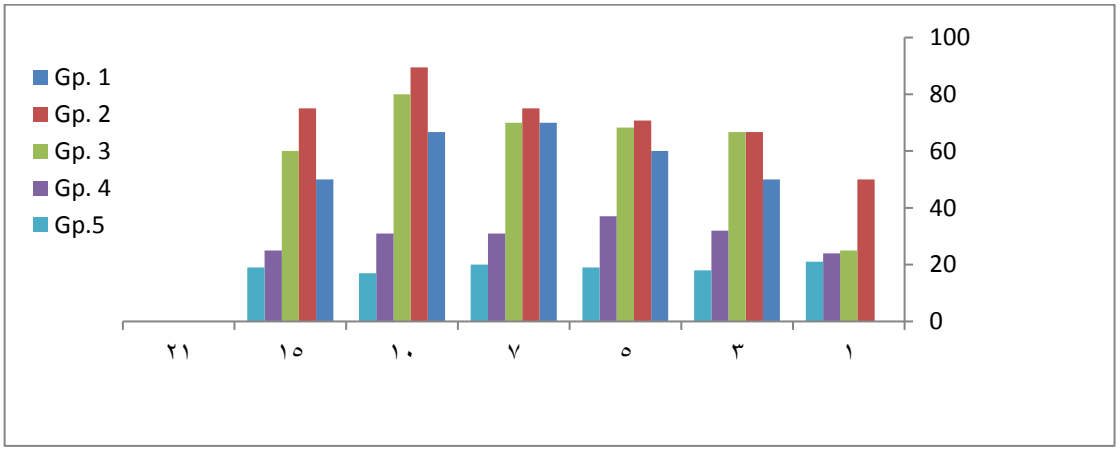

Figure (2). Results of phagocytic percentage obtained in different sheep groups after single vaccination with RVF prepared vaccines.

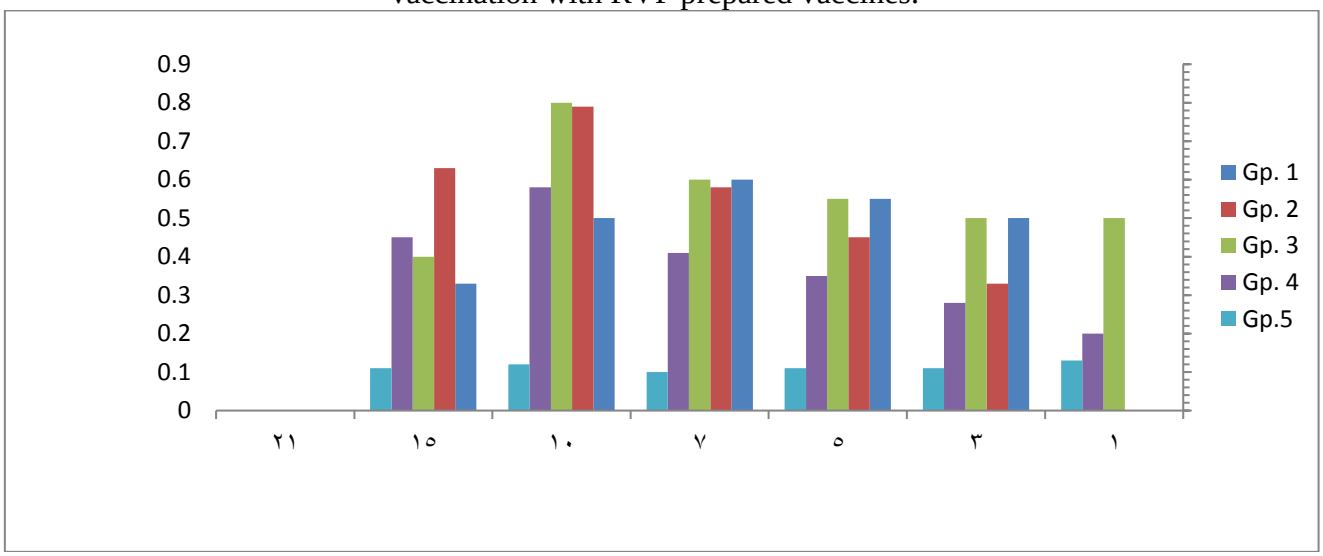

Figure (3). Results of phagocytic indices obtained in different sheep groups after single vaccination with RVF prepared vaccines.

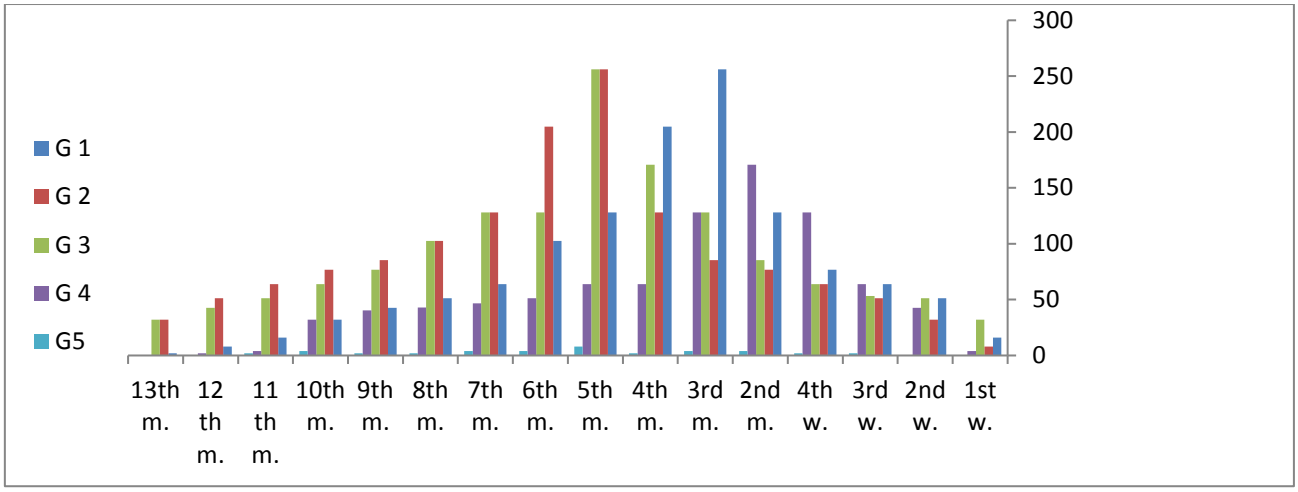

Figure (4). Mean values of neutralizing titers of sheep vaccinated with different forms of inactivated RVF vaccines:

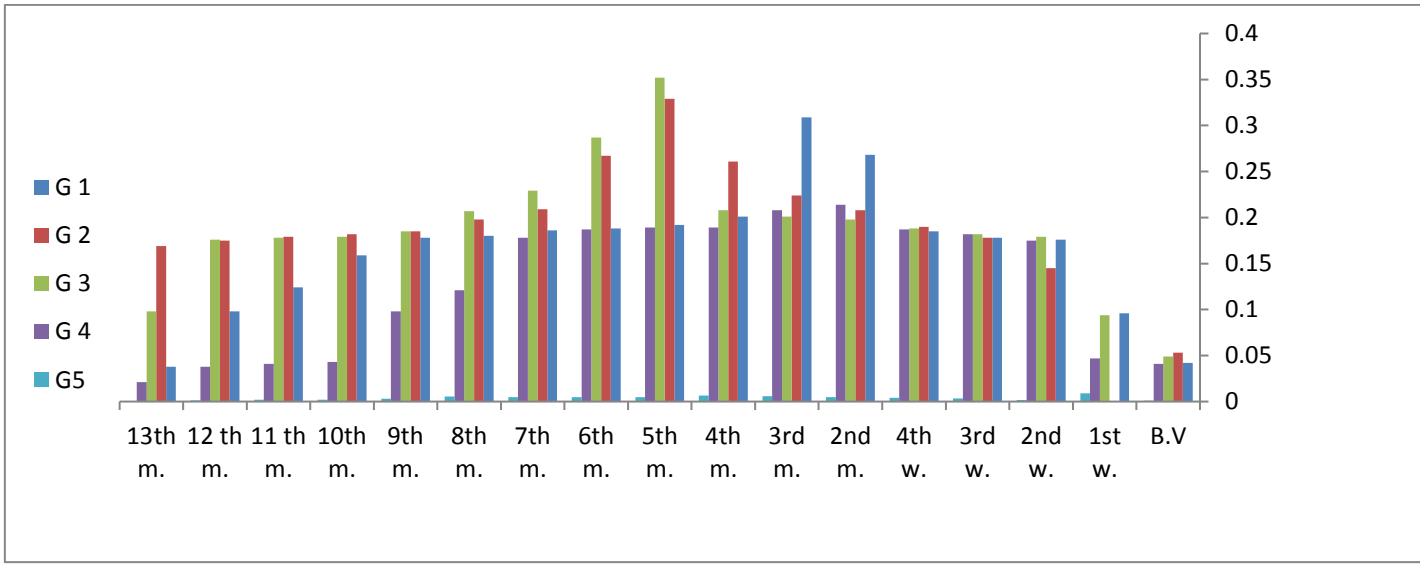

Figure (5). Mean values of ELISA optical density in sheep vaccinated with different forms of inactivated RVF vaccines

G1: RVF vaccine with poly (lactic-co-glycolic acid) 50:50 adjuvant.
G2: RVF vaccine with poly (lactic-co-glycolic acid) 85:15 adjuvant.
G3: RVF vaccine with mix of poly (lactic-co-glycolic acid) 50:50and 85:15 (1:1) adjuvant.
G4: RVF vaccine with alum. hydroxide gel.
G5: group of control non-vaccinated sheep.

DISCUSSION

Clinical examination of sheep vaccinated with the four different forms of inactivated RVF vaccine (Aluminum hydroxide gel PLGA50:50, PLGA85:15 and the mixture of PLGA 50:50,85:15 in a ratio (1:1) revealed no detectable signs of illness or local reaction at the site of injection over the investigation period. Likewise, Eman, (1995), El Nimr, (1980) and Hassan, (1998) reported that the inactivated RVF vaccine induced no adverse post-vaccinal reaction in inoculated animals.

The cell-mediated immune responses of the four vaccines were evaluated as follow: Cell proliferation was expressed by optical density. It was an early high significant value in sheep vaccinated with the inactivated RVFV adjuvanted with PLGA 85:15 and mixture of PLGA 85:15 and 50:50 in ratio 1:1; more than the inactivated RVFV adjuvanted with PLGA 50:50. Slight elevation was observed in sheep vaccinated with the local inactivated aluminum hydroxide gel-based RVF vaccine. The same results were obtained by both Lily, (1991) and Eman, (1995) they recorded the T-cell response in sheep vaccinated with the local inactivated aluminum hydroxide gel-based RVF vaccine 24-48 h post-vaccination and declined after the 7 dpv. to be very low till 21 dpv.

The phagocytic activity was expressed by phagocytic % and phagocytic indices in the four different groups. Early significant-high macrophage activity was observed in sheep vaccinated with the inactivated RVFV adjuvanted with PLGA 85:15 and mixture of PLGA 85:15 and 50:50 in ratio 1:1 more than

the inactivated RVFV adjuvanted with PLGA 50:50. While non-significant values was observed in sheep vaccinated with the local inactivated aluminum hydroxide gel-based RVF vaccine.

Evaluation of humoral immune response in vaccinated sheep was studied by SNT which showed that mean neutralizing titer (NI) in vaccinated sheep sera started to rise from 1st-week post-vaccination and increased to the protective level at the 2nd-week post-vaccination by the local inactivated RVF-vaccines with aluminum hydroxide gel adjuvant. These results are in agreement with El Nimr, (1980), Gihan, (1990) and Eman, (1995), who reported that the protective NI level obtained by the inactivated vaccines was 2 weeks' post-vaccination

The mean neutralizing titer in sera of sheep vaccinated with the inactivated PLGA 50:50 based vaccine reaches the protective level at the 2nd week and increased gradually till it reached the peak at the 3rd-month post-vaccination then the level decreased at the 9th-month post-vaccination then declined to a non-protective level, the mean neutralizing titer in sera of sheep vaccinated with the inactivated PLGA 85:15 based vaccine reach the protective level at 3rd week and was increased gradually till it reached the peak at the 5th-month post-vaccination then the level decreased at the 12th-month post-vaccination then declined to a non-protective level and the mean neutralizing titer in sera of sheep vaccinated with the inactivated PLGA mixture of 50:50, 85:15 (1:1) based vaccine reach the protective level at 3rd week and increased gradually till it reached the peak at the 5th-month post-vaccination then the level decreased at the 12th-month post-vaccination then declined to a non-protective level. The extended effect of inactivated PLGA mixture of 50:50, 85:15(1:1) based vaccine may be due to that PLGA is biodegradable in water by hydrolysis of its ester linkages. The presence of methyl-side groups in PLA makes it more hydrophobic than the PGA, therefore lactide rich PLGA copolymers are less hydrophilic, can absorb less water, and subsequently slower in degradability (Hirenkumar and Steven, 2011)

In sera of sheep vaccinated with the inactivated aluminum hydroxide gel-based vaccine, the mean neutralizing titer reached the peak at the 2nd-month post-vaccination then the level decreased at the 9th-month post-vaccination and then decline to a non-protective level. These results come in agreement with Sales-Junior et al. (2005) who reported that the W/O/W PLGA, elicited a superior immune response than the aluminum hydroxide gel vaccine, and the immune response development was quicker. The result of ELISA confirms that obtained by SNT. Similar results were obtained by Paweska, et al. (2005), Catherine et al., (2009) and Ali, et al., (2012) who used ELISA for the detection of IgG instead of SNT.

The results of the present study document the immunoenhancing effects of PLGA as promising adjuvant candidates towards promoting both humoral and cellular responses. The PLGA 50:50, PLGA 85:15, and mixture of PLGA 50:50, 85:15(1:1) based vaccine induced high potent immunological response including cellular and humeral immunity with longer duration extended for 9 months, 12 month and 12 months, respectively without toxicity. And with high significant statistical values in in the lymphocyte blastogenesis assay, phagocytic percentage, phagocytic indices, ELISA optical density assay, and neutralizing titers which appeared in sheep vaccination with RVF prepared vaccines containing PLGA 85:15 and PLGA 50:50, 85:15 in a ratio (1:1) as adjuvant than the sheep vaccination with RVF prepared vaccines with aluminum hydroxide gel only as adjuvant.

CONCLUSIONS

The results of the present study document the immuno-enhancing effects of PLGA as promising adjuvant candidates towards promoting both humoral and cellular responses. These vaccines will add value to improve the locally produced RVF vaccine, as PLGA 50:50 induces early immuno-response, PLGA 85:15 induces prolonged immuno-response, and the mixture of PLGA 50:50, 85:15 in a ratio (1:1) induce early and prolonged immuno-response. With high significant statistical values for G.2 and G.3 in the lymphocyte blastogenesis assay, phagocytic percentage, phagocytic indices, ELISA optical density assay and neutralizing titers which appeared in sheep vaccination with RVF prepared vaccines contains PLGA 85:15 and PLGA 50:50, 85:15 in a ratio (1:1) as adjuvant than the sheep vaccination with RVF prepared vaccines with aluminum hydroxide gel only as adjuvant.

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