

VACCINATION TRIAL IN CATTLE USING MP12 LIVE ATTENUATED RIFT VALLEY FEVER VIRUS

Marwa F.M. Abdel Monem; Gihan K. Mohammed;** Sami A.M.;
and Saber M.S.*

The present study was aimed to evaluate the live mutagenized RVF-MP12 vaccine in 4 months old calves. The used dose was 5 log₁₀ TCID₅₀/animal. The safety and potency of the vaccine was followed up as well as the duration of the immunity by ELISA and SNT through 12 months post vaccination. There was no evidence of untoward effects except slight elevation of body temperature in the calves through 14 days post vaccination. The virus was recovered from the sera of 3 calves on the 2nd, 3rd and 4th day post vaccination respectively 82,78 and 76, but there was no evidence to virus shedding in ocular, nasal nor rectal swabs. The immunity was detected as early as 7 days post vaccination to reach its peak by the 6th month and still in a protective level only 10th month post vaccination.

Key words:

*Faculty of Veterinary Medicine, Cairo University
**Veterinary Serum and Vaccine Research Institute.
P.O.Box 131, Abbasia, Cairo, Egypt.

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INTRODUCTION

Rift Valley Fever (RVF) is an acute arthropod born virus affecting many species including sheep and cattle with abortion in the pregnant and high mortality in the new born lambs as well as influenza like illness in human beings (Gerdes, 2002). The first isolation of RVFV among ewes and lambs was recovered in Kenya during 1930 (Daubney *et al.*,1931). In Egypt, RVFV was recorded for the first time in 1977-1978 with high human mortality and 25-50% of sheep and cattle were infected (Meegan,1979). The re-occurrence of RVF was reported in 1993 among animals as well as human beings in Aswan Governorate in May 1993 (El-Gabery *et al.*,1994).

RVF was controlled in Egypt by formalin-inactivated tissue culture vaccine (killed ZH₅₀₁) (Abd El-Ghaffar *et al.*, 1981). To avoid the multiple dose and slow onset of immunity with inactivated vaccine and abortion in lambs by Smithburn vaccine (Smithburn,1949; Weiss,1962 and El-Sawalhy *et al.*, 1997). This study was carried out on the mutagenized MP12 which induced rapid onset of immunity in 6-7

days with long duration for not less than 7 months with only one dose as well as no shedding in the colostrums (Morrill *et al.*, 1997a). It was immunogenic, non teratogenic and non abortogenic (Morrill *et al.*,1997a and morrill *et al.*,1997b). There was no evidence to reversion to virulent state (Saluzzo and Smith,1990 and Turell and Rossi,1991).

The aim of this work is to study of the immunogenicity of the live attenuated RVF-MP12 in Calves.

MATERIALS AND METHODS

1- Animals :-

Eight-cross breed four month-old calves were vaccinated with MP12 and other one calve were considered as control. They were tested to antibodies against RVF virus before vaccination by SNT and ELISA. Serum samples were collected on 7,14,21,28,60 days post vaccination then every month till 12 months.

2-Vaccine :-

Attenuated RVF virus MP12 vaccine strain ZH₅₄₈ prepared in Medical Council Research-5 (MCR-5) cell culture with a titer of 5 log₁₀ TCID₅₀ /ml was kindly supplied by NAMRU-3. It was used for experimental vaccination of calves using a dose of 10⁵ TCID₅₀ / animal inoculation S/C at the side of neck .

3- Virus :-

RVF virus strain ZH₅₀₁ of a titer 7.5 log₁₀ TCID₅₀ / ml was obtained from RVF Department, Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo. It was used in SNT and preparation of RVFV antigen for ELISA.

4-VERO cell culture:-

African green monkey kidney cell culture (VERO) was supplied by the Dep. Of Pet Animal Vaccine Research, Veterinary Serum and Vaccine Research Institute. These cells were used in SNT in trials of virus recovery.

5- Serum neutralization test (SNT) :-

It was performed according to (OIE Manual 1996).

6- Preparation of RVFV antigen

It was prepared according to (Elian and Botros 1997) with a final dilution 1:200

7- Conjugate :-

Antibovine IgG conjugate with horse radish peroxidase with dilution 1:91,000 was supplied by MP Biomedical, LLC and used in ELISA.

8- Indirect Enzyme-Linked Immunosorbent Assay (ELISA):-

It was carried according to (Voller et al., 1976)

RESULTS

1- Safety test of MP-12 RVF vaccine:-

It was found that vaccination of calves with the live attenuated MP12 RVF vaccine did not induce any abnormal signs, where there were no detectable local post injection signs. Also, the vaccinated animals did not show any clinical abnormalities and still healthy allover a period of 15 days post vaccination. Slight rise in body temperature was noticed on the 2nd day and till the end of observation

period. These results are tabulated in table (1) and demonstrated in figure (1).

2- Recovery of RVF virus from vaccinated calves:-

Trials of RVF Virus recovery from vaccinated calves with MP-12 vaccine were carried out on 28 samples. 4 samples of each serum, nasal, ocular and rectal swabs were taken daily from each calf all over a period of 7 days post vaccination. These trials were carried out through the infection of VERO cell culture and the presence of RVF virus was detected by the demonstration of its characterized CPE. It was found that RVF virus was detectable in serum samples obtained from the calf No. 82 on the 2nd day, from calf No. 78 on the 3rd day and from calf No. 76 on the 4th day. post vaccination. It was failed to recover the virus from nasal, ocular and rectal swabs all over the experimental period. These results are shown in table (2 & 3).

3- Potency test of live attenuated RVF-MP12 vaccine:-

The potency of RVF-MP12 vaccine was tested and the level of the induced immunity was followed up to 12 months post vaccination using SNT and indirect ELISA.

3.1. Serum neutralization test (SNT):-

SNT revealed that RVF MP12 live attenuated vaccine was able to induce detectable RVF serum neutralizing antibodies by the 1st week post vaccination in vaccinated calves with a mean titer of 4.5. These antibodies were reached the protective level at the 2nd month with a mean titer 64 and reached their peak titer by the 6th month post vaccination with a mean titer of 184. It was found that the induced neutralizing RVF antibodies were stable up to the 10th month then began to decrease gradually by the 12th month post vaccination to reach their lowest titer (7.5) by the 12th month post vaccination. The results are in details in table (4) and fig.(2). It was recommended that the protective titer of RVF neutralizing antibodies should not be less than >40.

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Table (1) RVF virus recovery from different vaccinated calves through 7 days post vaccination

Animal number	No. of examined Samples	Rate of samples showing CPE * in VERO cells			
		Serum	Nasal	Ocular	Rectal
		No.	No.	No.	No.
76	28	1/7	0/7	0/7	0/7
77	28	0/7	0/7	0/7	0/7
78	28	1/7	0/7	0/7	0/7
79	28	0/7	0/7	0/7	0/7
80	28	0/7	0/7	0/7	0/7
81	28	0/7	0/7	0/7	0/7
82	28	1/7	0/7	0/7	0/7
83	28	0/7	0/7	0/7	0/7
Control	28	0/7	0/7	0/7	0/7

* CPE= detection of RVF virus

Table (2) RVF virus recovery in the serum of vaccinated calves through 7 days post vaccination

Animal number	Days post vaccination						
	1st	2nd	3rd	4th	5th	6th	7th
76	(-ve)	(-ve)	(-ve)	(+ve)	(-ve)	(-ve)	(-ve)
77	(-ve)	(-ve)	(-ve)	(-ve)	(-ve)	(-ve)	(-ve)
78	(-ve)	(-ve)	+ve) ((-ve)	(-ve)	(-ve)	(-ve)
79	(-ve)	(-ve)	(-ve)	(-ve)	(-ve)	(-ve)	(-ve)
80	(-ve)	(-ve)	(-ve)	(-ve)	(-ve)	(-ve)	(-ve)
81	(-ve)	(-ve)	(-ve)	(-ve)	(-ve)	(-ve)	(-ve)
82	(-ve)	+ve) ((-ve)	(-ve)	(-ve)	(-ve)	(-ve)
83	(-ve)	(-ve)	(-ve)	(-ve)	(-ve)	(-ve)	(-ve)
Control	(-ve)	(-ve)	(-ve)	(-ve)	(-ve)	(-ve)	(-ve)

Number of animals showing viremia 3/8 = 37.5%

N.B. The obtained virus titer = $2.\log_{10}$ TCID₅₀/ml

Table (3) RVF neutralizing antibody titers in vaccinated calves with MP12 vaccine

AN.NO.*	RVF neutralizing antibody titers** post vaccination with RVF MP12 vaccine															
	Pre-v.***	1W PVo	2W PV	3W PV	4W PV	2M PV#	3M PV	4M PV	5M PV	6M PV	7M PV	8M PV	9M PV	10M PV	11M PV	12M PV
76	0	4	4	32	32	64	64	128	128	256	128	64	64	64	16	8
77	0	8	8	32	32	32	64	64	128	256	256	128	64	64	32	8
78	0	4	4	16	32	32	64	128	128	256	256	128	64	32	32	4
79	0	4	8	16	16	64	64	64	256	256	128	128	128	128	8	8
80	0	2	2	16	16	32	64	256	256	128	128	64	32	32	8	4
81	0	2	16	32	32	32	32	64	128	128	128	256	64	32	16	16
82	0	8	16	32	32	128	128	256	256	64	64	64	64	64	32	8
83	0	4	4	32	32	128	128	128	128	128	128	128	128	128	16	4
Mean	0	4.5	7.7	26	28	64	76	136	176	184	152	120	76	68	20	7.5
Control	0	0	0	2	2	4	2	4	4	2	8	4	4	2	2	4

** RVF neutralizing antibody titer = The reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100 TCID_{50%} of the virus.

*** Pre-v. = Pre-vaccination oWPV = Week post vaccination # MPV = Month post vaccination
 * N.B.: The protective level of RVF neutralizing antibody titer is >40 * AN.NO. = Animal Number

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Table (4) RVF ELISA antibody values in vaccinated calves with MP12 vaccine

An.No.*	RVF ELISA antibody values post vaccination with RVF MP12 vaccine																							
	Pre-v. **	1W PVo	2W PV	3W PV	4W PV	2M PV#	3M PV	4M PV	5M PV	6M PV	7M PV	8M PV	9M PV	10M PV	11M PV	12M PV								
76	0.03	1.2	1.61	1.9	2	2.2	2.37	2.45	2.62	2.87	2.45	2.5	2.5	2.5	2.5	2.34								
77	0.02	0.86	1.24	1.5	2.0	2	2.2	2.45	2.5	2.45	2	1.89	1.58	1.67	2.24	1.65								
78	0.04	1.0	1.3	1.2	1.5	1.87	2	2.1	2.3	2.6	2.0	1.89	1.48	1.58	1.98	1.34								
79	0.03	0.95	1.2	1.4	1.66	1.98	2.1	2.3	2.4	2.7	2.5	1.89	1.85	1.83	1.98	1.56								
80	0.03	1.0	1.23	1.2	1.12	1.43	1.87	1.98	2.35	2.5	2.4	2.3	1.61	1.91	1.92	1.43								
81	0.04	0.85	1.01	1.2	1.5	1.87	1.98	2.1	2.5	2.1	2	1.87	1.56	1.94	1.24	1.72								
82	0.02	0.76	0.95	1.04	1.75	1.9	2	2.1	2.2	2.5	2.5	2.1	1.98	1.5	1.43	1.23								
83	0.02	0.85	1.0	1.14	1.14	1.41	1.6	1.89	2.16	2.6	2.4	2	1.95	1.5	1.2	1.1								
Mean	0.03	0.93	1.29	1.32	1.58	1.83	2.01	2.17	2.38	2.54	2.28	2.06	1.81	1.76	1.68	1.55								
Control	0.01	0.03	0.01	0.02	0.02	0.01	0.04	0.02	0.01	0.03	0.03	0.01	0.02	0.04	0.03	0.01								

** Pre-v. = Pre-vaccination
 #MPV = Month post vaccination
 N.B. :- C.O. = 1.45

* AN.NO. = Animal Number
 o WPV = Week post vaccination

3.2. Indirect ELISA :

The Indirect ELISA applied on serum samples obtained from vaccinated calves showed that such animals exhibited protective levels of RVF/ ELISA antibodies starting from the 1st week post vaccination with a mean absorbance value of 0.93. This titer was increased gradually recording a peak value of 2.5 by 6th month and still with in high levels till the end of study as shown in table (5) and fig.(3). From table (4) and (5) it seems that the results of SNT were parallel to these of the indirect ELISA confirming each other and showing that the live RVF MP12 vaccine is protect calves up to 10 months post vaccination.

DISCUSSION

The experimental results showed that RVF ZH₅₄₈ MP12 vaccine did not show any untoward effects except slight elevation in body temperature (39.5 : 40°C) in the calves. These findings agree with Hubbard *et al.*,(1991), Morrill *et al.*,(1991), Baskerville *et al.*,(1992), Morrill *et al.*,(1997a) and Morrill and Peters (2003) and disagree with Vialat *et al.*,(2000) who showed

that MP12 has a significant pathogenic effect in cattle

Low virus titer was detected in serum samples of febrile and non febrile calves with a titer 2 log₁₀ TCID₅₀ / ml from 3 vaccinated calves number 76,78 and 82 respectively on 2nd, 3rd and 4th day (table 1& 2) in agreement with Morrill *et al.*,(1997a), Morrill *et al.*,(1997b) and Morrill and Peter (2003). The virus was not shed from rectal, nasal nor ocular swabs.

The immunity was recorded as early as 7 days post vaccination to reach a protective level at the 2nd month and reach the peak at the 6th month then still in such protective level till 10th month only (table 3). These finding agree with those Hubbard *et al.*, (1991), Morrill *et al.*,(1991), Morrill *et al.*,(1997a) and Morrill *et al.*,(1997b).

Regarding the results of ELISA, it was found that they were parallel to those of serum neutralization antibodies (table 4).

Challenge test of vaccinated animals did not carry out to avoid the public health hazard and the vaccine evaluation was depending on both of safety and potency tests which showed that such vaccine is safe and immunogenic and could be used to protect cattle against

RVF to a period not more than 10 months .

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