j.Egypt.vet.med.Assoc 77, no 4. 903 – 910 (2017)

TRIALS FOR LOCAL PRODUCTION OF RIFT VALLEY FEVER VACCINE FROM INACTIVATED SMITHBURN STRAIN

By

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ABTRACT

The vaccination campaign (using life Smithburn vaccine) performed during the outbreaks of 1996,1997 and 2003 did not stop the disease but it causes viremia in vaccinated animals which explain the fear of spread of the virus via mosquito's vectors and the concern about possible reasserting event with wild type viruses. The Egyptian GOVS in the 2008 demonstrated that (Rift Valley Fever) live vaccine (Smithburn strain) isn't used in Arab Repuplic of Egypt in the present time. Consequently, the RVF vaccination programs in Egypt are performed by inactivated (ZH-501) RVF vaccine. In this study Inactivated smithburn RVF vaccine was prepared and evaluated by serological tests (SNT and ELISA), compared with local vaccine. Results indicated that Smithburn RVF vaccine was more protective, induced high titer of antibodies. Furthermore, time of protection is longer than the local vaccine.

Keywords:

RVF, Vaccine, Evaluation, Smithburn, ZH-501.

INTRODUCTION

Rift Valley fever (RVF) is a vector-borne viral disease caused by RVF virus, a member of the Bunyaviridae family and Phlebovirus genus that primarily affects domestic ruminants, causing large epizootics with high mortality rates in young animals and abortions in affected female animals. **(Shabani *et al.,* 2015)**. Egypt is the most northern, and populous country to have suffered from RVF and the human illness and death during 1977-1978 epizootic. Since then, RVF outbreaks in Egypt have occurred in 1993, 1999, and most recently, 2003. In most cases these 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 animals into Egypt from the south. **(Hanafi *et al.,* 2011)**. Smithburn vaccine was named A modified live virus vaccine

(MLVV) was produced in 1971 by amplifying the Smithburn strain-derived viruses. It was used in African countries including South Africa, Kenya and Egypt. Athough the successes seen with the Smith-burn vaccine, problems regarding residual pathogenicity have been reported, and furthermore the potential for teratogenic and abortifacient effects following administration have been observed. This was reported by **Botros *et al.*, 2006** who said that administration of the vaccine to European breeds of cow (n = 100) led to abortion in 29 % of pregnant animals. The use of such vaccine during an outbreak may lead to the vaccination of a viraemic animal, providing an opportunity for reassortment between wild and vaccine viruses, which has the potential to increase the diversity of circulating RVFV strains **(Ikegami *et al.*, 2015).**The Egyptian GOVS in the 2008 demonstrated that RVF live vaccine (smithburn strain) doesn't used in Arab Repuplic of Egypt in the present time consequently. The RVF vaccination programs in Egypt are performed by the killed vaccine only. So in this study inactivated smithburn strain will used for production of anew inactivated smithburn rift valley fever vaccine**.**

MATERIAL AND METHODS

1. Virus:

1.1. Attenuated RVFV:

The Smithburn neurotropic strain which had been passaged for 104 passages in mice brain and 5 passages in tissue culture, and has final titer of $10^{7.5}$ MIPD50 / ml. It was used for preparation of the inactivated smithburn RVF vaccine.

1.2. Virulent RVFV (ZH-501) :

The original virus was that isolated from a human patient in Zagazig, Sharquia provinces. It was twice passaged i/c into suckling mice and has a final titer of $10^{7.5}$ MIPD50 / ml.

1.3. Tissue Culture Cells:

Baby Hamster Kidney cells (BHK_{21}). The cells were grown and maintained according to **Macpherson and Stocker (1962)**. The cells were used for propagation, titration of RVF virus, vaccine production and evaluation.

2. Experimental Animals:

2.1 Mice:

2.1.1Baby mice:

Groups of 3 5 days old suckling mice were used for testing safety of the inactivated smithburn RVF virus.

2.1.2Adult mice:

Groups of weaned mice, 21days old were used for titration of (ZH-501) virus, and testing the potecy of the prepared inactivated Smithburn RVF vaccine.

2.2. Sheep:

Eeleven adult local breed sheep 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. They were housed under strict hygienic measures in insect proof stables receiving balanced ration and adequate water. Also these animals were examined and proved to be apparently healthy and free from external and internal parasites.

2.3. Lambs:

Lambs (2-3) weeks old were used for safety test of the prepared inactivated smithburn RVF. vaccine.

4. Adjuvants:

4.1. Aluminum hydroxide gel:

The gel obtained from (Alliance Bio Company, USA), and used in concentration 15% for local vaccine preparation.

5. Conjugate:

Antisheep horse-reddish peroxidase (HRPO) labelled antispecies IgG; it was supplied by Sigma Company, USA.

6. Vaccine:

ZH-501 inactivated RVF vaccine supplied by veterinary serum and vaccine research institute. RVF department.

Preparation of the vaccine:

The attenuated smithburn RVF virus was inactivated by binary ethylamine according to **Eman (1995)** and then alum hydroxide gel was added to the inactivated virus with percentage 15%.

Evaluation of the vaccine:

1. Sterility test:

It was done according to **OIE (2000).**

2. Saftey test:

I Baby mice:

They were inoculated I/C according to **Eman (1995).**

II Lambs:

Two lamb of (5-10) day s old used for safety test of the prepared RVF vaccine. It was performed according to **EL-Nimr (1980) and Eman (1998).**

3. Potency test:

It was carried out according to **(Randall *et., al* 1964).**

Experimental Design:

11 sheep were divided into 3 groups:

Group 1(G1): Four animals, each vaccinated subcutaneously (S/C) with 1ml of inactivated Smithburn RVF vaccine.

Group 2 (G2): Four animals, each vaccinated subcutaneously (S/C) with 1ml of inactivated ZH-501 RVF vaccine.

Group 3 (G3): Three animals kept as non-vaccinated (control negative).

Serological tests:

1. Serum neutralization test:

It was done according to **(Walker, 1975).**

2. Enzyme Linked Immunosorbant Assay (ELISA):

It was done according to **Voller *et al.* (1976).**

RESULTS

Table (1): Results of sterility, safety and potency of the prepared vaccine.

vaccine	Sterility	Safety in		Potency
		baby mice*	Lamb**	ED50/ml
Inactivated Smithburg RVF vaccine	Sterile	0/8	0/2	0.00093

*** The minimum permissible limit of ED50/ml is .02ml.**

***Saftey test in baby mice performed of inactivated smithburb RVF virus without adjuvant.**

****Saftey in lamb: No thermal or clinical reaction or manifestations.**

Table (2): Neutralization Index of sheep vaccinated with the 2 different RVF vaccines as well as non-vaccinated control.

Animal group	No.of animals	Before Vaccine.	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.	11th m.	12th m.
Gp. 1 yaccinated with inactivated smithburn RVF vaccine	4	0.4	0.6	1.8	2.1	1.8	2.1	2.4	2	2	1.8	1.5	1.5	1.2	0.9	0.9	0.3
		0.3	1.3	1.8	1.8	2.1	2.4	2.1	1.8	1.8	1.8	1.5	1.2	1.2	1.2	0.9	0.6
		0.5	1.5	1.2	1.8	1.8	2.7	2.4	2	2	2	2	1.8	1.5	0.9	0.6	0.6
		0.3	0.6	1.8	1.8	2.1	2.4	2.7	2.4	2.1	2.1	2.1	1.5	1.5	1.5	1.2	0.9
		mean	0.4	0.6	1.8	1.9	2.1	2.4	2.4	2.1	2	1.9	1.7	1.5	1.3	1.1	0.9	0.6
Gp. 2 vaccinated with inactivated RVF vaccine (zH-501)	4	0.4	0.6	1.8	1.8	1.8	1.8	2.1	2.1	2.1	2	1.8	1.3	1.1	0.9	0.9	0.8
		0.3	0.3	1.2	1.5	1.8	2.1	2.1	2	1.5	1.5	1.3	1	0.7	0.5	0.5	0.4
		0.5	1.5	1.8	1.8	1.2	2.1	2.2	1.8	1.8	1.8	1.5	1.3	0.9	0.6	0.6	0.5
		0.3	0.6	1.2	1.5	1.8	1.8	2.2	2	1.8	1.5	1.5	1.3	1	0.9	0.9	0.8
		mean	0.4	0.6	1.5	1.6	1.9	2	2.2	2	1.8	1.7	1.5	1.2	0.9	0.7	0.7	0.6
GP.3 Non-vaccinated group	3	.02	0.1	0.3	0.4	0.3	0.2	0.3	0.4	0.3	0.3	0.3	0.2	0.2	0.2	0.3	0.3
		0.1	0.2	0.1	0.2	0.1	0.3	0.1	0.2	0.3	0.2	0.4	0.1	0.1	0.3	0.2	0.2
		0.1	0.2	0.3	0.2	0.1	0.3	0.4	0.5	0.2	0.3	0.5	0.1	0.1	0.2	0.1	0.2
		mean	0.1	0.1	0.2	0.3	0.2	0.3	0.4	0.3	0.3	0.4	0.1	0.1	0.2	0.2	0.2

Protective neutralizing index is 1.5

The mean protective level for the neutralizing antibody indices is(1.8) for inactivated smithburn RVF vaccine and(1.5) for (ZH-501) RVF vaccine according to *Randall, et al., (1964).

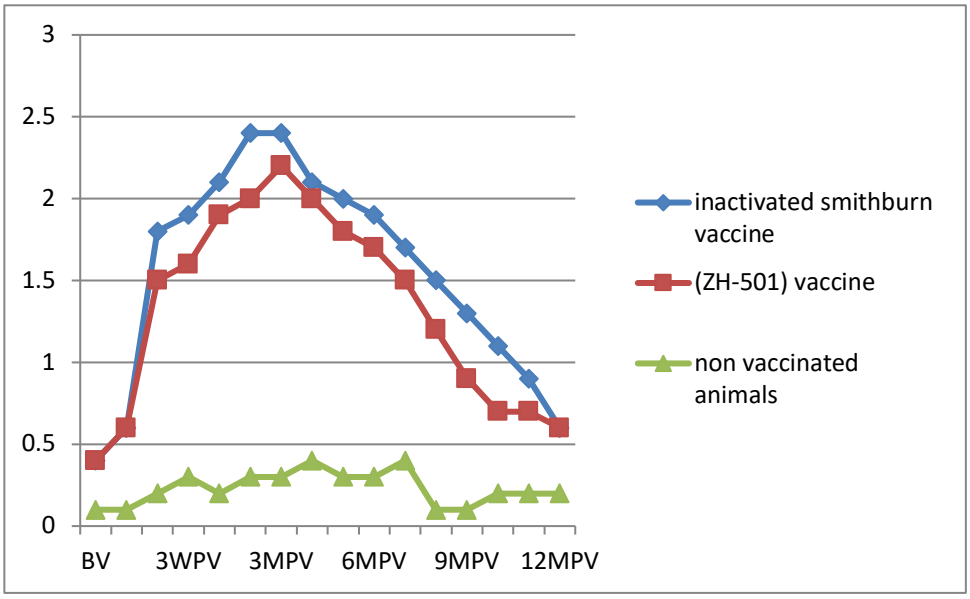

Fig.(1): Neutralizing indices of group of sheep vaccinated with inactivated smithburn RVF vaccine and another group vaccinated with (ZH-501) RVF vaccine and non-vaccinated group.

***Bv: before vaccination.**

***Wpv: week post vaccination**

***Mpv: month post vaccination.**

3. Enzyme Linked Immunosorbant Assay (ELISA):

The result of ELISA test is parallel to SNT result.

DISCUSSION

Rift Valley fever (RVF) is a vector-borne viral disease caused by RVF virus, a member of the Bunyaviridae family and Phlebovirus genus that primarily affects domestic ruminants, causing large epizootics with high mortality rates in young animals and abortions in affected female animals. **(Shabani SS *et al.*, 2015)**. Rift Valley Fever has wide range of hosts, Domesticated ruminants such as sheep, cattle, and goat are the predominant hosts and it was early established that, the newborn lamb was particularly susceptible **(Dabney R *et al.*, 1931).** In epizootic outbreaks of RVF, the use of live attenuated Smithburn vaccine is recommended **(WHO, 1983)** but limitation to be used in pregnant animals due to fear from teratogenic or aborteogenic effect **(Kathryn *et.al*, 1991)**. In addition to reversion to virulent state as well as it is pathogenic to human **(Davies and Martin, 2006; Sall *et al.*, 1998)** but it gives prolonged period of immunization more than 2 years in vaccination of sheep **Elian *et al.*, (1987)**. So this study was trial for production of RVF vaccine from the attenuated smithburn strain. As shown

in (Table 1). The prepared vaccine was sterile and safe when inoculated in baby mice and lambs showed no variation of body temperature of lambs or no signs of illness or deaths were observed in mice and lambs. In addition, the vaccine was potent, its ED_{50}/ml was 0.00093 /ml .So it was within permissible limit as cited by **Randall *et., al.* (1964)** who said that, the ED_{50} must not more than 0.02/ml . These result agree with **Gehan (1990) and Eman (1995)**. The immune response of vaccinated sheep was tested by SNT (Table 2) and Fig.(1) that show the neutralizing indices of all groups of sheep.it was noted that,the mean neutralizing index in sheep vaccinated with inactivated smithburn RVF vaccine reached above the protective level (1.5NI) at the 2nd week post vaccination (1.8NI) and increased gradually till reached the peak (2.7NI) at 2nd month and continue to 3rd month post vaccination then the level decreased to be (1.8NI) at the 9th month post vaccination and then decline to a non-protective level .While the mean neutralizing index in sheep vaccinated with inactivated (ZH-501) RVF vaccine reached the protective level (1.5NI) at the 2nd week post vaccination (1.5NI) and increased gradually till reached the peak (2.3NI) at the 3rd month post vaccination then the level decreased to be (1.5NI) at the 8th month post vaccination and then decline to a non-protective level. The results of (ZH-501) RVF vaccine were in agreement with those obtained by **El -Nimr, (1980), Gihan, (1990) and Eman, (1995)**. Result of ELISA test was correlated with that obtained by SNTtest. From the previous data, the Smithburn RVF vaccine was more protective than (ZH-501) RVF vaccine; induce higher titre of antibodies and longer time of immunity than the local vaccine.

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