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TRIALS FOR INACTIVATION OF RIFT VALLEY FEVER VIRUS USING ULTRA-VIOLET RAYS FOR PRODUCTION OF INACTIVATED RVF VACCINE

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SUMMARY

In this study, a trial to improve the alteady produced inactivated RVF vaccine. This vaccine was inactivated by using ultra-violet rays. The results obtained in the present investigation could be indicated the optimum time for inactivation of RVF virus using ultra violet. It is clear that ultraviolet completely inactivated RVF virus particles after 20 minutes from exposure with no residual infective virus, meaning a much more safe and potent vaccine. The immune response of this vaccine has been tested by vaccinating susceptible sheep and in seroconverted these animals by carrying serum neutralization test. Neutralizing antibodies were detected at the first week post vaccination with an index of 0.8. Then it reached a level of 2.5 by the 4th week post vaccination. When these vaccinated animals were challenged with virulent RVFV, the animals did not reveal any thermal reaction. On the contrary, the non vaccinated challenged control revealed high thermal reaction.

From these results, we can conclude that ultra-violet inactivated vaccine was a safe and potent vaccine as well as adequately immunogenic and efficient for vaccination of susceptible animals as it induced a good immune response when measuring the humeral immune response.

INTRODUCTION

RVFV is an arbovirus, with single stranded RNA virus, belonging to family Bunyaviridae (WHO), 1982 and Connie, 1996). One of the problems facing countries threatened by RVF is that it could be found somewhere in the country in dormant state during the interepizootic period . Therefore, the best tool for protecting our animal

populations and indirectly human beings is the use of a safe and potent vaccine. The Egyptian veterinary researchers succeeded in preparing a safe and potent alum adjuvant inactivated RVF vaccine to protect sheep and cattle against the disease (El-Nimr, 1980). Other studies were conducted by Taha et al., (1984) to improve the vaccine quality and to raise its efficiency. Many disadvantages appeared following application of formalin as an inactivating agent for RVF virus (Bahnemann, 1975). Formalin lifting residual virus as it reacts with many chemical groupings of proteins of the virus leading to a phenomenon of the "membrane effect" in which the reactions close the outer protein shell of the virus before the nucleic acid of the infectious genome has been destroyed. So, the infectious nucleic acid can emerge and lead to replication of the virulent virus. This causes a subelinical infection or even leads to disease in the vaccinated aniamls (Gard et al., 1957). Other substances were studied for the inactivation of RVFV among which the aziridine derivatives especially binary ethylenimine (BEI) Bahnemann, 1975). They have the disadvantage of requiring the handling of a highly loxic and carcinogenomic materials. On the same time, these chemical inactivants have effect on the epitopes of RVF virus. Glycoprotein on the inactivated virus showed a reduced activity against specific monoclonal antibodies (Blackburn et al., 1991).

Due to the effect of chemical inactivants on the epitopes of RVFV glycoproteins, ultra-violet rays were used as inactivant for the virus in a trial for production of safe and immunogenic inactivated RVF vaccine (Brown et al., 1963).

In this study, different times of exposure were examined and the suitable time of inactivation was used as a method of inactivating the virus and producing safe, optent and immunogenic inactivated RVFV.

MATERIAL AND METHODS

1. Material:

1. Experimental animals:

a. Mice:

Baby mice: 3-5 days old suckling mice were used for the safety test and detection of residual RVF virus inactivated by ultra-volet light.

Adult mice: 21-30 days old mice were used for the potency test of the vaccine.

b. Sheep:

Two lambs of 5-10 days old were used for the safety test of the prepared vaccine.

Six adult sheep were used to study the immue response of the vaccine as well as the challenge test.

2. Virus:

Rift valley fever virus used in this work was designated as ZH501 and had a final titre of $10^{7.8}$

$TCID_{50}$/ml following the techniques recommended by El-Nimr (1980) and Taha (1982).

3. Cell culture: Monolyaer BHK cell culture were grown and maintained for propagation and titration of RVF virus according to the method applied by El-Nimr (1981).

4. Ultra violet lamp: The used ultra violet lamps has the following technical features:

1. Model :ultra violet, Direct 38+1.
2. Dimension: 1010 X, 100 X, 230 mm.
3. Deabsorption watt : 40.

METHODS:

1. Inactivation of RVF virus : The virus was exposed to the ultra violet lamp from a distance of 50 cm with different times of exposure using large quantity of about one litre. Its action is sharp reduction of germs in the environment and on the surfaces reached by the radiation.

2. Determination of the optimum inactivation time of RVF virus by ultra violet lamp:

Virus samples were collected at different intervals and inoculated into BHK cell culture as well as in adult mice to detect the residual infectivity and the cytopathic effect indicating the optimum time for inactivation and this also done in adult mice.

3. Evaluation of the vaccine:

1. Sterility test:

The prepared vacccine was tested for its sterility and purity from any bacterial or fungal contaminants.

2. Safety test:

a. In baby mice:

Safety of the vaccine was checked by I/C inoculation of 10 suckling mice (3-5 days old) each with 0.03 ml of inactivated virus. The mice were kept under observation for a period of 10 days and the vaccine was considered as safe if all inoculated mice survived during the observation period.

b. In lambs:

Two lambs of 5-10 days old were also used for the safety test of the prepared vaccine by inoculating each lamb with 10ml of the inactivated vaccine (Half the dose intraperitoneally and the other half subcutaneously). Then these animals were observed for 10 days for any signs of RVF disease or deaths (EL Nimr, 1980).

3. Potency test:

This test was done according to the method adopted by El-Nimr (1980) and Taha (1982) and finally the ED_{50} in ml was calculated according to the method adopted by Randall et al., (1964).

4. Vaccination of sheep:

Group (I): Three sheep were vaccinated S/C with 1ml of ultra violet inactivated vaccine.

Group (II): Two sheep considered as non vaccinated control were challenged S/C with 1 ml of 10^5 $TCID_{50}$/ml virulent RVF virus then observed again 10 days for any sings of disease and also for seroconversion.

Group (III): One sheep was left as test control.

Table (1): Virus infectivity in tissue culture and mice for RVF inactivated virus at different intervals

Exposure time/second	Titre expressed in log_{10} $TCID_{50}$/ml in BHK				Death % in mice
	PI	P2	P3	Mean	
0 time	7.8	7.5	7.8	7.5	100%
5 time	7.2	7.2	7.2	7.2	100%
10 time	5.8	5.8	5.5	5.7	100%
15 time	3.5	3.8	3.8	3.7	100%
20 time	0.0	0.0	0.0	0.0	0.0
25 time	0.0	0.0	0.0	0.0	0.0
30 time	0.0	0.0	0.0	0.0	0.0

Death % in mice: Signs of RVF disease or deaths.

Table (2): Safety test of ultra violet inactivated RVF vaccine in both susceptible lambs and baby mice.

Vaccine	Results of inoculation of ultra violet incativated RVF vaccine in both lambs and baby mice.			
	Susceptible lambs		Baby mice	
	PI Dead	P2 Alive	P2 Dead	P3 Alive
Ultra violet inactivated RVF vaccine	0/2*	2/2	0/10**	10/10

* Number of dead lambs / number of living lambs
** Number of dead mice/number of living mice.

Table (3): Results of potency test in mice.

Type of vaccine	ED_{50}/ml
Ultra violet inactivated RVF vaccine	0.0012/ml

Fig. (1): Temperature degrees of sheep vaccinated with ultra violet inactivated RVF vaccine and challenged with virulent RVF virus.

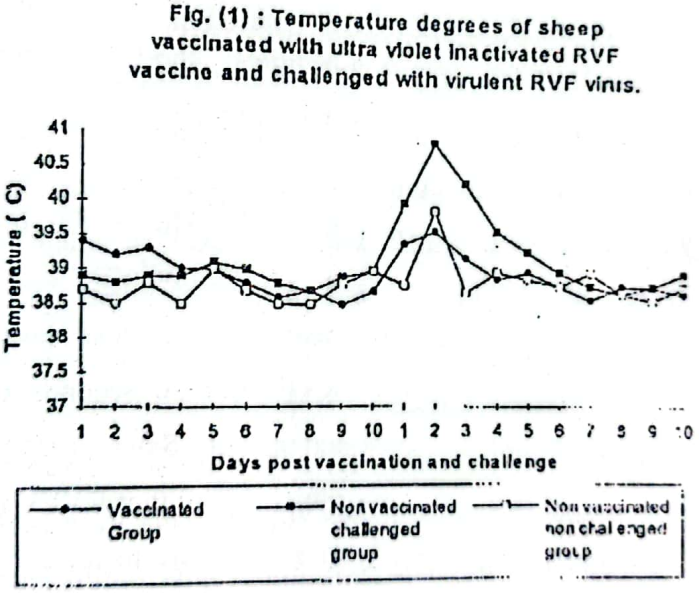

Table (4):NI's of vaccinated and challenged sheep (with UV inactivated RVF vaccine), control non vaccinated challenged sheep and non vaccinated non challenged control sheep.

Number of sheep	Treatment	*	NI at different weeks post vaccination				NI at different days post challenge with RVF vaccine				
			1	2	3	4	1st day	3rd day	5th day	7th day	10th day
1	Ultra-violet inactivated RVF vaccine	0.2	0.8	1.2	2.0	2.8	2.5	2.0	2.2	2.5	3.1
2		0.2	0.8	0.8	1.8	2.5	2.2	1.8	2.0	2.2	2.8
3		0.5	0.5	1.2	1.8	2.2	2.2	1.8	2.0	2.0	2.5
Mean		0.3	0.7	1.1	1.9	2.5	2.3	1.9	2.0	2.2	2.8
1	Non vaccinated challenged control sheep	0.2	0.2	0.5	0.2	0.2	0.2	0.5	0.8	1.2	2.0
2		0.2	0.5	0.5	0.2	0.5	0.5	0.8	1.2	1.8	2.2
Mean		0.2	0.4	0.5	0.2	0.4	0.4	0.7	1.0	1.5	2.1
1	Non vaccinated non challenged	0.2	0.2	0.5	0.2	0.2	0.5	0.2	0.2	0.5	0.5

* Before vaccination.

DISCUSSION

Rift Valley fever is one of the seriuous viral zoonotic diseases in Egypt, the first outbreak appeared in 1977 Zuckerman et al., (1978). Recently live attenuated vaccine was added to the vaccination programe of animals beside inactivated one. Many studies were performed to improve the produced vaccines and to increase their effeiciency and duration of immune response. In this study, trials to produce and new inactivated RVF vaccine using other inactivators than fomalin or binary ethyleneimine (BEI). Ultra violet rays are considered one of the effective methods used for inactivation as there's no use of chemicals in the inactivation process and it acquires short duration in the process of inactivation.

Tabel (1) shows the influence of time on the infectivity of RVFV using ultra violet rays which revelaed that these rays completely inactivated RVF after 20 seconds of exposure. This result agrees with Sawa (1955) and Brown et al (1963) which found that RVFV is completely inactivated after exposure to ultra violet rays for 30 seconds while formalin inactivates the same virus after 18 hours of exposure El-Nimr, (1980) and its deleterious effects on the viral antigen causing denaturation of the viral proteins and consequently degradation of the virion into subunits. Moreover, inactivation with formalin is

not linear, beside it doesn't produce a first order inactivation kinetics of RVFV and formalinized vaccine might still contain some infective virus or viral particles Blackburn et al., (1991) also binary ethyleneimine (BEI) completely inactivates RVFV after six hours of exposure with less destructive effect on viral epitopes Eman, (1995).

Table (2): shows the results of safety test in both lambs and baby mice indicating that the produced vaccine was safe as there is no clinical symptoms or deaths denoting RVF infection due to residual infectivity in the prepared vaccine.

Table (3) showed the results of the potency test applied on the prepared vaccine indicating that the vaccine gave a dood protection in mice with an ED_{50} of 0.0012/ml. These results were similar to that obtained by El-Nimr (1981) and Taha (1982) when using formalin as inactivant and Eman (1995) when using binary ethyleneimine (BEI) as inactivator.

To study the efficiency and immunogenic properties of the prepared ultra violet inactivated vaccine laboratory tests (animal inoculation and serum neutralization test) were conducted by inoculating each susceptible sheep S/C with 1ml (field dose) of the prepared vaccine. Body temperatures were recorded daily as well as blood samples were collected at predetermined intervals. There was no post vaccinal reaction in the form of RVF symptoms or rise of temperature (viraemia). Temperature recording of vaccinated aniamsl fluctuated between 39.4°C and 38.5°C as shown in fig. (1). Vaccinated animals were challenged after 4 weeks with 10^5 $TCID_{50}$/ml of the virulent RVF virus. Temperature was recorded after challenge revealed that the vaccinated challenged group did not manifest any thermal reaction. While, the two non vaccinated control sheep manifested high temperature when challenged up to 41°C in the same fig. Seroconversion test for vaccinated sheep was conducted using serum neutralization test on serum samples collected from vaccinated sheep. Table (5) showed a rise in the neutralizing indices from the 3rd week (1.9) till the 4th week (2.5). Following challenge, there was a drop in the value of the neutralizing indices and then it started to rise being at higher level of 2.8 by the 10th day. These results support those of Nagano et al. (1949) who reported that RVF virus inactivated by ultra violet light was injected I/V in mice interferes with the growth of the virulent virus and Sawa (1955) found that mice inoculated S/C with the inactivated ultra violet RVF virus for 30 seconds did not die and resisted an injection of fully virulent virus either immediately after the first injection or up to 4 days.

The results of the present work indicate the

possible use of ultra violet rays as inactivator for inactivation of RVF virus which could be used as antigen for inactivated vaccine or as a diagnostic for antigen reagent.

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