

STUDIES ON THE STABILITY OF RVF VIRUS

By

EMAN, M. SHELKAMY ; LILY, S. SALAMA AND ELIAN, K. ALY

Veterinary Serum and Vaccine Research Institute , Abbasia , Cairo

SUMMARY

Rift Valley Fever virus is one of the weak viruses. So this study was made to know the degree of stability of the virus at different temperatures, how to deal with it and how to keep it for a long time without great loss in its titre by using different stabilizers at different temperatures especially at the diagnostic and vaccine production Laboratories .

In this study , we titrated a lot of the virus and it was $8.5 \log_{10} \text{TCID}_{50}/\text{ml}$. We divided it into groups and kept at 37°C , 4°C , 20°C and -70°C without using any stabilizer then another groups were kept at -20°C and -70°C using different percentages of peptone with sucrose, lact albumin with sucrose , and skimmed milk . It was found that the titre of the virus decreased about $2 \log_{10} \text{TCID}_{50}/\text{ml}$ after 2 months at -20°C but using skimmed milk, the loss in the virus titre after 12 months was not so great . While at -70°C , the virus lost about $2 \log_{10} \text{TCID}_{50}/\text{ml}$. after 6 month of preservation . It was found that 37°C , the virus lost about $2 \log_{10} \text{TCID}_{50}/\text{ml}$ after 6 month

of preservation . It was found that 37°C , is not suitable to keep the virus for not more than 2 hours as its titre was $5.8 \log_{10} \text{TCID}_{50}/\text{ml}$. While at 4°C the virus was still suitable for use up till 3 days as its titre was $6.1 \log_{10} \text{TCID}_{50}/\text{ml}$.

From this study , it was recommend to use 3% skimmed milk as a stabilizer to preserve the virus at -20°C , for 6 months and for twelve months at -70°C , without great loss in its titre . Also it was found that 37°C , is not suitable to keep the virus more than 2 hours although the virus was still present but with very low titre ($0.7 \log_{10} \text{TCID}_{50}/\text{ml}$.) after seven days of Preservation.

INTRODUCTION

Rift Valley Fever is an acute febrile mosquito-borne viral disease .It is a zoonotic disease , highly infectious and fatal among livestock and human beings . Zuckerman and Simpson (1978), stated that an outbreak of Rift Valley Fever occurred for the first time in Egypt at 1977 - 1978. The virus could be preserved in oxalate carbol

glycerin solution (Findlay and Daubney 1931). They found that it became less virulent after 40 minutes at 56°C. At room temperature and pH 6.6, the virus was partially destroyed while in incubator at the same pH complete destruction occurred. Higher pH values as 8 destroyed the virus at both room temperature and incubator. They added that after 8 months at 4°C a slight virulence still remained. The virus in blood was viable for 147 days in oxalate carbol glycerin solution at pH 8 (Weiss 1957). The virus was most stable when kept at -70°C, The virus in mouse blood plus normal serum and dried in vacuum over H₂SO₄, then frozen remain viable for 8 months. Also he mentioned that stabilizers such as 5% sucrose, 1% solution of 21 amino acids, 5% peptone and saturated solution of lactalbumin hydrolysate were efficient. Soad (1986) used lact albumin (3%, 5%) and skimmed milk (3%, 5%) as stabilizers to fowl pox vaccine. In lyophilized RVF attenuated vaccine (Elian 1998) and (WHO 1973) mentioned that 5% lact albumin and 2.5% sucrose, 5% peptone and 2.5% sucrose were efficient as stabilizers.

MATERIAL AND METHODS

1- Virus :-

Rift valley fever virus used in this work was designated as ZH501 and had a final titre of 10⁸ TCID₅₀/ml following the techniques recommended by (El-Nimr 1980) and (Taha 1982).

2 - Cell culture :-

Monolayer BHK cell culture were grown and maintained for virus titration according to El-

Nimr et al. (1981).

3 - Stabilizers :-

The different types and concentrations of stabilizers were added to the titrated virus as follow :-

- a - Peptone 5% and sucrose 2.5%, then from this mixture 3% and 5% were used.
- b - Lact albumin 5% and sucrose 2.5% then from this mixture 3% and 5% were used.
- c - skimmed milk 3% and 5% were added to the stock virus.

Experimental design :-

Samples of the virus with different stabilizers mentioned before were kept at various temperatures as the following design :

- a - At 37°C the virus was preserved and the titration was measured after 2 hours, 4 hours then daily for a week.
- b - At 4°C : the virus was preserved and the titration was carried daily for a week.
- c - At 20°C : the virus was preserved and the titration was made every two months for a year.
- d - At -70°C : the virus was preserved and the titration was made every two months for a year.

RESULTS AND DISCUSSION

RVF disease is an economically important disease of animals. So it is very important to control it by several methods, one of these methods is the vaccination of animals by using either inactivated or attenuated RVF vaccines. So it is very important to study how to deal with RVF virus in the laboratories especially which make huge quantities of vaccines or antigens.

Table (1) Thermostability of RVFV (ZH501) strain at 37 °C and 4 °C .

Time				
	37 °C	Log ₁₀ reduction	4 °C	Log ₁₀ reduction
0 time	8.0	0.0	8.0	0.0
2 hr *	5.8	2.2	ND ***	-
4 hr	5.4	2.6	ND	-
1 D **	3.8	4.2	6.7	1.3
2 D	3.5	4.5	6.5	1.5
3 D	3.1	4.9	6.1	1.9
4 D	2.8	5.2	5.9	2.1
5 D	2.5	5.5	5.5	2.5
6 D	1.8	6.2	5.2	2.8
7 D	0.7	7.3	4.8	3.2

* hr = hours

** D = Days

*** ND = Not Done

Table (2) Thermostability of RVFV (ZH501) strain at - 20 °C and - 70 °C without stabilizer .

Time				
	- 20 °C	Log ₁₀ reduction	- 70 °C	Log ₁₀ reduction
0 time	8.0	0.0	8.0	0.0
2 M *	6.0	2.0	7.8	0.2
4 M	4.5	3.5	7.0	1.0
6 M	4.1	3.9	6.0	2.0
8 M	4.0	4.0	5.5	2.5
10 M	3.8	4.2	5.1	2.9
12 M	3.5	4.5	4.8	3.2

* M = Months

Table (3) Thermostability of RVFV (ZH501) strain at - 20 C using different stabilizers .

Time	Skimmed milk		Pepton + Sucrose			Lact Albumin + Sucrose		
	3 %	Log reduction	5 %	Log reduction	3 %	Log reduction	5 %	Log reduction
0 time	8.0	0.0	8.0	0.0	8.0	0.0	8.0	0.0
2 M	7.8	0.2	7.4	0.6	7.1	0.9	6.8	1.2
4 M	7.1	0.9	6.5	1.5	6.8	1.2	5.8	2.2
6 M	6.1	1.9	5.5	2.5	5.5	2.5	5.5	2.5
8 M	5.8	2.2	5.2	2.8	5.0	3.0	4.7	3.3
10 M	5.0	3.0	4.8	3.2	4.5	3.5	4.5	3.5
12 M	4.8	3.2	4.0	4.0	4.2	3.8	3.8	4.2

$Log_{10} TcID_{50} / ml$



Table (4) Thermostability of RVFV (ZH501) strain at - 70 ° C using different stabilizers .

Time	Skimmed milk				Pepton + Sucrose			Lact Albumin + Sucrose				
	3 % reduction	Log reduction	5 % reduction	Log reduction	3 % reduction	Log reduction	5 % reduction	Log reduction	3 % reduction	Log reduction	5 % reduction	Log reduction
0 time	8.0	0.0	8.0	0.0	8.0	0.0	8.0	0.0	8.0	0.0	8.0	0.0
2 M	8.0	0.0	8.0	0.0	8.0	0.0	8.0	0.0	7.5	0.5	8.0	0.0
4 M	7.8	0.2	7.1	0.9	7.8	0.2	7.0	1.0	6.1	1.9	7.8	0.2
6 M	7.6	0.4	6.6	1.4	6.9	1.1	6.3	1.7	6.0	2.0	6.9	1.1
8 M	7.5	0.5	6.3	1.7	5.5	2.5	6.0	2.0	5.8	2.2	6.5	1.5
10 M	7.5	0.5	6.0	2.0	5.3	2.7	5.2	2.8	5.5	2.5	6.0	2.0
12 M	7.4	0.6	5.8	2.2	5.1	2.9	5.0	3.0	5.5	2.5	5.9	2.1

*Log*₁₀ TCID₅₀ / ml

The purpose of the present work is to study the stability of the virulent RVFV (ZH501), the strain used for vaccine production, using different stabilizers. The stock seed virus has an original titre of 10^8 TCID₅₀/ml after passing this virus in BHK cells and collecting the harvest. Results of thermostability of RVFV kept at 37 °C without using stabilizers showed a gradual drop of the virus titre until reaching 0.7 TCID₅₀/ml after 7 days (Table 1). Also in the same table we noticed that the stability of the virus at 4 °C showed also gradual decrease of the virus titre until reaching 4.8 TCID₅₀/ml after 7 days. This agrees with Donated et al. (1967) who found that the storage of RVF-infective tissue culture fluids at 4 °C for up to 30 days without loss of infectivity or antigenicity has been shown to be a practical procedure.

Also (Findlay 1932 a) found that infective blood preserved in oxalate carbol glycerine retained slight virulence for mice when held at 4 °C for 8 months. Elian et al. (1998) found that attenuated RVF vaccine which reconstituted and kept 24 hours at 4 °C, its titre was decreased in \log_{10} TCID₅₀/ml by 3.4. So we detected from these results that both temperature 37 °C and 4 °C are not suitable for the preservation of the virulent RVFV for vaccine production but still has a viable virus.

Concerning results of the thermostability of the virus at much lower temperatures (-20 °C & -70 °C) without any stabilizer for a year. Table (2) revealed that the drop in the virus titre is more at -20 °C than at -70 °C after 12 months of storage.

From this table, we notice the log reduction at -20 °C was more in the first 4 months after preservation but then it reduced gradually till reached 3.5 TCID₅₀/ml after 12 months of storage, while at -70 °C the drop in virus titre was slight after the first four months and still the log reduction was not so great, up till the 12 months reaching 4.8 TCID₅₀/ml. This agrees with (Mims 1956 a) who found that RVFV stored at -20 °C retained its titre at least for a month and small quantities of the virus were present after 8 months. So we detected from these results that -70 °C is more suitable for preservation of the virus than -20 °C after 6 months of preservation. This agrees with (Weiss 1957) who found that the virus was most stable when kept at -70 °C.

Results of thermostability of RVFV at -20 °C for a period of a year using different stabilizers as mentioned in Table (3) revealed that the drop in the titre was gradually. The table showed that the best stabilizer of choice is skimmed milk (3%) as the titre reached 4.8 after 12 months. This agrees with Wassel et al. (1992) who found that inactivated RVF vaccine using 10% skimmed milk as a virus stabilizer gave high ED₅₀ in mice reaching (0.0018) and (2.5) NI titre in sheep after 6 weeks of vaccination.

Result of the thermostability of RVFV at -70 °C for a period of a year using different stabilizers in Table (4) showed that for the first 2 months, there was slight drop in the titre of the virus preserved in lact albumin and sucrose 3% for \log_{10} till reaching 7.5 \log_{10} TCID₅₀/ml. From the men-

tioned data in Table (4) we noticed that there was no change could be observed in the titre of RVFV preserved in the other stabilizers after 2 months . Then there was somewhat decrease in titre in both peptone & sucrose (3% & 5%) and lact albumin & sucrose (3% & 5 %) up till the 12 months .

Also in the same table , skimmed milk 3% revealed that there was slight drop in the titer of the virus with about $(0.6) \log_{10} \text{TCID}_{50}/\text{ml}$. reduction after 12 months from the original titre which was $(10^8 \text{TCID}_{50}/\text{ml})$. But skimmed milk (5%) revealed a drop in the titre of the virus with reduction of $2.2 \log_{10} \text{TCID}_{50}/\text{ml}$ after the same period.

From the above results we can conclude that the best stabilizer of choice is skimmed milk 3% . Also we can store the RVFV at -70°C at with slight change in its titre for 12 months and we cannot preserve the virus at 37°C .

REFERENCES

Donald, E. Craig, William, J. Thomas and Armand N. De Sauctis (1967): Stability of Rift Valley Fever Virus at 4°C . American society for Microbiology, 15 : 446 - 447 .

Elian, K. A. A.; Gihan, R. M. and Eman , M. Shalkamy (1998): Production and keeping quality of attenuated RVF vaccine. Assuit Veterinary Medical J. , (under press).

El-Nimr, M.M. (1980): Studies on the inactivated vaccine against RVF virus. Ph. D. Thesis (Microbiology) Fac. Vet. Med. Assuit University , Egypt .

El - Nimr, M. M.; Abdel Gaffar, S. ; Mohsen, A. Y.; El Debegy , A.; El Danaf, N. A; El Nakashly, S.; Mohamed , Z. and E. Nafie (1981): Infection of BHK cells with RVFV. Bull of Int. Epiz., 93 (11-12) : 1351 - 1359 .

Findlay , G. M. and Daubney , R. (1931): The virus of Rift Valley Fever or enzootic hepatitis. Lancet , 22 : 1350 - 1351 .

Findlay , G. M. (1932 a) : Rift Valley Fever or enzootic hepatitis. Trans . Roy . Soc. Trop. Med. and hyg . 25, 229 - 265 .

Mims, C. A. C. (1956 a) : Rift Valley Fever in mice I General features of the infection . Brit. J. Exp. Path., 37 : 99 - 109 .

Soad , M. S. (1986) : Immunological and virological studies on fowl pox vaccine . Fac. Vet. Med. Cairo University .

Taha, M. M. (1982) : Studies on inactivated vaccine against Rift Valley Fever virus . Ph. D. Thesis, Microbiology Dept., Fac . Vet. Med., Cairo University .

Wassel, M. S., Taha, M. M.; Elham. A. El.Abiary, Z. E. (1992) : Preliminary studies on freeze - dried inactivated Rift Valley Fever vaccine in Egypt. Assuit Vete. Med. J. , 26 ; 76 - 82 .

Weiss, K. E. (1957) : " Rift valley fever " A review . Bull . Epiz . Dis . Afr., 5 : 431- 458 .

W. H. O. Technical Report series no. 530 , (1973)

Zuckerman , A. J. and Simpson D. I H. (1978) : Rift Valley Fever. Nature , 271 (5643) : 308 .