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Attenuated Rift Valley Fever Virus (NMP18) Chemically For Vaccine Development

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Serial treatment of RVF virus ZH501 with nitrous acid and 5-flourouracil has been employed to derive an attenuated strain of *Rift Valley Fever virus* (NMP18) for use as alive virus vaccine besides studying its safety, potency and the duration of immunity in pregnant and non-pregnant sheep and cattle as well as remaining its antigenic and immunogenic power in animals using its protein analysis and Western blotting.

INTRODUCTION

Rift Valley Fever (RVF) is a peracute or acute disease of domestic ruminants in Africa, caused by a mosquito borne virus causing high mortality rate in newly born animals and abortion in pregnant ones. It is a zoonotic disease and humans become infected from contact with tissues of infected animals or by mosquito bite causing sever influenza-like illness, encephalitis and haemorrhagic diseases occur and deaths (Swanepoel, 1976 and Gerdes, 2002).

Immunization of susceptible animals is considered the most effective to control the disease (Meslin, 1993). Attenuated vaccines developed for control measure against RVF infection especially during outbreak. Smithburn (1949) succeeded to prepare attenuated RVF vaccine for non-pregnant animals to protect them against abortion and their young for a period of several months and poorly immunogenic in cattle (Swanepoel, 1981).

The laboratory-attenuated minute plaque variant vaccine of ZH501 (Moussa *et al.*, 1982) is avirulent for adult mice when inoculated by i.p. route. However, Peters and Anderson (1981) have found no constant correlation between plaque size and virulence. The mutagenized Rift Valley Fever virus (MVP12) attenuated and immunogenic in cattle and sheep has the ability to cause teratogenic effects in pregnant sheep with foetal malformation of the central nervous and musculo-skeletal system were mostly consistent with those observed in sheep (Hunter *et al.*, 2002).

Our aim of work is to prepare and evaluate a newly attenuated vaccine from local strain RVF ZH501 which could be administrated to farm animals safe and potent for sheep and cattle.

MATERIALS AND METHODS

Virus

Rift Valley Fever virus used in this study (ZH501) (Taha, 1982) was kindly supplied by RVF Department, Veterinary Serum and Vaccine Research Institute (VSVRI).

Cell culture

Monolayer BHK cell culture were grown and maintained as described by El-Karamany (1981).

Animals

Three susceptible pregnant sheep and 6 non-pregnant sheep, native breeds 2 years old, 6 susceptible cattle mixed breed 12 months old and Swiss albino mice used in this study were used for vaccine potency and safety tests.

Seroconversion

a. Serum neutralization test (SNT) using tissue culture system according to Wassel *et al.* (1992).

b. Enzyme linked immunosorbent assays (ELISA) (Voller, 1976 and Nirmeen, 2002).

SDS-PAGE analysis

The viral protein was extracted by triazol reagent and resuspended in 50ul of SDS-PAGE loading buffer and fractionated at 12% running gel at constant voltage for 2 hours as described by Laemmli (1970).

Western blotting against known monoclonal antibodies of RVF virus

Fractionated protein was electroblotted onto a nitrocellulose membrane, blocked with 1% bovine serum albumin (BSA), sheep anti-RVF virus antibody was used as primary antibody at 1:1000 dilution followed by addition of anti-sheep peroxidase for 2 hours then the membrane washed twice with phosphate buffer saline-Tween (PBST) and signal was developed as the method described by Sambrook *et al.* (1989).

Attenuation of RVF virus (NMP18)

RVF virus ZH501, initially propagated in BHK cell line was treated with 100ul/litre 0.0001 mol nitrous acid pH 6 for 12 hours at 37°C and passage again in BHK cell line for 10 time (Wassel *et al.*, 2003), then a series of mutagenesis using 5-fluorouracil 200ug/ml of maintenance media for 18 passage in BHK cell line as described by Cleweley and Bishop (1979) and Caplen *et al.* (1985), then attenuated RVF virus passaged again in BHK cell line for 10 time and its titre reach 7.5 $\log_{10}$ $TCID_{50}$/ml (Reed and Muench, 1938).

Preparation of attenuated RVF vaccine (NMP18)

Freeze-dried live attenuated RVF vaccine contain 10% sucrose and 5% lactose albumen, equal amount of each to the stock virus (Wassel *et al.*, 1992). No preservative was used (OIE, 1996).

Evaluation of vaccine:

Purity and sterility

The vaccine must be free from bacteria, mycoplasma and fungi contamination according to the United State Code of Federal Regulation (1987) 9 CFR testing 113.26.

a. Virus identification:

It was done according to WHO (1983) using DFA test as described by Nirmeen (2002).

b. Safety test:

According to OIE (1996), 4 susceptible hamster, 30 Swiss mice, 2 susceptible sheep native breed 2 years old, 2 susceptible cattle 12 months old native breed were used in this experiment and kept under observation for 21 days.

c. Potency test:

According to OIE (1996), 2 susceptible sheep with 1 field dose S/C and 2 susceptible cattle with 1

field dose S/C, 2 animals from each species were left as a control and antibody response were measured by SNT and ELISA after 6 months post vaccination.

RESULTS AND DISCUSSION

The use of nitrous acid for attenuation of viral protein of RVF virus causes addition of hydroxyl group to amino group (oxidation) with release of nitrogen and formation of hydroxiamine also reaction with phenolic acid chain of tyrosine to give 3-nitrotyrosine (oxidation of thyol group) these engines or activates the binding activity of RVF viral protein at pH 6 (Walker. 2002 and Wassel *et al.*, 2003).

The use of 5-fluorouracil causes oligonucleotide change through inhibiting aminoisobutyrate-puryvate aminotransferase enzyme (Caplen *et al.*, 1985 and Cleweley and Bishop. 1979).

The result of FA for identity test against RVF virus (Table 1) where strongly positive for RVF virus agreed with WHO (1983).

The attenuated RVF NMP18 vaccine was free from any contaminants (Table 1) and was safe for all vaccinated non-pregnant and pregnant sheep as well as cattle (Table 2) as they remain healthy with normal clinical symptom and with normal rectal temperature as well as no abortion in early pregnant sheep. These agreed with WHO (1983), OIE (1996) and Nirmeen (2002).

The seroconversion of inoculated sheep and cattle for 6 months (Tables 3, 4) showed a neutralizing antibody titre of > 40 by SNT and > 2000 by ELISA in sera of vaccinated sheep and cattle post vaccination with attenuated RVF NMP18 vaccine (highly immunogenic in sheep and cattle). These agreed with Hassan (1994) and Nirmeen (2002). Where the attenuated Smithburn strain, is poorly immunogenic in cattle (Swanepoel *et al.*, 1976). Our results showed that no teratogenic effect of attenuated RVF NMP18 vaccine in early pregnancy. These agreed with Nirmeen (2002), where the attenuated RVF of MPV12 vaccine has a teratogenic effect in early pregnancy (Hunter *et al.*, 2002).

Photo (1) showed the viral protein profile analysis of attenuated RVF NMP18 following treatment with nitrous acid and 5-fluorouracil (Table 5 and Photo 1) when compared with the viral protein of the original RVF ZH501 indicated that changes in L protein of the origin ZH501 of molecular weight 504.4 kDa while in NMP18 split into two bands of molecular weight 386 and 255 (Rinonucleo-protein which are coding for RNA-dependent RNA polymerase) (Nora *et al.*, 1995). The M-segment each for enveloped glycoprotein, G1 and G2 and non-structure protein mostly no big changes between the original ZH501 and attenuated NMP18 virus (Nora *et al.*, 1995) but in the S-segment which code for N-protein and non-structural protein (Sall *et al.*, 2002) is present in original RVF ZH501 viral protein but not present in viral protein of attenuated RVF NMP18. These S-proteins are important for early diagnosis of RVF infection (Sall *et al.*, 2002).

Table (1): Evaluation of attenuated RVF (NMP18) vaccine by purity and identity test

Type of vaccine	Titre	Purity and sterility					Identity by DFA
		Aerobic bacteria	Anaerobic bacteria	Mycoplasma	Fungi	Extraneous viruses	
Attenuated RVF (NMP18) vaccine	7.5 $\log_{10}$ $TCID_{50}$/ml	Free	Free	Free	Free	Free	RVF virus

DFA: Direct Fluorescent Assay
1X dose contain 4 $\log_{10}$ $TCID_{50}$/ml of attenuated RVF (NMP18) vaccine

Table (2): Safety test for attenuated RVF (NMP18) vaccine in different animals for 21 days post vaccination

Animals vaccination and route of vaccination	Hamster 1/10 field dose I/P	Pregnant sheep 1 month pregnancy		Cattle	Mice	
		1x dose S/C	1x dose I/V	10x dose S/C	0.03x dose I/C	0.2x dose I/P
Number	4	2	2	2	10	10
Clinical findings	Safe	Safe	Safe	Safe	Safe	Safe
Control non-vaccinated	Safe	Safe	Safe	Safe	Safe	Safe

1x dose contain 4 log10 TCID50/ml of attenuated RVF (NMP18) vaccine.
N.B. The control animals did not show any signs of illness.

Table (3): Potency test as measured by SNT and ELISA in sera of vaccinated sheep and cattle inoculated S/C with field dose of attenuated RVF (NMP18) vaccine

Type of vaccine	Main SNT titre								Main ELISA titre							
Attenuated RVF (NMP18) vaccine	Sheep sera				Cattle sera				Sheep sera				Cattle sera			
	Weeks post vaccination								Weeks post vaccination							
	0 day	2	3	5	0 day	2	3	5	0 day	2	3	5	0 day	2	3	5
Vaccinated	2	16	32	64	2	8	32	64	120	650	950	2200	130	520	910	2120
Control non-vaccinated	2	2	2	2	2	2	2	2	120	130	131	130	136	130	132	120

rotective level of SNT is > 40
Protective level of ELISA is > 2000

Table (4): Serotitres collected from vaccinated sheep and cattle with RVF (NMP18) as measured by SNT and ELISA following

Type of vaccine	Main SNT titre								Main ELISA titre							
Attenuated RVF (NMP18) vaccine	Sheep				Cattle				Sheep				Cattle			
	Months post vaccination								Months post vaccination							
	2	3	4	6	2	3	4	6	2	3	4	6	2	3	4	6
Vaccinated	128	128	128	128	64	128	128	128	2940	3600	3610	3640	2160	2920	3500	3600
Control non-vaccinated	2	2	2	2	2	2	2	2	120	130	130	120	130	130	130	130

Protective level of SNT is > 40
Protective level of ELISA is > 2000

Table (5): Viral protein analysis for RVF ZH501 and attenuated NMP18 virus vaccine

Lanes: Bands	Standard		RVF virus ZH501		Attenuated RVF NMP18	
			Origin		Vaccine	
	Mol. Wt.	Amount	Mol. Wt.	Amount	Mol. Wt.	Amount
1	200	4.6191	504.4	9.1343	386.12	9.5029
2	97.4	7.0014	176.79	11.526	255.95	5.7284
3	68	7.264	138.14	2.4776	176.79	3.8989
4	43	15.584	114.81	5.3076	143.94	2.2019
5	29	10.845	68	5.0638	117.19	4.6816
6	18.4	7.6545	59.654	2.8974	60.781	2.7503
7			58.003	6.3942	56.399	4.2179
8			42.034	9.5181	43.404	7.9257
9			37.519	13.189	39.264	2.7813
10			13.597	13.944	33.236	6.7473
Sum		52.968		79.452		50.436
In Lane		100		100		100

From profile of viral protein pattern of attenuated RVF NMP18 we can say that this new strain is replicable and non-infectious and still immunogenic when compare it with the original ZH501 virus in Western immunoblotting (Photo 2) when using a reference RVF monoclonal antibodies. These also agreed with SNT and ELISA results and the safety of attenuated RVF NMP18 in mice, pregnant sheep and cattle.

From the above all mentioned, we could say that attenuated RVF NMP18 vaccine is safe and potent vaccine in vaccination of all sheep and cattle and could be used during outbreaks for control measure of RVF disease in Egypt.

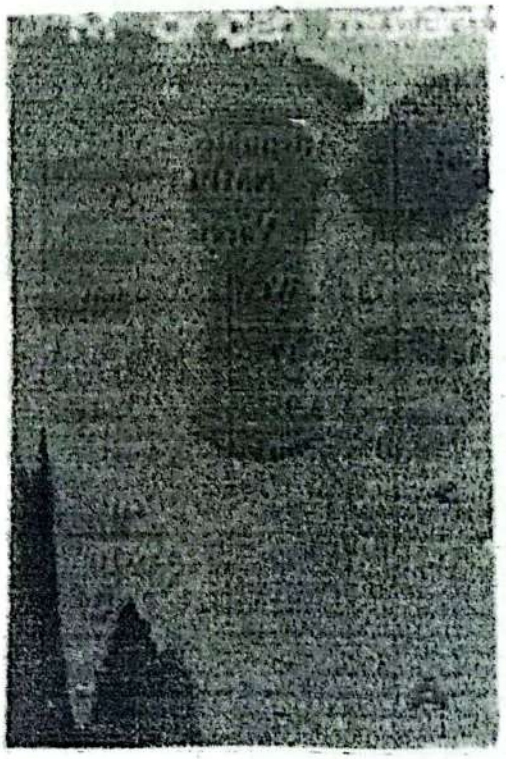

Fig (1): Viral protein profile of RVF virus ZH501 lane 2 and attenuated RVF NMP18 vaccine lane 3 and Marker lane 1 using SDS Polyacrylamide gel electrophoresis (SDS-PAGE)

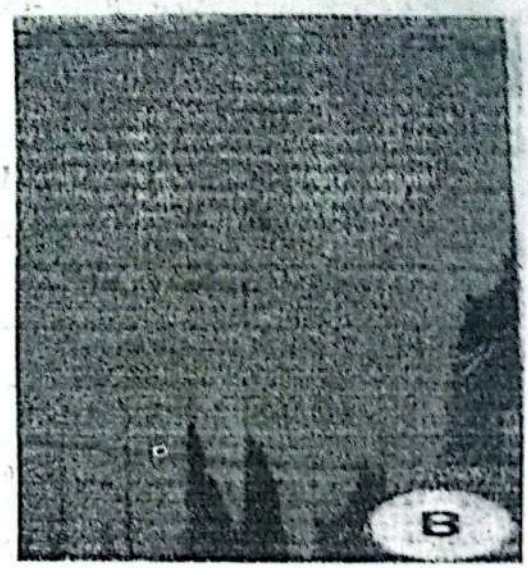

Fig (2): Western blot for attenuated RVF NMP18 virus against reference RVF monoclonal antibodies

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