Zag. Vet. J. (ISSN. 1110-1458) Vol. 43, No.1 (2015) pp. 110-116

Preparation and Evaluation of Antigens of Rift Valley Fever Virus

Mona AM El-Manzlawy and Effat L El sayed
*Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo.

ABSTRACT

This work aim to prepare and evaluate Rift Valley Fiver (RVF) antigen for detection of RVF antibodies in infected and vaccinated animals. Three types of antigen (whole cells suspension , cell lysate and supernatant) were prepared and to be proved sterile and safe when inoculated in tissue culture and mice, and revealed positive reaction in AGPT with specific reference antiserum and checkerboard ELISA was used for titration of the prepared antigens.

The obtained results revealed that the whole cell antigen had a titer of 1/160, and the cell lysate antigen had a titer 1/200 while the supernatant antigen was of a titer 1/20.

Application of these antigens with the serum samples obtained from vaccinated animals with RVF inactivated vaccine, revealed that the cell lysate antigen is more potent followed by the whole cell suspension one for detection of anti RVF IgG than other supernatant antigen.

From the previous results, it can be concluded that the cell lysate antigen is the best and can be used to detect the anti-bodies of the RVF in the animal vaccines.

INTRODUCTION

Rift Valley Fever (RVF) is a viral zoonosis that primarily affects animals and humans. Infection can cause severe disease in both animals and humans where it results in significant economic losses due to death and abortion among the infected livestock.

RVF is a mosquito borne zoonotic endemic to Africa, and was introduced for the first time in Egypt in 1977 with about 82 million Egyptian pounds economic losses *(1,2)*.

In May 1993 RVF infection reoccurred in Aswan governorate characterized by increase in number of abortions among cattle and buffaloes and visual impairment among human beings *(3,4)*.

Limited foci of RVF occurred between April and August 1997, the signs of infected animals (cattle and sheep) were high fever, icterus, bloody diarrhea and abortion *(5)*.

Although historically, it was limited to Africa and Madagascar, more recently severe outbreaks of the disease were recorded in 2000 on the Arabian Peninsula and in 2008 on the Archipelago of Comoros, including the French department of Mayotte *(6)*.RVF cases in 2000, were confirmed in Saudi Arabia and Yemen, making the first report occurrence of the disease outside the Africa *(7)*.

RVF can be diagnosed using several different serological methods such as: neutralization test, complement fixation test, haemagglutination inhibition test, Immune fluorescent technique, Eliza and agar gel diffusion test *(8)*.

Enzyme –linked immunosorbant assays (ELISA) is the highly sensitive and specific technique based on the use of gamma irradiated antigen for the detection of antibody to RVF and has the advantage of differentiating between immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies to RVF virus *(9&10)*. Since IgM antibodies persist in the blood for 4-7 weeks following vaccination or natural infection and IgG antibodies persist for few years *(10& 11)*, sandwich ELISA was more sensitive in detection of the earliest immunological response to infection or

vaccination with RVF virus compared to virus neutralization and haemagglutination inhibition test *(12)*.

Prepared RVF antigens (CLA, SA)using ultra centrifuge and inactivated by beta propulacton for detection of RFV antibody(IgM and IgG) using ELISA *(13)* .

The aim of the present work is to produce safe and highly potent RVF diagnostic antigens (cell lysate, supernatant and whole cell suspension) by using simple technique for rapid and accurate detection of RVF antibodies following infection or vaccination, with choice of the best antigen for ELISA and AGPT tests.

MATERIAL AND METHODS

Laboratory animals

Suckling mice: 20 Swiss albino suckling mice (3-5 days old) were used for safety test of the prepared antigens supplied by veterinary serum and Vaccine Research Institute, Abbassia.

Adult mice: 40 weaned mice of 21 days old were used for titration of RVF virus.

Virus Strain

The original Zagazig human strain (ZH_{501}), isolated during the RVF outbreak in (1977-1978) was propagated in Vero cell line and after freezing and thawing and centrifugation the supernatant was dispensed into small aliquots and kept at -70°C for further use. This virus was titrated according to *(14)* and the titer was calculated according to *(15)*. The virus was supplied by the Department of RVF Research, Veterinary Serum and Vaccine Research Institute, Abbasia.

Tissue Culture

African green monkey kidney (VERO) cell line was used for the propagation of RVF virus using roux bottle *(16)*.

Samples

Serum samples were collected from vaccinated sheep with inactivated RVF vaccine (1-4 week post vaccination). All sera sample were inactivated at 56°C for 30 min. and kept at -20°C until applying ELISA and AGPT tests for evaluation of the three prepared antigens.

Biological materials

Anti-sheep IgG (whole molecule) horse reddish peroxidase conjugate (Sigma) used for ELISA.

Reference anti-sera supplied by RVF Department, Veterinary Serum and Vaccine Research Institute, Abbassia used forAGPT.

Preparation of Antigen

Titration of RVF virus

The virus was titrated in mice as well as in tissue culture according to *(14)*.

Preparation and inoculation of VERO tissue culture

Raux VERO cell line was prepared according to *(16)*.

Two ml of RVF virus, Z H501 strain (10^5 $TCID_{50}$/ml) was added for each confluent VERO raux bottle. Then incubated at 37°c and allowed for adsorption of the virus to the cells for 1 hour, then 100ml of MEM without serum was added (pH 8). The pH was adjusted by sodium bicarbonate solution 7.5%, and then incubated at 37°c.

Virus harvest

The time of virus harvest was differed from complete CPE ,about 48hr for SA, to 70% CPE(24-36hr) for CLA and WCA according to the type of antigen.

Antigens preparation

It was done according to *(13)* for preparation of cell lysate(CLA) and supernatant antigens (SA) and according to *(17)* for whole cell antigen (WCA). The antigens were inactivated by binary ethaline amine (BEI) according to *(18)*

Quality control of the prepared antigen

Sterility test

It was done according to (19).

Safety test

In mice

It was done for each antigen in two groups of mice (3-5) days old, where the first group was inoculated I/ C with 0.02 ml/mouse and the other group was inoculated with PBS I/C and kept as control .

In addition each antigen was inoculated in two groups of adult mice I/P with 0.3 ml/mouse in one group and the other inoculated by the same dose and rout with PBS as control. All mice were observed for 14 days for detection signs of illness or death.

In tissue culture

Antigen safety was tested in tissue culture by inoculating 10 ml of each antigen on vero cell culture in Raux Tissue culture flask bottle. Other flasks were kept as animal control.

Inoculated cell cultures were observed daily for 5 days for cytopathic effect (CPE).

Identity

The prepared antigens were identified by Agar Gel Precipitation. Test (AGPT) according to the method described by *(20)* using reference antigen and anti-sera as control.

Antigenicity

Titration of the prepared antigens was done by the checkerboard method according to *(21)*.

Serological tests

Indirect Enzyme linked immunosorbant assay was carried using the prepared antigens for RVF antibody detection according to *(22)*.

Agar gel precipitating test, using the prepared antigens for RVF antibody detection according to *(23)*.

RESULTS AND DISCUSSION

Rift Valley Fever (RVF) is an acute highly infectious febrile viral disease. It is a zoonotic disease, highly fatal among livestock. It is responsible for great losses in animal live stock due to abortions in pregnant animals and high mortality in young animals *(24,25)*.

Immunity to Rift Valley fever (RVF) occurs in animals after natural infection with RVF virus as well as after vaccination. This immunity is demonstrated by detection of antibodies to RVF virus in serum samples of infected or vaccinated animals. Our aim in this study is to prepare and evaluate specific antigen to be used as a tool for determination of specific RVF antibodies, for usual periodically interval survey all over the countries to avoid any new spots of infection and to follow up the status of field immunity due to the vaccination complain.

The obtained results revealed that the titer of the originally seed RVF ZH_{501} in tissue culture was 8.5 log $_{10}$ $TCID_{50}$/ml and in mice (inoculated I/P in weaned mice) 7.8 log10 $MIPD_{50}$/ml. This result agree with that obtained by *(16)* who proved that the virus could be propagated easily in different tissue culture.

Regarding to the evaluation measures including sterility, identity and safety tests (Table 1); the obtained results revealed that all the prepared antigens were sterile and safe where all of them was free from bacteria and with absence of specific CPE for RVF virus in tissue culture as well as no deaths or symptoms of illness in both inoculated suckling or adult mice, in agreement with *(13,19)*, who used baby and adult mice for safety test of cell culture antigen (Table 1,2). Identity test was conducted by using reference hyper immune serum and reference RVF antigen, the result proved that all antigens identified were RVF virus, and agree with *(26)* who used hyper immune sera for detection of RVF antigen .

The checkerboard ELISA test was applied on the prepared antigen using reference anti-serum. The results revealed that the optimal dilution of these antigen were 1/160, 1/200 and 1/20 for whole cell suspension, cell lysate and supernatant respectively as shown in (Table 1).

Regarding to the use of the prepared antigens in indirect ELISA to monitor RVF antibodies in serum samples obtained from vaccinated animals, the results in table (3) indicated that some serum samples were found to be positive when tested by the three types of antigens but in different percent where the cell lysate antigen gave higher positive percent (100%) at the 2nd, 3rd& 4th,week post vaccination and the whole cell suspension antigen revealed also high percent (80%) of positive samples recorded at 3rd and 4th week post vaccination while the supernatant antigen revealed a low positive percent (20%) at the 4th w.p.v. These data came in agreement with those of (*13;23*) who indicated that EISA technique either using cell lysate or cell supernatant is a useful tool as monitoring method for vaccination program. Accoding to AGPT test it was clear that the positive results came in a parallel to the results obtained by ELISA when more ppt lines were obtained by the cell lysat antigen the obtained by the whole cell suspension antigen while the supernatant antigen did not showe clear line the rsults show in table (4).

From all mentioned results it is clear that the three prepared antigens can be used as highly sensitive, specific and safe diagnostic antigens specially cell lysate antigen in ELISA technique for detection of IgG antibodies.

Table 1. Quality parameters of the prepared RVF antigens

Type of Antigens	Sterility	Safety in			Antigenicity (Titer)
		CPE in T.C.	Baby mice I/C	Adult mice I/P	
Whole cell suspension (WCA)	Free from foreign contamination	No detected CPE	No deaths or RVF symptoms	No deaths or RVF symptoms	160
Cell Lysate (CLA)					200
Supernatant (SA)					20

CPE: Cytopathic Effect. TC: Tissue Culture.

Table 2. Evaluation of difference antigens using AGPT

Type of Ag	AGPT
Whole Cell (WCA)	Moderate positive (++)
Cell Lysate (CLA)	Strong positive (+++)
Supernatant (SA)	Weak positive (+)

Table 3. Detection of Antibody against RVF virus in sera of vaccinated sheep using indirect ELISA

Number of Samples	Week Post Vaccination	Type of Antigens					
		Cell lysate (CLA)		Whole Cell (WCA)		Supernatant (SA)	
5	1st	+ve	3/5 (60%)	+ve	0/5 (0%)	+ve	0/5 (0%)
5	2nd	+ve	5/5 (100%)	+ve	2/5 (40%)	+ve	0/5 (0%)
5	3rd	+ve	5/5 (100%)	+ve	4/5 (80%)	+ve	0/5 (0%)
5	4th	+ve	5/5 (100%)	+ve	4/5 (80%)	+ve	1/5 (20%)

Table 4.Detection of Antibody against RVF virus in sera of vaccinated sheep using AGPT

Number of Samples	Week Post Vaccination	Type of Antigens		
		Cell lysate (CLA)	Whole Cell (WCA)	Supernatant (SA)
5	1st	+ve 0/5 (0%)	+ve 0/5 (0%)	+ve 0/5 (0%)
5	2nd	+ve 1/5 (20%)	+ve 0/5 (0%)	+ve 0/5 (0%)
5	3rd	+ve 2/5 (40%)	+ve 1/5 (20%)	+ve 0/5 (0%)
5	4th	+ve 4/5 (80%)	+ve 2/5 (40%)	+ve 1/5 (20%)

REFERENCES

1.Meegan J M (1979): "Rift Valley Fever epizootic in Egypt 1977-1978. Descriptions of the epizootic and virological studies." Transaction of the Royal Society of Tropical Med. and Hyg. 73: 618 – 623.

2.Imam Z E I, El-Karamany R and Darwish M A (1979): An epidemic of Rift Valley Fever in Egypt. 2. Isolation of the virus from animals. Bulletin of the WHO 57: 441-444.

3.Arther R R, El-Sharkawy M S M, Cope S E, Bot-reos BA, Oun S, Mo-rrill, J C, Hibbs, R G Darwish. MA and Imam I Z (1993): "Reoccurence of Rift Valley fever in Egypt." Lancet, Nov. 6, 342 (8880): 1149-1150.

4.El-Gabery GH, Nawal MA, Hadia A, Fathia MM and Ayoub NN (1994): "Unclassical picture of RVF in man and animals in Aswan Governorate in May 1993." Vet. Med. J. Giza. Vol. 42(1) 1994: 135-138.

5.Abdel Rahim (1999): "An epizo-otic of Rift Valley Fever in Egypt in 1997." Rev. Sc.Tech. Dec, 18 (3): 74- 81.

6.Sissoko D, Giry C, Gabrie D, Tanrantola A, Pettinelli F, Collet L, D ortenzio E, Renault P and Pierre V (2009): Rift Valley fever, Mayotte, 2007-2008. Emerg. Infect. Dis. 15, 568-570.

7.Shoemaker, Boulianne C and Vincent MJ (2002): Generic analysis of viruses associated with emergence of Rift Valley Fever in Saudi Arabia and Yemen, 2000 – 2001 Emerg . Infect . Dis. ; 8:1415 – 1420.

8.Shope RE and Sather GE (1979): Arboviruses, In diagnostic procedures for viral, rickettsial and chlamydial infectious. Edited by Lennette, E. H. and Schmidt, N. J., 5th Ed. Puplish. Am. Pub. H. Ass., 26: 767-814.

9.Niklasson B, Peters Cd, Grandien M and Wood O (1984): Detection of human IgG and IgM antibodies to Rift Valley Fever v by ELISA d. Clin . Micro ., 19 (2) : 225 – 229.

10.Paweska J T, Burt F J, Anthony F, Smith S J, Grobbelaar A A, Croft J E, Ksiazek T J and Swanepoel R (2003): IgG-sandwich and IgM-capture enzyme-linked immunosorbent assay for the detection of antibody to Rift Valley fever virus in domestic ruminants. Journal of Virological Methods, November, 113 (2): (103-112).

11.Abd-Elfadil A A, Musa S M and Ali S S (2005): Antibody response of sheep and goats in Saudi Arabia to the live attenuated Rift Valley fever vaccine. Journal of Science and Technology, Vol. 6(1), (126-133).

12.Paweska JT, Felicity J and Burt Robert Swanepoel (2005a): Validation of IgG-sandwich and IgM-capture ELISA for detection of antibody to Rift Valley Fever

inhuman. Journal of Virological Methods. 124:173-181.

13.Elian KA and Botros B(1997): Production and evaluation of RVFdiagnostic antigens by Elisa technique. 3rd Arab Vet . Med. Cong. March 22-26.1997 Cairo.

14.El–Nimr M M (1980): "Studies on the inactivated vaccine against RVF." Ph. D. Thesis (Microbiology), Fac. Vet. Med. Assiut Univ. Egypt.

15.Reed L J and Muench H (1938): "A simple method for estimating 50 percent end point." Am. J. Hyg., 27; 493-497.

16.Taha M M (1982): "Studies on inactivated vaccine against RVFV." Ph. D. Thesis (Microbiology), Fac. Vet. Med., Cairo Univ. Egypt.

17.Hieholzer and stockes A (1996): Preparation of antigen in virology methods manual edited by brain, W.J. Mohyad Hillor O. Kangro. Academic press Ltd. P: 47-69

18.Eman MS(1995): Studies on Rift Valley Fever Vaccine inactivated with Binary. PH.D. thesis, Micro biology , Face of Vet. Med , Cairo Unv.

19.OIE (2000): Rift Valley Fever

20.Ozawa Y and Hafez SM (1973): Serological relationip between AHS and BT viruses Bull.Off.Int.Epiz., 77(11-12):1612-1613.

21.Rose NR, Fviodman H and Faney G L (1986): Manual clinical laboratory Immunology 3rd Ed. Am. Soc. For Mic. Washington, D.C.:107-109.

22.Voller A and Bidwell OD (1980): The enzyme linked immunosorbent assay (ELISA). A review of recent development with abstract of microplate application. P. 1-126 Microsystems Ltd., Guernsey, channel Island.

23.Taha M, Elian K, Gihan K, Hassan K and Fatma S(2002):Standardization of both Rift Vally fever cell lysate and supernatant antigens used in ELISA comparing with SNT and AGPT.Vet.Med.J.,Giza.Vol.50 no.4.:987-992.

24.Digoutte J P and Peter C J (1989): General aspect of the 1987 RVF epidemic in Mauritania." Res. Virol. (1989 Jan.-Feb) 140 (1): 27-30.

25.Swanepoel R and Cotezer JAW (1994): "Rift Valley Fever, infectious diseases of livestock with species reference to South Africa.", edited J. A. W. Coetzer, G. F. Thomson & R. C.Tustin.

26.OIE (1989): Rift Vally Fever: Recommended diagnostic techniques and requirement for biological products OIE manual:Vol1

الملخص العربي

تحضير و تقييم انتيجينات لفيروس حمي الوادي المتصدع

مني علي محمد فلا المنزلاوي، عفت لطفي السيد
معهد بحوث و انتاج الأمصال و اللقاحات البيطريه

تهدف هذه الدراسة لتحضير انتيجينات لفيروس حمي الوادي المتصدع للكشف عن الأجسام المناعيه المضاده له في امصال الحيوانات المحصنه و المصابه طبيعيا.

حيث تم تحضير ثلاث انواع من الانتيجينات و هي الأنتيجن الكامل و الأنتيجن الخلوى و الأنتيجن الحر وقد وجد ان التخفيف المثالي للانتيجينات المحضره بـأستخدام الطريقه الشطرنجيه هو: 1/160 , 1/200 , 1/20 للانتجينات الثلاث علي التوالي .

كما اثبتت اختبارات النقاوه والامان والنوعيه للانتيجينات المحضره بحقنها في كل من خلايا الزرع النسيجي و الفئران انها خاليه من الملوثات وامنه وأعطت نتائج ايجابيه للثلاث انتيجينات مع اختبار الترسيب في الاجار.

وبالتطبيق العملي للانتيجينات المحضره بأستخدام عينات سيرم من حيوانات محصنه بلقاح حمي الوادي المتصدع الميت بأجراء اختبار الاليزا الغير مباشر والترسيب ف الاجار و أظهرت النتائج ان الانتيجين الخلويي يعطي اعلي ايجابيه من باقي الانتيجينات الاخري ويليه الانتيجين الكامل.

نستخلص من النتائج السابقة ان الانتجين الخلوى المحضر هو الافضل و يمكن استخدامه للكشف عن الاجسام المناعية لحمى الوادى المتصدع فى امصال الحيوانات