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Hemorrhagic Fever Caused by Rift Valley Fever Virus: Rapid Detection Based on Viral Small (S) RNA Genome Segment

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Abstract: A Reverse Transcriptase (RT) Polymerase Chain Reaction (RT-PCR)-based assay was developed and evaluated for detection of Rift Valley Fever Virus (RVFV) ribonucleic acid (RNA). A pairs of oligoribonucleotide primers (RVFV1 and RVFV2), selected from the Small (S) RNA genome segment of RVFV virus, were designed and used as a target for PCR amplification. The primers RVFV1 and RVFV2 resulted in amplification of a primary 490 base pair (bp) PCR product. The PCR products were amplified from RNAs extracted from RVFV field isolates and vaccine strains, propagated in Vero cell cultures. Amplification products were not detected when the RT-PCR-based assay was applied to RNA from other related hemorrhagic fevers viruses including, Crimean Congo Hemorrhagic Fever (CCHF); dengue virus; epizootic hemorrhagic disease virus and total nucleic acid extracts from uninfected Vero cells. The described RT-PCR-based assay provides a rapid, sensitive and specific assay for detection of RVFV in cell culture and should be recommended for inclusion during an outbreak of the disease among humans and susceptible livestock.

Key words: Viral hemorrhagic fevers, RVFV, sRNA, RT-PCR

INTRODUCTION

Viral hemorrhagic fevers including, Rift Valley Fever (RVF), dengue fever, Crimean Congo hemorrhagic fever constitute one of the major unresolved public health hazards in developing countries including the Sudan. Clinical manifestations of RVFV infection are indistinguishable from those caused by other hemorrhagic fever viruses and hence cannot reliably identify infected animals (Abdelwahab *et al*., 1978). RVFV, a member of the phlebovirus genus in the family Bunyaviridae, is an arthropod-borne virus, which emerges periodically through Africa and recently Asia (Daubney *et al*., 1931; Arthur *et al*., 1993; Abdelhakeem *et al*., 1999). The virus genome is single-stranded and has 3 segments, which code for viral structural and non structural proteins (Sall *et al*., 1999). The virus may infect both humans and animal populations and hence the disease is of public health importance. Strains of RVFV have been isolated in many parts of African and Mediterranean countries including Kenya, Sudan, Egypt, Somalia, Mauritania, Saudi Arabia and Yemen (Daubney *et al*., 1931; Eissa *et al*., 1977; Olaleye *et al*., 1969; Imam *et al*., 1997). The economic impact of the disease is mainly attributed to morbidity and mortalities among animals and humans. The direct losses are usually associated with clinical disease resulting in abortions, fetal malformation and reduced reproductive performance. Unfamiliarity with the ecology, biology and molecular epidemiology has led to restrictions on the international trade of livestock and their and germplasm (Shope *et al*., 1980).

Currently, diagnosis of the disease is by conventional virus isolation and serum neutralization test (Eissa *et al*., 1980; Anderson *et al*., 1989). Serology may not identify an active infection and cross-reactions at the serogroup level are likely to occur with other members of the pheleboivirus genus (Eissa, 1984; Meegan *et al*., 1987). In general, virus isolation is time consuming, expensive and labor intensive. These limitations encourage the development of reliable, sensitive, inexpensive and rapid methods for virus detection. Sensitive assays are critical to the success of

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any monitoring program, such as prevention and control programs. RT-PCR assay for detection of RVFV in cell culture was described by (Mohamed and Imadeldin, 2006; Sall *et al*., 2001; Ibrahim *et al*., 1997). In the present study, we developed and evaluated RT-PCR for specific detection of RVFV RNAs based on the small sRNA genome segment of RVFV.

MATERIALS AND METHODS

Cell culture and virus propagation: Four Vaccine strains of RVFV were obtained from Institute of Animal Health (Onderstepoort, South Africa); EHDV serotypes 1 and 2 were obtained from (Institue of Animal Health, Pirbright, UK) and dengue virus and Crimean Congo Hemorrhagic Fever (CCHF) virus RNA were obtained from (Berhard-Nocht Institute of Tropical medicine, Hamburg, Germany). The viruses were isolated and processed as described previously (Anderson *et al*., 1989). All viruses were propagated on confluent monolayers of Vero cells. The infectious material was harvested and centrifuged at 1,500×g for 30 min and the supernatant was used for viral RNA extraction.

Extraction of viral nucleic acid from infected cell culture: Viral RNAs were extracted from the infected cell cultures using QIAamp viral RNA kit (QIAamp, GmHb, Germany) as per manufacturer's instructions. Briefly, 140 μL of virus suspension were added to 560 μL AVL buffer containing carrier RNA into a 1.5 mL micro-centrifuge tube and mixed by pulse-vortexing for 15 sec followed by incubation at room temperature for 10 min. 560 μL of absolute ethanol were added and mixed by pulse-vortexing for 15 sec. 630 μL of the mixture were transferred to QIAamp spin column mounted on 2 mL collection tube and centrifuged at 6000×(8000 rpm) for one minute. The column was then transferred to another collection tube and the other 630 μL of the mixture were passed through it. The column was then washed twice with 500 μL of washing buffers WB1 and WB2, respectively. Finally, RNAs were carefully eluted by 60 μL of buffer AVE equilibrated to room temperature. Total nucleic acid was quantified using a spectrophotometer at 260 nm wavelength.

Primer selection: The outer pair of primers (RVFV1 and RVFV2) were selected from the published sequence of Small (S) RNA genome segment, which codes for nucleoproteins and non structural proteins of RVFV (Giorgi *et al*., 1991) and used for the synthesis of the primary PCR amplification product. RVFV1 included bases 101-120 of the positive sense strand of small RNA genome segment. (5)-TTT AGA GGA GAT GGT CCT CC-(3). RVFV2 included bases 571-590 of the complementary strand: (5)-TGA GAT CAA AGC CTG GCA AC -(3). Primers RVFV1 and RVFV2 would result in amplification of a 490-bp primary PCR product. All primers were synthesized on a DNA synthesizer (Milliigen/Biosearch, a division of Millipore Burlington, MA) and purified using oligo-pak oligonucleotide purification columns (Glen Research Corporation, Sterling, VA.) as per manufacturer's instructions.

Reverse Transcriptase (RT) Polymerase Chain Reaction (RT-PCR) protocol: One μL l of 80 mM methyl mercuric hydroxide was used to denature 5.0 μL of target RVFV RNA and 2.0 μL of primers RVFV1 and RVFV2, such a concentration of 12.5 methyl mercuric hydroxide, 20 μL M of each primer in a total volume of 8.0 μL l per tube were obtained. The denaturation mixture was then incubated at 25°C for 10 min. 10 μL l of neutralization mixture containing 1 uL of 1 M 2-mercaptoethanol, 1 μL of 20 U μL^{-1} l RNAse inhibition and 2 uL of each dNTP (10 μL M dATP, 10 μL M dTTP,10 μL M dGTP, 10 μL M dCTP), were added. A reverse transcriptase mixture of 8.8 uL containing 5 μL of 25 mM magnesium chloride, 2.7 μL l of 10×PCR buffer and 1.1 uL of 50 U μL^{-1} M-MLV Reverse Transcriptase (RT) was added immediately after neutralization and the reaction was incubated at 42°C for 30 min followed by incubation at 99 C for 5 min. 73.2 uL of a PCR mixture containing (7.3 μL l of 10×PCR buffer, 8 μL Mg cl_2, 56.9 double distilled water and 1 μL l of Tag DNA polymerase (Perkin-Elmer Cetus, The Perkin Elmer Corporation, Norwalk, CT.) at a concentration of 5.0 U μL^{-1} l was added to each PCR tube. All PCR amplifications were carried out at a 100 μL l volume per PCR tube. The thermal cycling profiles were as follows: A 2-min incubation at 95°C, followed by 40 cycles of 95°C for 1 min, 55°C for 30 sec and 72°C for 45 sec and a final incubation at 72°C for 10 min. Thermal profiles were performed on a Techne PHC-2 thermal cycler (Techne, Princeton, NJ). Following amplification, 20 μL from each PCR tube containing amplified product were loaded onto gels of 1.5% SeaKem agarose (FMC Bioproduct, Rockland ME) and electrophoresed. The gels were stained with ethidium bromide and the PCR products were visualized using UV light.

RESULTS AND DISCUSSION

The RT-PCR-based assay afforded sensitive and specific detection of all vaccine strains and field isolate of RVFV strains used in this study. The Primary 490-bp PCR product was visualized onto ethidium bromide-stained gel from as little as 100 fg of viral RNA (Fig. 1).

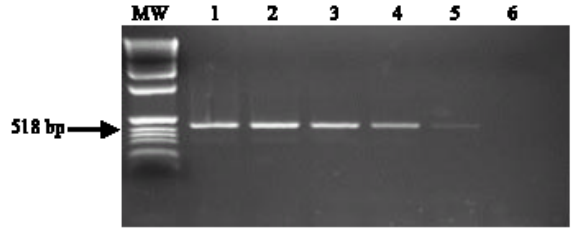

Fig. 1: Sensitivity of the PCR for detection of the 490 bp PCR product of RVFV using the outer pair of primers (RVFV1 and RVFV2). MW: Molecular weight marker (1Kb DNA ladder); Lane (1-5): RVFV RNA extracted from RVFV infected Vero cells at concentrations of, 1 ng, 100 pg, 10 pg, 1pg, 100 fg, 10 fg; respectively; Lane 6: total nucleic acid extracted from non-infected Vero cells (negative control sample)

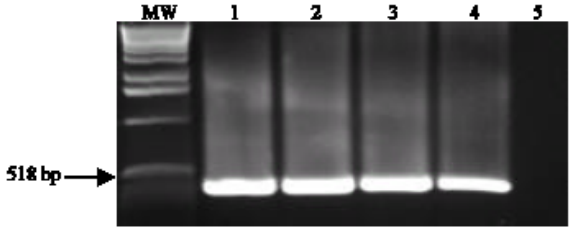

Fig. 2: Visualization of the specific 490-bp PCR products from 10 pg RNA extracted from different strains of RVFV using primers (RVFV1 and RVFV2). MW: Molecular weight marker (1K DNA ladder); Lane 1-4: different strains of RVFV; Lane 5: total nucleic acid extracted from non-infected Vero cells (negative control sample)

The 490-bp specific PCR product was detected from 1.0 pg of RNA target from vaccine strain and field isolates of RVFV using ethidum bromide-stained agarose gel electrophoresis (Fig. 2). The specificity studies indicated that, the amount of 1.0 pg RNA extracted from Crimean Congo Hemorrhagic Fever (CCHF) virus; dengue virus and Epizootic Hemorrhagic Disease (EHD) virus and total nucleic acid extracts from uninfected Vero cells failed to demonstrate the primary 490 bp PCR product (Fig. 3). Clinical manifestations of RVFV infection are indistinguishable from those caused by other viral hemorrhagic fever and hence cannot reliably identify infected animals (Abdelwahab *et al*., 1978). Serology is useful to identify past infection. The Agar Gel Immunodiffusion Test (AGID), a group specific test is complicated by cross-reaction between members of the phelebo virus genus (Ayoub and Allam, 1981). Polymerase Chain Reaction (PCR) affords a means to

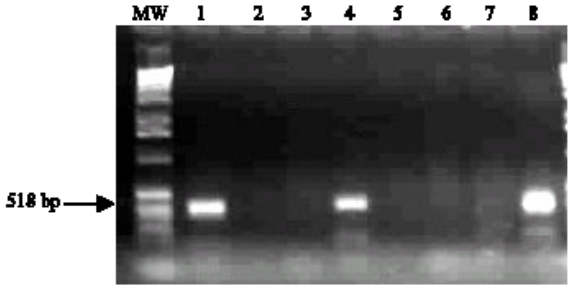

Fig. 3: Specificity of the PCR for detection of the primary 490 bp PCR product from RVFV. MW: Molecular weight marker; Lane 1: RNA extracted from RVFV vaccine strain (positive control); Lane 2: RNA extracted from dengue fever virus; Lane 3: RNA Extracted from Crimean Congo hemorrhagic fever; Lane 4: RNA extracted from RVFV vaccine strain; Lane 5-7: RNA extracted from Epizootic hemorrhagic disease virus serotype 2 (negative controls). Lane 8: RNA extracted from RVFV vaccine strain (Positive control)

amplify defined target nucleic acid sequence before detection by NAH protocols. The development of a rapid, sensitive, specific and inexpensive method for the detection of RVFV serogroup is advantageous for a variety of circumstances including clinical disease investigations and provision of valuable information on the epidemiology and ecology of these viruses (Ibrahim *et al*., 1997; Sall *et al*., 2001).

In humans and animals, viremia is well documented, providing virus for insect transmission to more susceptible ruminants (Abdelwahab *et al*., 1978). In Sudan, infection with RVFV is of concern to dairy producers and wildlife managers because of a possible epizootic among susceptible domestic livestock and wildlife populations (Eissa and Obeid, 1977). In addition, international movement of livestock and/or their associated germplasm may be restricted unless the animals are certified free of infection by virus isolation or serology (Shope *et al*., 1980).

In the present study, we optimized RT-PCR for detection of RVFV in cell culture. Selection of the primers was based on the observation that the S RNA genome has the most conserved nucleotide sequences among cognates of RVFV strains (Giorgi *et al*., 1991). The specificity studies indicated that the specific 490-bp PCR product was not amplified from a concentration of 1.0 pg RNA from dengue virus, CCHF virus, EHDV-2, or total nucleic acid extracts from Vero cell controls, under the same stringency condition described in this study. This RT-PCR-based assay could serve as a supportive

diagnostic assay to the time consuming and cumbersome conventional virus isolation laboratory procedure. The rapidity, sensitivity and specificity of the RT-PCR assay would greatly facilitate detection of RVFV infection during an outbreak of the disease among humans and susceptible animals. The described RT-PCR is a rapid procedure as the time required from submission of samples to final results of definitive diagnoses could be affordable within the same working day. The described RT-PCR-based detection assay does not required nucleic acid hybridization confirmation, which usually takes overnight. In addition, the RT-PCR assay does not required sophisticated equipments and could be easily performed in developing countries. The use of single amplification renders this RT-PCR assay a rapid and an inexpensive assay. The RT-PCR does not required hybridization confirmation and hence removes the hazardous and cumbersome radioactive laboratory procedures of working with P^{32} or P^{33} (Aradaib *et al*., 2003). Sample preparation and DNA extraction using QIAamp extraction kit was a simple procedure, which takes 1 h. The thermal cycling profiles for production of the primary and the nested PCR products were consistently 4 h. Running of the agarose gel and electrophoresis usually takes 1 h. Thus, confirmatory diagnosis of RVFV could be obtained with in the same working day.

CONCLUSION

In conclusion, the described RT-PCR assay, using primers derived from the S RNA genome segment of RVFV, provides a simple, rapid, sensitive and specific diagnostic method for detection of RVFV during epidemiological surveys and outbreaks of the disease among humans and susceptible ruminants. Application of this RT-PCR for direct detection of RVFV in clinical sample is in progress.

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REFERENCES

Abdelhakeem, I.E., U. Abdelhakeem and M. Hussien, 1999. An epizootic of Rift Valley fever in Egypt in 1997. Rev. Scientific Technique, 18: 741-748.

Abdelwahab, K.S.E., L.M. Elbaz, E.M. Eltayeb, H. Omer, M.A.A Osman and W. Yassin, 1978. Rift Valley fever virus infection in Egypt. Pathological and virological findings in man. Trans. Royal Soc. Trop. Med. Hyg., 72: 392-396.

Andersons, G.W., J.F. Saluzzo, T.G. Ksiazek, J.F. Smith, W. Ennis, D. Thureen and C.J. Peters, 1989. Comparison of *In vitro* and *In vivo* systems for propagation of Rift Valley Fever clinical specimens. Res. Virol., 140: 129-38.

Aradaib, I.E., W.S. Smith, J.S. Cullor and B.I. Osburn, 2003. A multiplex PCR for simultaneous detection and differentiation of North American serotypes of bluetongue and epizootic hemorrhagic disease viruses. Comparative Immunol. Microbiol. Infect. Dis., 26: 77-87.

Arthur, R.R., M.S. El-sharkawy, S.E. Cope, B.A. Botros, S. Oun and J.C. Morrili *et al*., 1993. Recurrence of Rift Valley Fever in Egypt. Lancet, 6: 1149-50.

Ayoub, N.N.K. and I.H. Allam, 1981. The immunodiffusion test in Rift valley fever. J. Egyptian Public Health Assoc., 56: 459-462.

Daubney, R., J.R. Hundson and P.C. Gernham, 1931. Enzootic hepatitis or Rift Valley fever: An undescribed virus disease of sheep, cattle and man from East Africa. J. Pathol. Bacteriol., 34: 545-579.

Eisa, M. and H.M.A. Obeid, 1977. Rift Valley fever in the Sudan. II. Isolation and identification of the virus from a recent epizootic in Kosti District, 1973. Bull. Anim. Hlth. Prod. Afr., 24: 349-355.

Eisa, M., E.D. Kheirelseed, A.M. Shommein and J.M. Meegan, 1980. An outbreak of Rift Valley fever in the Sudan-1976. Trans. Royal Soc. Tropic. Med. Hyg., 74: 417-419.

Giorgi, C., L. Accardi, L. Nicolleti, M.C. Gor and K.H. Takehara *et al*., 1991. Sequence and coding stratige for the S RNAs of Toscana and Rift Valley fever viruses compared to those of Punta Toro Sicilia sand fly fever and Uukaniemi viruses. Virology, 180: 738-753.

Ibrahim, M.S., J.M. Turell, K.F. Knauert and R.S. Lofts, 1997. Detection of Rift valley fever virus in mosquitoes by RT-PCR. Molecular and Cellular Probes, 1: 49-53.

Imam, I.Z.E., R. Elkaramany and M. Darwish, 1979. An epizootic of Rift Valley fever in Egypt: Isolation of Virus from Animals. Bull. World Health Organ., 57: 441-443.

Meegan, J.M., R.J. Yedloutschnig, B.A. Peleg, C.J. Peters, J.S. Walker and R.F. Shope, 1987. Enzyme-linked immunosorbent assay for detection of antibodies to Rift Valley Fever in ovine and bovine sera. Am. J. Vet. Res., 48: 1138-1141.

Mohamed, E.A. and I.E. Imadeldin, 2006. A simple and rapid detection of Rift Valley fever virus in cell culture using RT-PCR. Int. J. Trop. Med., 1: 44-47.

Olaleye, O.D., O. Tomori and H. Schmitz, 1996. Rift valley fever in Nigeria: Infections in domestic animals. Rev. Scientific Techniques, 15: 937-946.

Sall, A.A., J. Thomon, O.K. Sene, A. Fall, M. Ndiaya, B. Baudez, C. Mathiot and M. Bouloy, 2001. Single-tube and nested reverse transcriptase-polymerase chain reaction Valley fever virus in human and animal sera. J. Virol. Methods, 91: 85-92.

Shope, R.E., C.J. Peters and J.S. Walker, 1980. Serological relation between Rift valley fever virus and viruses of Phlebotomus fever serogroup. Lancet, 1: 886-887.