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A Simple and Rapid Method for Detection of Rift Valley Fever Virus in Cell Culture Using RT-PCR

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Abstract: Rift Valley fever is a disease which affects both humans and animals and hence the disease is of public health importance. In the present study, a Reverse Transcriptase (RT) polymerase chain reaction (RT-PCR)-based assay, for detection of Rift Valley Fever Virus (RVFV) ribonucleic acid (RNA) in cell culture, was developed. A pairs of oligoribonucleotide primers (RVFV1 and RVFV2), selected from the small (S) RNA genome segment of RVFV virus, was used as a target for PCR amplification. Using primers RVFV1 and RVFV2, the RT-PCR resulted in amplification of a 240-bp PCR product from RNA samples from RVF vaccine strains, propagated in Vero cell cultures. Amplification product was not detected when the RT-PCR-based assay was applied to RNA from other related phleboviruses; or other related hemorrhagic fevers viruses including, Crimean Congo Hemorrhagic Fever (CCHF) and Epizootic Hemorrhagic Disease (EHD) of deer virus and total nucleic acid extracts from uninfected Vero cells. The described RT-PCR-based could be used as a simple and rapid diagnostic assay for detection of RVFV infection in humans and susceptible animal populations.

Key words: RVFV, molecular diagnostic, RT-PCR

INTRODUCTION

Rift Valley Fever Virus (RVFV), a member of the phlebovirus genus in the family Bunyaviridae, is an arthropod-borne virus, which emerges periodically through Africa and recently Asia[1-3]. The virus genome is single-stranded and has 3 segments, which code for viral structural and non structural proteins[4]. 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[1,5-7]. Recently an epizootic of the disease was reported in South boarder of Saudi Arabia with Yemen. In animals, the economic impact of the disease is mainly attributed to morbidity and mortalities among animals and humans, direct losses associated with clinical disease resulting in abortions and fetal malformation, 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.[8].

Currently, diagnosis of the disease is by conventional virus isolation and serum neutralization test [5-9]. 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[5,10]. 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 any monitoring program, such as prevention and control programs. Nucleic Acid Hybridization (NAH) can improve the existing techniques. However, a low level of the virus may be below the threshold of NAH sensitivity and hence a prior amplification step may be necessary[11,12]. The objective of this study is to develop a rapid, sensitive, specific and inexpensive diagnostic assay for detection of RVFV in cell culture using a Reverse Transcriptase (RT) polymerase chain reaction (RT-PCR) amplification technology.

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, 2 and 4 were obtained from (Institue of Animal health, Pirbright , UK)and ; CCHF RNA were was obtained from (Berhard- Nocht Institute of Tropical medicine, Hamburg,

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Germany). The viruses were isolated and processed as described previously[9]. All viruses were propagated on confluent monolayers of Vero cells. The infectious material was harvested and centrifuged at 1,500 x g for 30 min and the supernatant was used for viral RNA extraction.

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

Primer selection and synthesis of the probe : 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[13]and used for the synthesis of the PCR amplification product. RVFV1 included bases 281-300 of the positive sense strand of the S RNA gene (5)-GCG AAG CCT TTT CAG AGA CT-(3). RVFV2 included bases 501-520 of the complementary strand: (5)-TAA GCC AGC AAA GGA GTC CA -(3). RT-PCR using primers RVFV1 and RVFV2 would result in a 240-bp PCR product. All primers were synthesized on a DNA synthesizer (Milliigen/Biosearch, a division of MilliporeBurlington, MA) and purified using oligo-pak oligonucleotide purification columns (Glen Research Corporation, Sterling, VA.) as per manufacturer's instructions.

Reverse transcriptase polymerase chain reaction: The PCR amplification of the 240-bp PCR product was produced using our previously described PCR protocol[14]. 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 60°C for 10 min. Thermal profiles were performed on a Techne PHC-2 thermal cycler (Techne, Princeton, NJ.). Following amplification, 20 microliters from each PCR reaction 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 under UV light.

RESULTS AND DISCUSSION

The RT-PCR-based assay afforded sensitive and specific detection of all isolates of RVFV strains used in this study. The specific 240-bp PCR product was visualized on ethidium bromide-stained gel from as little as 1.0 pg of viral RNA (Fig. 1).

The 240-bp specific PCR product was detected from 1.0 pg of RNA target from four vaccine strains of RVFV using ethidum bromide-stained agarose gel electrophoresis (Fig. 2). The specificity studies indicated

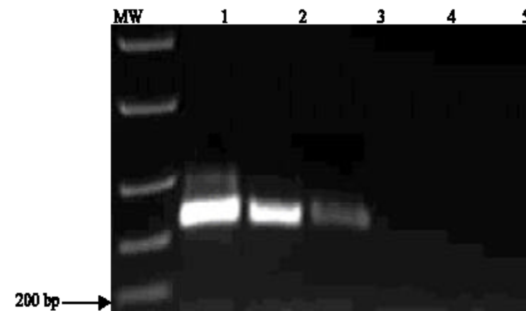

Fig. 1: Sensitivity of the Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) for detection of the specific 240 bp-PCR product from vaccine strain of Rift valley fever virus. Lane MW: molecular weight marker; Lanes 1-5: 100 pg, 10 pg, 1.0 pg, 100 fg and 10 fg of RNA extracted from RVFV vaccine strain, respectively. Lane 6: Vero cells total nucleic acid extract

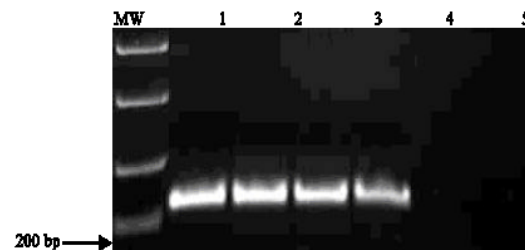

Fig. 2: The specific 240 bp-PCR product from 1.0 pg RNA of different vaccine strains of RVFV. Lane MW: molecular weight marker; Lane 1-4: 1.0 pg RNA extracted from RVFV Vaccine strains, Lane 5: Vero cell total nucleic acid extract

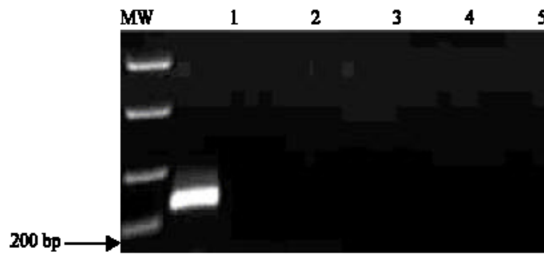

Fig. 3: Specificity of the RT-PCR for detection of the 240 bp PCR product from RVFV RNA . Amplification product was not detected from a concentration of 1.0 pg of RNA from EHDV serotype 1, 2 and 4; or total nucleic acid extracts from Vero cells. Lane MW: molecular weight marker; Lane 1: 1.0 pg of RNA from RVFV vaccine strain (positive control); Lane 2-4: EHDV serotypes 1, 2 and 4, respectively; Lane 5: Crimean congo hemorrhagic fever virus; Lane 6: Vero cell total nucleic acid extract. Using primers RVFV1 and RVFV2

that, the amount of 1.0 pg RNA extracted from other other phelebo viruses; Crimean Congo Hemorrhagic Fever (CCHF) virus and Epizootic Hemorrhagic Disease (EHD) of deer virus serotypes and total nucleic acid extracts from uninfected Vero cells failed to demonstrate the specific 240 bp-PCR product (Fig. 3). All RVFV vaccine strains which were PCR positive were also positive by conventional virus isolation.

RVFV may constitute one of the major unresolved public health hazards in many parts of the African and Mediterranean countries including the Sudan, Egypt, Somalia, Mauritania, Senegal, Saudi Arabia and Yemen. Clinical manifestations of RVFV infection are indistinguishable from those caused by other hemorrhage viruses and hence cannot reliably identify infected animals[15]. 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[16]. Polymerase Chain Reaction (PCR) affords a means to 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[11,12].

In humans and animals, viremia is well documented, providing virus for insect transmission to more susceptible ruminants[15]. In Sudan, infection with RVFV is of concern to dairy producers and wildlife managers because of a possible epizootic among susceptible animal populations[5]. 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[8].

Recently, we have reported on development of Reverse Transcriptase (RT) polymerase chain reaction (RT-PCR) assays for simultaneous detection and differentiation of two viral hemorrhagic fevers in domestic and wild animals. These RT-PCR assays proved highly sensitive and specific method for detection of BTV and EHDV viral RNAs[14]. In the present study, we evaluated RT-PCR for detection of RVFV in ell culture.

Selection of the primers was based on the observation that the S RNA genome has the most conserved nucleotide sequences from among cognates of RVFV strains[13]. The specificity studies indicated that the specific 240-bp PCR products were not amplified even from a concentration of 1.0 pg of RNA from CCHF virus RNA samples; EHDV prototype serotype 1 and 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.

CONCLUSION

This RT-PCR assay, using primers derived from S RNA genome segment of RVFV could provide a simple, rapid, sensitive and specific diagnostic method for detection of RVFV in cell culture.

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