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A PCR-Generated cDNA Probe for Detection of Sudanese Serogroup of Epizootic Hemorrhagic Disease Virus

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Abstract: A complementary DNA (cDNA) probe, derived from genome segment 6 (NS1) of epizootic hemorrhagic disease virus (EHDV) serotype 1 (EHDV-1), was synthesized by polymerase chain reaction (PCR) and evaluated for detection of Sudanese EHDV serogroup. A pair of primers (P1 and P2) was designed from NS1 genome segment of EHDV-1 and used for synthesis of a 387-bp cDNA probe. The cDNA probe hybridized with dsRNA from Sudanese EHDV serotypes including EHDV serotypes 4 and an untyped isolate designated (EHDV-318). However, dsRNA from blue tongue virus serotype 1, 2, 4 and 16 failed to hybridize with the cDNA probe. The result of this study indicated that, the developed cDNA probe could be used for rapid detection and differentiation of EHDV serogroup in cell culture.

Key words: EHDV, cDNA probe, Hybridization, Sudan

Introduction

Epizootic hemorrhagic disease of deer virus (EHDV) is a double stranded RNA orbivirus of the family Reoviridae (BORDEN *et al*., 1971). EHDV may cause an often-subclinical infection in goats and cattle (Osburn *et al*., 1994; Aradaib *et al*., 1994). There are at least ten serotypes of EHDV serogroup, distributed world wide, identified by serum neutralization and plaque inhibition test (Gorman, 1992). Of the 10 serotypes of EHDV serogroup, at least EHDV serotypes 4 and EHDV-318 are enzootic in the Sudan (Mohamed, 1987). The Sudanese serotypes of EHDV causes subclinical infection in Sudanese breed of goats and cattle and the disease potential of EHDV serotypes remains unknown (Abdalla *et al*., 2002; Aradaib *et al*., 1994; Abdalla *et al*., 2000). EHDV has a genome composed of 10 double-stranded RNA (dsRNA) segments (Hammami and Osburn., 1992). The genome segments code for the viral proteins (BORDEN., 1971). There are 3 nonstructural proteins (NS1, NS2 and NS3) and seven structural proteins, which are incorporated into the double layer protein coat (Aradaib *et al*., 1997). The nonstructural proteins NS1, NS2 and NS3 are coded for by genome segments 6, 8 and 10 respectively. The major protein of the outer coat, VP2, coded for by genome segment 2, is associated with serotype specificity and induction of neutralizing antibody (Mohammed, 1987).

In the present investigation, a complementary DNA (cDNA) probe, derived from genome segment 6 (NS1) of EHDV-1, was synthesized by polymerase chain reaction (PCR) and evaluated for detection of Sudanese serotypes of epizootic hemorrhagic disease virus (EHDV) serogroup.

Materials and Methods

Cell Culture and Virus Propagation: The two Sudanese EHDV serotypes 4 and 318 were used. Vero cells were prepared in minimal essential medium (MEM) containing 100 units penicillin/ml and 100 mcg streptomycin/ml, 10% tryptose phosphate broth and 10% fetal bovine serum (FBS) that was heat inactivated at 56 C for 30 minutes. Cell cultures were incubated at 37 ^{0}C in a humidified incubator until confluent monolayer was obtained (usually 2-3 days).

Virus Isolation and Identification: Virus isolation procedure was described previously (Aradaib *et al*., 1995). Briefly, Vero cell monolayers were inoculated with EHDV isolates. After incubation at 37 ^{0}C for 1 hour, the inoculated cell cultures were supplemented with MEM containing 2% fetal bovine serum (FBS). The cell cultures were again incubated at 37 ^{0}C and observed daily until cytopathic effect was 80% complete. All cytopathic agents were identified by serum neutralization test (Aradaib *et al*., 1994). The infectious material was harvested and centrifuged at 3,000 RPM for 30 minutes and the cell pellet was used for dsRNA extraction.

Nucleic Acid Extraction: dsRNA was extracted from infected cells. Total nucleic acid was extracted as described previously (Aradaib *et al*., 1994). Briefly, the cell pellet was lysed in a lysing buffer containing (proteinase K and 10 % SDS). The dsRNA was extracted with phenol, and ds RNA was precipitated by absolute ethanol. The dsRNS

was then partially purified with 10% lithium chloride. The dsRNA is then vaccum dried and resuspended in 100 microliter distilled water and quantified using spectrophotometer at 260 nm wave length.

Synthesis of the cDNA Probe: A pair of primers (P1 and P2) was designed from NS1 genome segment of EHDV-1 and used for synthesis of a 387-bp cDNA probe. P1 included bases 175-194 of the positive sense strand of genome segment 6: (5)-TCGAAGAGGTGATGAATCGC-(3). P2 included bases 543-562 of the complementary strand: (5)-TCATCTACTGCATCTGGCTG-(3). EHDV PCR using primer P1 and P2 would result in a 387 bp product. All primers were synthesized on a DNA synthesizer and purified using oligo-pak oligonucleotide purification columns as per manufacturer's instructions. The amplification product produced by P1 and P2, was purified using DNA binding beads according to the manufacturer's instructions and used as a cDNA probe for dot blot chemiluminescent hybridization.

Visualization of the cDNA Probe: Following amplification, 20 microliters from each PCR reaction containing amplified product were loaded onto 1.5% SeaKem agarose and electrophoresed. The gels were stained with ethidium bromide, and the specific 387 bp cDNA probe was visualized under UV light.

Dot Blots Hybridization: Dot blot blot hybridization was performed for detection of EHDV dsRNA. Briefly, dsRNA were dot blotted in a nylon membrane were then denatured and neutralized under vacuum at 50 mm Hg. Dot blotted nucleic acids were UV cross linked to the nylon membrane. Dot blot hybridization was performed using chemiluminescent detection kit according to the manufacturer's instructions. The cDNA probe prepared by PCR was labeled with peroxidase in the presence of gluteraldehide. Dot blots were prehybridized with hybridization buffer containing 5% blocking agent and 0.85 M NaCl at 42 C for 1 hr. The labeled probe was added to the hybridization buffer and the membranes were hybridized at 42° over night. After post hybridization washing, detection reagents were applied to the membranes for 1 minute. The membranes were then wrapped in saran wrap and exposed to X-ray film for 1- 60 minutes with an intensifying screen.

Results and Discussion

The developed EHDV cDNA probe, using a fragment from segment 6 of EHDV, which codes for NS1, reproducibly and specifically detected EHDV RNA in infected cell cultures. Selection of the probe was based on the observation that the NS1 is the most conserve genome among cognates of EHDV serogroup (Aradaib *et al.*, 1994; Abdalla *et al.*, 12002). In addition, this probe requires small number of infected cells to produce positive hybridization signal compared to other nucleic acid probes (Aradaib *et al.*, 1994; Aradaib *et al.*, 1995). Using dot hybridization, the cDNA probe hybridized with Sudanese EHDV RNA samples tested. Dot blot hybridization was a simple procedure that efficiently detected all EHDV serotypes under the stringency condition used in this study. It was easier when compared to conventional virus isolation procedures, many of which are lengthy and cumbersome (Mohamed, 1987; Abdalla *et al.*, 2000). It does not require a complementary DNA (cDNA) synthesis step from the dsRNA templates and subsequent amplification by PCR technology (Abdalla *et al.*, 2002).

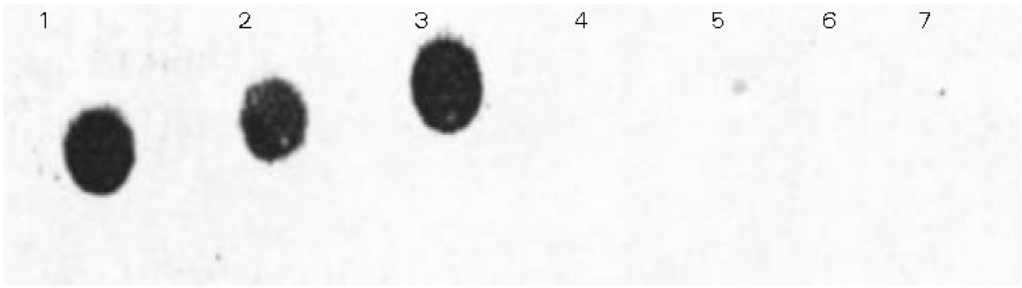

Fig. 1: Dot blots hybridization showing hybridization signals produced by the EHDV cDNA probe from dsRNA of Sudanese serotypes of EHDV serogroup. Lane 1: EHDV serotype 1 (positive control); Lane 2: (Sudanese EHDV serotype 4); Lane 3: Sudanese EHDV-318; Lane 4, 5, 6, and 7: Sudanese Blue tongue virus srotypes 1, 2, 4 and 16, respectively.

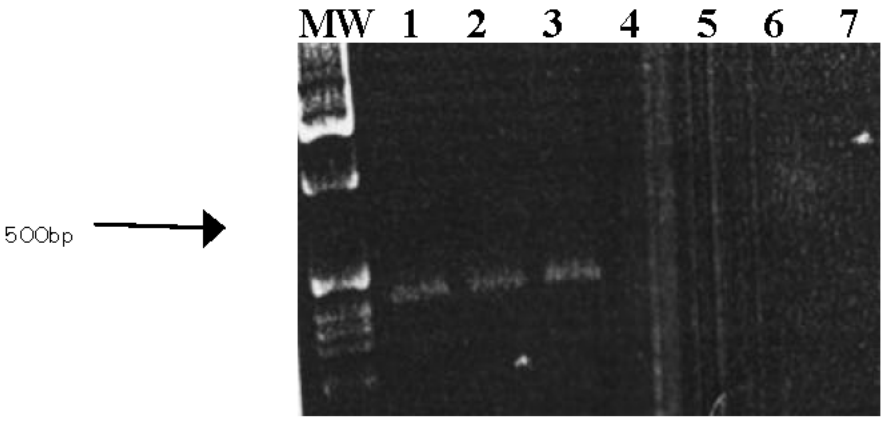

Fig. 2: Amplification of the 387-bp cDNA probe from Sudanese serotypes of EHDV serogroup. Lane 1: EHDV-1 (positive control); Lane 2: EHDV-4; Lane 3: EHDV-318; Lane 4, 5, 6 and 7; blue tongue virus serotypes 1, 2, 4 and 16

The time required for hybridization was approximately 16 hours. Excellent correlation of results from PCR amplification and dot blot with chemiluminescent hybridization renders this cDNA probe a rapid diagnostic alternative to conventional virological examination. The dot blot hybridization can replace the need for the lengthy cumbersome nucleic acid sequencing procedures, which is commonly used for detection of EHDV infection (Wilson., 1994; Aradaib *et al.*, 1995). The time required from sample submission to interpretation of the final results was by far much shorter than that required by conventional virus isolation and identification procedure. The rapidity, sensitivity and specificity of the dot hybridization assay with cDNA probe would greatly facilitate detection of EHDV infection in an outbreak among susceptible ruminants. Because dot blot hybridization is an extremely sensitive procedure care must be taken to avoid cross-contamination between tubes during pipetting of the cDNA probe. Negative and positive controls should be included in each hybridization assay to estimate the lower limit of specificity and the higher limit of sensitivity.

In conclusion, the described EHDV cDNA probe using dot blot hybridization assay could be used as an alternative to conventional virus isolation identification. In addition, the probe provides a valuable tool to study the molecular epidemiology of EHDV serogroup.

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