VETERINARSKI ARHIV 73 (3), 133-139, 2003

PCR detection of Sudanese isolates of bluetongue virus serogroup

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ABDALLA, M., S. SULIMAN, A. KARRAR, S. IDRIS, I. ARADAIB: PCR detection of Sudanese isolates of bluetongue virus serogroup. Vet. arhiv 73, 133-139, 2003.

ABSTRACT

The potential use of the recently reported reverse transcriptase (RT) polymerase chain reaction (RT-PCR)-based assay for detection of North American isolates of bluetongue virus (BTV) ribonucleic acid in cell culture was evaluated for detection of Sudanese isolates of BTV. Two pairs of oligoribonucleotide primers, selected from non-structural protein 1 (NS1) gene of BTV-17, were used for RT-PCR amplification. The BTV RT-PCR produced an 826 base pair (bp) amplification product. RNA from Sudanese BTV serotypes 4 and 16, propagated in cell cultures, were detected by this RT-PCR-based assay. Amplification product was not detected when the nested BTV RT-PCR-based assay was applied to RNA from closely related Sudanese *Orbivirus*, epizootic hemorrhagic disease virus (EHDV) serotype 4 and total nucleic acid extracts from uninfected Vero cells. The results of this study indicated that our previously described BTV RT-PCR assay could be used for detection of the Sudanese BTV isolates and possibly other serotypes of BTV serogroup from different continents.

Key words: orbiviruses, bluetongue virus, reverse transcriptase-polymerase chain reaction, Sudan

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ISSN 0372-5480
Printed in Croatia

Introduction

Bluetongue virus (BTV) is a double-stranded (ds) RNA *Orbivirus* in the family *Reoviridae*. The virus may cause an acute febrile disease in sheep and a fatal hemorrhagic infection in North American white-tailed deer (*Odocoileus virginianus*) (HOFF and TRAINER, 1974; SHOPE et al., 1960). The association of BTV with clinical disease in cattle is rare and the infection is typically asymptomatic (MACHLACLAN and OSBURN, 1983). Twenty-five serotypes of BTV are distributed worldwide (DAVIES et al., 1992). BTV serotypes 1, 2, 4, 5 and 16 have been isolated from sentinel cattle herds at the University of Khartoum Farm, Shambat Area, Khartoum, Sudan (MOHAMMED, 1987).

BTV has a genome composed of 10 dsRNA segments. The genome segments code for viral proteins (VP). Three non-structural and seven structural proteins are incorporated into the double layer protein coat. In previous studies, RT-PCR assays were developed and evaluated for detection of BTV serotypes based on nucleotide sequences of different genome segments. The development of a simple and rapid diagnostic test for detection of Sudanese BTV serotypes would be advantageous for a variety of circumstances, including clinical disease investigations, molecular epidemiological studies, ease of diagnosis and for a better understanding of the biology of BTV serotypes.

In the present study we evaluated the previously described BTV RT-PCR (ARADAIB et al., 1998) for detection of Sudanese BTV serotypes 4 and 16 in cell culture based on NS1 genome sequence analysis of BTV-17.

Materials and methods

Virus and cells. The BTV serotypes 4 and 16 present in the Sudan and the Sudanese serotype of EHDV-4 were studied. All viruses were propagated on confluent monolayers of Vero cells as described previously (ARADAIB et al., 1994). The infectious material was harvested and centrifuged at 1,500 x g for 30 min at 25 °C and the cell pellet was used for dsRNA extraction.

Viral nucleic acid extraction from infected cell monolayers. The BTV and EHDV dsRNAs were extracted from the infected cells as previously

described (ARADAIB et al., 1994). Total nucleic acid was ethanol-precipitated. Viral dsRNA was purified by differential lithium chloride precipitation, and re-suspended in 100 microliters (µl) double distilled water, and quantified using a spectrophotometer at 260 nm wavelength (ARADAIB et al., 1995).

Primer selection. Primers design for BTV RT-PCR assay was described in detail in a previous study (ARADAIB et al., 1998). 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 (RT) polymerase chain reaction (RT-PCR). The amplification of the 826-bp PCR product was produced using our previously described PCR protocol (ARADAIB et al., 1998). 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 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

The described BTV RT-PCR-based assay for detection of North American BTV serotypes (ARADAIB et al., 1998) afforded sensitive and specific detection of the Sudanese BTV serotypes 4 and 16. The specific 826-bp PCR product was visualized on ethidium bromide-stained gel from the United States, as well as the Sudanese BTV serotypes used in this study. However, total nucleic acid extracts from uninfected Vero cells failed to demonstrate the specific 826 bp-PCR product (Fig. 1).

The specificity studies of the described BTV RT-PCR indicated that the amount of 1.0 ng RNA from Sudanese EHDV serotypes 4, an *Orbivirus*

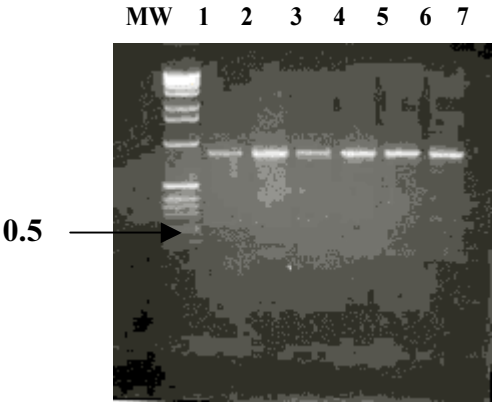

Fig. 1. Ethidium bromide-stained agarose gel electrophoresis showing the specific 826 bp-PCR product from 1.0 pg of RNA extracted from United States as well as Sudanese BTV serotypes. Lane MW: molecular weight marker; Lane 1-2: BTV-4; BTV-16; Lane 3-6: United States serotypes 10, 11, 13 and 17, respectively; Lane 7: Vero cells total nucleic acid extract (negative control).

closely related to BTV, failed to demonstrate the specific 826 bp-PCR product (Figure 2).

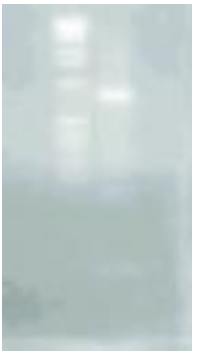

Fig. 2. Specificity of the RT-PCR for detection of BTV RNA. Amplification product was not detected from 1.0 ng of RNA from Sudanese EHDV-4. Lane MW: molecular weight marker; Lane 1: 1.0 pg of RNA from BTV-4 (positive control); Lane 2: (EHDV-4).

Discussion

RT-PCR based detection assays have been described for detection of bluetongue virus infection in susceptible ruminants, as well as the biting midge (McCOLL and GOULD, 1991; WADE-EVANS et al., 1991; WILSON and CHASE, 1993). In our laboratory, most of the current research is directed toward improved *Orbivirus* diagnosis (ARADAIB et al., 1994; ARADAIB et al., 1995; ARADAIB et al., 1998; ABDALLA et al., 2002). We recently reported a multiplex RT-PCR for simultaneous detection and differentiation of North American serotypes of bluetongue and epizootic hemorrhagic disease viruses (ARADAIB et al; 2003). The BTV RT-PCR-based assay using primers derived from genome segment 6 of BTV-17, which codes for NS1, reproducibly and specifically detected the Sudanese isolates of BTV in cell cultures. This finding indicated that the previously described BTV RT-PCR is capable of detecting additional serotypes of the BTV serogroup, including BTV serotypes 4 and 16. Using this BTV RT-PCR we validated the detection of North American BTV serotypes 10, 11, 13, and 17, as well as the Sudanese BTV serotypes 4 and 16. It is probable that this BTV RT-PCR would detect all the remaining serotypes of the BTV serogroup. However, additional research is necessary to confirm this assumption.

The BTV RT-PCR was a simple, rapid, sensitive and specific technique which did not require hybridization assay, which is time consuming and cumbersome. The specificity studies indicated that the specific PCR products were not amplified from RNA from Sudanese isolates of EHDV-4, or total nucleic acid extracts from Vero cell controls under the same stringency conditions described in this study. The BTV RT-PCR-based assay could serve as a supportive diagnostic assay to replace the time consuming and cumbersome conventional virus isolation laboratory procedure (WADE-EVANS et al., 1991; ARADAIB et al., 1998; ARADAIB et al., 2003).

It is worth mentioning that this BTV RT-PCR is group-specific and can be used for detection of BTV serogroup infection but not for BTV serotype-specific identification. It is well documented that segment 2 (L2) is the most variable genome among cognates of BTV serotypes. Therefore, a fragment of this genome could be targeted for designing BTV serotype-specific primers, which could be used for BTV serotyping. Application of

RT-PCR assays for specific identification of the Sudanese BTV serotypes is currently under way.

In conclusion, the previously described BTV RT-PCR assay, using primers derived from genome segment 6 of BTV-17 could provide a simple, rapid, sensitive and specific diagnostic method for detection of Sudanese serotypes of BTV in cell culture and could be used as a valuable tool to study the epidemiology of BTV during an outbreak of the disease among susceptible animal populations.

Acknowledgements

We would like to thank Professor Suliman M. El-Sanousi, Institute for Promotion of Animal Export, University of Khartoum, Sudan, for providing financial support for this study. We would also like to thank Dr. Adil ElGarai for his assistance during the preparation of the draft manuscript.

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Received: 27 January 2003
Accepted: 22 May 2003

ABDALLA, M., S. SULIMAN, A. KARRAR, S. IDRIS, I. ARADAIB: Dokaz sudanskih izolata serološke skupine virusa bolesti plavog jezika pomoću lančane reakcije polimerazom (PCR). Vet. arhiv 73, 133-139, 2003.

SAŽETAK

Lančana reakcija polimerazom uz reverznu transkripciju (RT-PCR) prethodno je razvijena za dokazivanje ribonukleinske kiseline sjevernoameričkih izolata virusa bolesti plavog jezika (BPJ) umnoženih u staničnoj kulturi te je ujedno rabljena i za dokazivanje sudanskih izolata virusa BPJ. Za RT-PCR amplifikaciju odabrana su dva para oligonukleotidnih početnica na genu koji kodira za nestrukturni protein 1 (NS1) virusa BPJ-17. Pomoću početnica dobiven je amplifikacijski proizvod od 826 parova baza (pb). Ovim RT-PCR postupkom dokazana je RNA sudanskih serotipova 4 i 16 umnoženih u staničnim kulturama. Amplifikacijski proizvod nije bio dokazan kad je metoda bila primijenjena za dokazivanje RNA usko srodnog serotipa 4 sudanskog orbivirusa epizootske hemoragijske bolesti i nukleinske kiseline izdvojene iz neinficiranih stanica Vero. Rezultati ovog istraživanja pokazuju da se ranije opisani RT-PCR za virus BPJ može koristiti za dokazivanje sudanskih izolata virusa BPJ i moguće drugih serotipova serološke skupine virusa BPJ s različitih kontinenata.

Ključne riječi: orbivirusi, virus bolesti plavog jezika, RT-PCR, Sudan