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Detection of Bluetongue Virus Serogroup in Cell Culture Using RT-PCR

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Abstract: A reverse transcriptase polymerase chain reaction (RT-PCR) protocol was evaluated for detection of bluetongue virus ribonucleic acid in cell culture. BTV serotypes 1, 2, 4, 5, 10, 11, 13, 16 and 17 were studied. RNAs from these BTV serogroup, propagated in cell cultures, were detected by the described RT-PCR-based assay. The specific 519 bp PCR products were visualized on ethidium bromide-stained agarose gel using a pair of primers derived from segment 6 of BTV 11. Amplification product was not detected when the RT-PCR-based assay was applied to RNA from epizootic hemorrhagic disease virus (EHDV) or palyam virus serogroups; or total nucleic acid extracts from uninfected Vero cells. The results of this study indicated that the described RT-PCR assay could be applied for detection of BTV serogroup. In addition, the described BTV RT-PCR assay could be used as a supportive diagnostic assay to the current conventional virus isolation procedures used for detection of BTV in cell cultures.

Key words: Bluetongue virus, serogroup, RT-PCR

Introduction

Bluetongue virus (BTV), is a double stranded (ds) RNA orbivirus of the family Reoviridae (Borden *et al.*, 1971 and Fenner *et al.*, 1974). The virus has a worldwide distribution and exists in at least twenty five distinct serotypes (Davies, 1992). BTV serotypes 1, 2, 4 and 16, are known to be enzootic in the Sudan (Mohammed; 1987). BTV serotypes 2, 10, 11, 13 and 17 are enzootic in North America (Osburn *et al.*, 1990). BTV causes a fatal febrile infection in sheep. However, infections caused by BTV in goats and cattle are usually inapparent and no evidence of clinical hemorrhagic disease has been reported (Gard *et al.*, 1988; Maclachlan *et al.*, 1990 and Work *et al.*, 1992). However, indirect losses associated with loss of body weight and condition, drop in milk production and poor subsequent reproductive performance were thought to have greater economic effect than occasional overt disease (Mohammed and Mellor, 1990). The North American BTV serotypes 2, 10, 11, 13 and 17 may cause an often fatal hemorrhagic disease in the white-tailed deer (*Odocoileus virginianus*) of North America (Shope *et al.*, 1955).

In addition, because of unfamiliarity with the biology and ecology of the virus, there is the potential of restriction on the international movement of livestock and associated germplasm from countries suspected to harbor the disease to BTV free countries unless the animals are certified free of infection by conventional virus isolation or serology (Pearson *et al.*, 1992). Current diagnosis of BTV infection by conventional virus isolation and serology is time consuming, labor intensive and expensive (Osburn *et al.*, 1994). The development of a rapid, sensitive, specific and inexpensive method for detection of BTV from different geographical locations would be advantageous for a variety of circumstances including clinical disease investigations, molecular epidemiological studies for understanding the biology of the virus, and enhancement of international trade of livestock and associated germplasm. Recently, we have described a simple, rapid, sensitive and specific RT-PCR assay for detection of palyam viruses and EHDV serotypes in cell culture (Abdalla *et al.*, 2002 and Aradaib *et al.*, 2003). In the present study, the potential of BTV RT-PCR assay was evaluated for detection of BTV using primers derived from genome segment 6 (NS1) of BTV serotype 11.

Materials and Methods

Cell Culture and Virus Propagation: The four BTV prototypes serotypes 1, 2, 4, 5 and 16; and palyam virus isolates present in the Sudan, and the BTV prototypes serotypes 2, 10, 11, 13, 16 and 17 present in North America were used in this study. EHDV prototypes serotypes 1, 2, 4 and isolate 318 (EHDV-318) were included as negative controls. The viruses were isolated and processed as described previously (Aradaib *et al.*, 1994a). 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 cell pellet was used for the dsRNA extraction.

Extraction of Viral Nucleic Acid from Infected Cell Culture: The BTV and EHDV and palyam viruses dsRNA were extracted from the infected cells as previously described (Aradaib *et al.*, 1994b). Total nucleic acid was ethanol-

precipitated. Viral dsRNA was purified by differential lithium chloride precipitation, and resuspended in 100 ul double distilled water, and quantified using a spectrophotometer at 260 nm wavelength.

Primer Selection: BTV primers (B1 and andB2) were designed based on the nucleotide sequences of genome segment 6 of BTV serotype 11 (BTV-11). This genome segment codes for non-structural proteins 1 (NS1) and was reported to be highly conserved (Aradaib *et al*, 1998). Primer B1 included bases 311-330 of the positive sense strand of NS1 genome (5):TACGAGGAGGATGTCGAAGG (3). Primer B2 included bases 811-830 of the complementary strand (5): TTCCGAAGAGCTGAAGTACA (3). BTV primers would result in amplification of a 519 bp PCR product. All primers were synthesized on a DNA synthesizer and purified using oligo-pak oligonucleotide purification columns as per manufacturer's instructions.

Reverse Transcriptase Polymerase Chain Reaction(RT-PCR): The RT-PCR protocol used in this study was basically as previously described (Aradaib *et al.*, 1995). Five units /ul of the Taq DNA polymerase were used per reaction. 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 62° C for 10 min. Thermal profiles were performed on a Techne PHC-2 thermal cycler.
Twenty microliters from each PCR reaction containing amplified product were loaded onto 1% SeaKem agarose and electrophoresed. The gels were stained with ethidium bromide, and the specific PCR products were visualized under UV light.

Results

All BTV isolates, which were positive by conventional virus isolation, were also positive by the described BTV RT-PCR assay. The described BTV RT-PCR-based assay afforded sensitive and specific detection of BTV serotypes and field isolates. The sensitivity studies indicated that the specific 519 bp PCR product was visualized on ethidium bromide-stained gel from 1.0 pg RNA of the Sudanese isolates of BTV serotypes 1, 2, 4, and 16 as well as North American BTV serotypes 2, 10, 11, 13 and 17 (Fig.1).

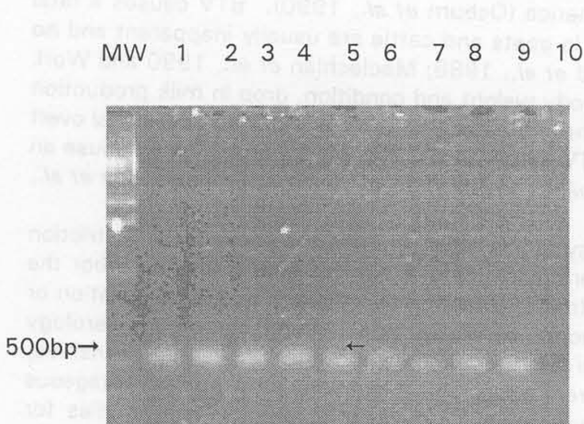

Fig. 1: Detection of (BTV) by the reverse transcriptase polymerase chain reaction (RT-PCR)-based assay.
Visualization of the 519-bp specific-BTV PCR product on ethidium bromide-stained agarose gel from 1.0 pg of RNA of the Sudanese BTV serotype 1, 2, 4 and 16; and North American BTV prototypes serotypes 2, 10, 11, 13 and 17. Lane MW: molecular weight marker; Lane 1: BTV-1; Lane 2: BTV-2; Lane 3: BTV-4; Lane 4: BTV-5; Lane 5: BTV-16; Lane 6: BTV-10; Lane 7: BTV-11; Lane 8: BTV-13; Lane 9: BTV-17; Lane 10: non infected Vero cell control.

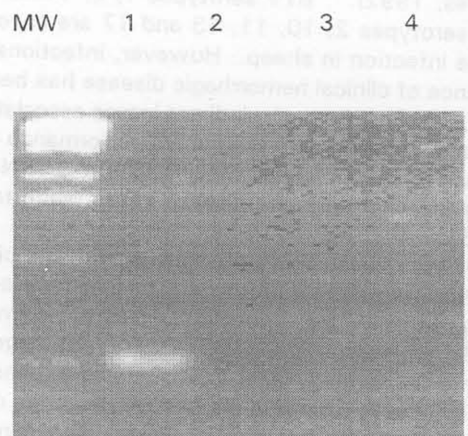

Fig. 2: Specificity of the RT-PCR for detection of BTV RNA. Amplification product was not detected from a high concentration of 1.0 ng RNA different EHDV serotypes, palyam viruses; or total nucleic acid extracts from non infected Vero cells Lane MW: molecular weight marker; Lane 1: 1 pg BTV-4; Lane: 2-3 EHDV prototypes serotypes 1; Lane 3: palyam virus isolate ; Lane 4: Vero total nucleic acid extract.

The specificity studies indicated that the amount of 1.0 ng RNA from closely related orbiviruses including EHDV, palyam virus; and total nucleic acid extracts from uninfected Vero cells failed to demonstrate PCR products (Fig.2).

Discussion

Outbreaks of bluetongue virus infections among sheep were observed in different part of the world. At least four serotypes of BTV designated BTV-1, BTV-2, BTV-4 and BTV-16 are known to be enzootic in the Sudan (Mohammed and Mellor., 1990). Very little information is available about orbiviruses originally isolated in the Sudan. Further studies on Sudanese BTV serogroup are necessary to determine their biology, ecology and molecular epidemiology. In addition the disease potential of these BTV serotypes is unknown (Mohammed and Taylor, 1987). The BTV serotypes used in this study represented a range of topotype viruses, isolated from a diverse geographic location in North America and Central Africa, recovered from different animal species. The described BTV RT-PCR assay using serogroup primers derived from segment 6 of BTV-11, which codes for NS1 (Gould *et al.*, 1992), reproducibly and specifically detected BTV RNA in infected cell cultures. Selection of the primers was based on the observation that the NS1 genome specific probe is the best of all BTV genome segments to produce positive hybridization signal compared to other nucleic acid probes (Aradaib *et al*, 1994a). The specific 519 bp PCR products, visualized on ethidium bromide-stained agarose gel, were obtained from all BTV RNA samples tested. The BTV RT-PCR assay was a simple procedure that efficiently detected all BTV isolates under the stringency condition used in this study. In the present study, the amplification procedure was rapid and the assay does not involve the use of hazardous and cumbersome radioactive laboratory procedures of working with radiolabled materials.

In previous report, the sensitivity studies of the EHDV RT-PCR protocol indicated that the PCR assay was capable of detecting the amount of 0.1 fg of total EHDV genomic dsRNA. The total molecular weight of the EHDV genome is 11.44×10^6 Da, and 0.1 fg of EHDV RNA corresponds to 5 viral particles (Aradaib *et al.*, 1994a; Abdalla *et al.*, 2002). The BTV RT-PCR amplification technology was approximately a million times more sensitive than the dot-blot hybridization using BTV cDNA probe, where at least a few nanograms of the dsRNA are required to produce a positive hybridization signal (Aradaib *et al.*, 1995).

The specificity studies indicated that the specific 519 bp PCR product was not amplified from a relatively high concentration of 1.0 ng of RNA from EHDV, palyam virus isolates or total nucleic acid extracts from Vero cell controls under the same stringency condition described in this study. Temperature and time for denaturation, primer annealing and extension, enzyme and $MgCl_2$ concentration, and number of cycles of the three temprature per time segments were very important for maintaining sensitivity and specificity of the PCR reaction.

The sample size used in this study represents all BTV isolates, so far, known to be enzootic in the Sudan and North America. The BTV RT-PCR assays provide supportive diagnostic techniques to the lengthy cumbersome conventional virus isolation procedures. The rapidity, sensitivity and specificity of the RT-PCR assay would greatly facilitate detection of BTV infection in an outbreak among susceptible ruminants. In the present study, of the 25 serotypes of BTV, we validated the detection of BTV serotypes 1, 2, 4, 10, 11, 13, 16 and 17 by the described BTV RT- PCR-based detection assay. All BTV isolates used in this study were positive to the PCR assay using the same oligonucleotides primers. The described RT-PCR assay will probably detect the remaining serotypes of BTV serogroup. However, additional research is necessary to validate the detection of the remaining serotypes of BTV serogroup by this RT-PCR assay.

The development of RT-PCR-based assays for detection of Central African and North American isolates of BTV, described in this study, provides the basis for future diagnosis of BTV. Further studies are in progress to determine the capability of the described BTV RT-PCR assay to detect additional serotypes of BTV serogroup, and to evaluate its potential as a sensitive and specific diagnostic assay through comparison with current diagnostic techniques used for detection of BTV infection.

In conclusion, the described serogroup-specific BTV RT-PCR assay using primers derived from genome segment 6 of BTV-11 provides rapid characterization of an unknown isolate of BTV from Sudan or North America.

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References

Aradaib, I. E., G. Y. Akita and B. I. Osburn, 1994a, Detection of epizootic hemorrhagic disease virus serotypes 1 and 2 in cell culture and clinical samples using polymerase chain reaction. J. Vet. Diag. Invest., 6: 143-147.

Aradaib, I. E., M. M. Sawyer and B. I. Osburn, 1994b, Experimental epizootic hemorrhagic disease virus infection in calves: Virologic and Serologic studies. J. Vet. Diagn. Invest., 6: 489-492.

Aradaib, I. E., G. Y. Akita, J. E. Pearson and B. I. Osburn, 1995, Comparison of polymerase chain reaction and virus isolation for detection of epizootic hemorrhagic disease virus in clinical samples from clinically infected deer. J. Vet. Diag. Invest., 7: 192-200.

Abdalla, M. A., I. E. Aradaib and B. I. Osburn, 2002. Evaluation of RT-PCR detection of Sudanese serotypes of epizootic hemorrhagic dsease virus serogroup. Veterinarski. Arhiv., 72: 53-63.

Borden, E. C., R. E. Shope and F. A. Murphy, 1971, Physicochemical and morphological relationships of some arthropod-borne viruses to bluetongue virus-a new taxonomic group. Physicochemical and serological studies. J. Gen. Virol., 3: 261-271.

Davies, F. G., J. N. Mungai and A. Pini, 1992. A new bluetongue virus serotype isolated in Kenya. Vet. Microbiol., 31: 25-32.

Fenner, F., H. G. Pereira and J. S. Porterfield, *et al.*, 1974, Family and generic names for virus approved by the international committee on taxonomy of viruses. Intervirology, 3:193-194.

Gard, G. P., R. P. Weir and S. J. Walsh, 1988, Arbovirus recovered from sentinel cattle using several virus isolation methods. Vet. Microbiol., 18:119.

Gould, A. R., K. A. McColl and L. I. Prichchord, 1992, Phylogenetic relationship between bluetongue virus and other orbiviruses, In bluetongue African horse sickness and related orbiviruses, ed. Walton TE, Osburb BI , CRC press, Boca Raton, Fl. p: 452-460.

Karrar, A. E., I. E. Aradaib, A. A. Majid, S. M. Elamin, Salih and B. I. Osburn, 2003. Detection of Sudanese isolates of Palyam serogroup of orbiviruses using reverse transcriptase polymerase chain reaction. J. Animal and Veterinary Advan., 12: 316-343.

Maclachlan, N. J., G. Jagels, P. V. Rossitto, P. F. Moore and H. W. Heidner, 1990, The pathogenesis of experimental bluetongue virus infection of calves. Vet. Pathol., 27: 223-229.

Mohammed E. H. M. and P. S. Mellor, 1990, Further studies on bluetongue-related Orbiviruses in the Sudan. Epidemiology and infection, 105: 619-632.

Mohammed, M. E. H. and W. P. Taylor, 1987, Infection with Bluetongue and related orbiviruses in the Sudan detected by the study of sentinel calf herds. Epidemiol. and Infect., 99: 533-545.

Osburn, B. I., I. E. Aradaib and C. Schore, 1994. Comparison of Bluetongue, and epizootic hemorrhagic disease complex. Proceedings of the 13th International Symposium of the World Association of Veterinary Microbiologist, Immunologist and Infectious Disease Specialist, perugia, Montova, Italy.

Pearson, J. E., G. A. Gustafson, A. L. Shafer and A. D. Alstad, 1992, Diagnosis of Bluetongue virus and epizootic hemorrhagic disease. Proceedings of the 2nd International Symposium on Bluetongue, African horse sickness and related Orbiviruses, ed. Walton TE, Osburn BI, CRC Press, Boca Raton, Fl. p: 533-546.

Shope, R. E., L. G. Macnamara and R. Mangold, 1995. Report on the deer mortality, Epizootic hemorrhagic disease of deer, New Jersey Outdoors, 6: 17-21.

Work, T. M., D. A. Jessup and M. M. Sawyer, 1992, Experimental bluetongue and epizootic hemorrhagic disease virus infection in California black-tailed deer. J. wildlife Diseases, 4:623-628.