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RESEARCH ARTICLE

CHARACTERIZATION OF A BLUETONGUE VIRUS ISOLATE FROM SHEEP OF MARATHWADA REGION IN MAHARASHTRA

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ABSTRACT

Bluetongue is an arthropod borne viral disease of ruminants. Bluetongue virus was isolated from clinically affected sheep of Marathwada region in Maharashtra. In initial studies inoculation of the isolate in chicken egg embryo by yolk sac route revealed characteristic cherry red discoloration and death of the chicken embryo. The isolate was adapted on BHK-21 cell line. The bluetongue virus isolate gave characteristic cytopathic effect (CPE) consisting of rounding of cells and cell detachment from 24 hours post inoculation. Further to confirm the bluetongue virus isolate by molecular characterization, the viral RNA was extracted and purified. The extracted RNA was subjected to electrophoresis in 1 % agarose gel. A total of 10 characteristic bands were observed upon electrophoresis thereby confirming the isolate. The results warrant continuous surveillance of sheep in Marathwada region of Maharashtra.

INTRODUCTION

Isolation and identification of virus is essential to confirm role of pathogen in infectious diseases. Researchers have isolated and identified bluetongue virus in India from various outbreaks (Mehrotra, 1991 and Deshmukh and Gujar, 1999). The BHK-21 cell line is used to isolate and maintain bluetongue virus isolates. Physical and chemical characterizations of isolates are of great help in confirming the isolate (Kale, 2002). However, use of molecular techniques like RNA profiling of bluetongue virus helps in confirmation and these techniques are successfully used (Mehrotra *et al*., 1995 & Roy, 1996). In the present investigation bluetongue virus isolate was used in electrophoresis study.

MATERIALS AND METHODS

History

Clinical samples were collected from sheep suffering from bluetongue disease at the height of temperature. Typical symptoms of oedema of eyelids, head and torticollis are observed in the affected sheep. The bluetongue virus isolate was inoculated in chicken embryonated eggs to observe characteristic cherry red discoloration of the chicken embryo. It was also adapted on BHK-21 cell line.

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Maintenance of bluetongue virus isolate

The bluetongue virus isolate was maintained in the Department of Microbiology, College of Veterinary and Animal Sciences, Parbhani and further passaged in BHK-21 cell line prior to its use in the present investigation.

Extraction of viral RNA

The dsRNA of bluetongue virus isolate was extracted (Roy, 1996). Viral supernatant was collected at 60 % CPE and centrifuged at 4000 rpm for 15 min at 4 ^{o}C. After washing and centrifugation the cell pellet was dissolved in 2 mM Tris EDTA and homogenized. Supernatant was collected after centrifugation at 2000 rpm for 5 min at 4 ^{o}C, 4 M NaCl and 10 % SDS were added to a final concentration of 0.4 M and 1 % respectively and extracted with buffer saturated phenol-chloroform-isoamylalcohol and centrifuged at 9000 rpm for 10 min. 2.5 volume of chilled ethanol added to aqueous phase and kept overnight at 4 ^{o}C and centrifuged at 9000 rpm for 30 min and to the pellet 1 mM EDTA was added. In this 4 M LiCl was added to final concentration of 2 M and kept overnight at 4 ^{o}C and centrifuged at 9000 rpm for 10 min at 4 ^{o}C. Supernatant was collected and 8 M LiCl was added to the final concentration of 4 M and kept for overnight at 4 ^{o}C. This was centrifuged at 9000 rpm for 30 min and pellet was collected and suspended in 2 mM Tris EDTA to which 4 M NaCl was added to a final concentration of 0.4 M. In this 2.5 volume of chilled ethanol was added and centrifuged at 9000 rpm for 30 min at 4 ^{o}C and

washed with 70 % ethanol. Dried pellet was suspended in nuclease free water.

Agarose gel electrophoresis of dsRNA

The dsRNA of bluetongue virus isolate was subjected to electrophoresis using 1 % agarose in 0.5 x TBE buffer stained with ethidium bromide. (Pedley *et al*., 1988). The process was allowed to run for 2.30 hours at 55 volts. The viral dsRNA was seen under ultraviolet transilluminator.

RESULTS

Maintenance of isolate

The isolate of bluetongue virus was maintained on BHK-21 cell line for its molecular characterization during the study period. A total of five serial passages for the bluetongue virus isolate were made on the BHK-21 cell line. A 5^{th} passaged virus was used for the present study. The CPE was observed at 12, 24, 36, 48, 72, 84 and 96 hours post inoculation. The isolate showed 50 % CPE from 36 hours post inoculation on 10^{th} passage. The CPE consisted of rounding and detachment of the cells. Similar observations were reported by Kale, 2002.

RNA profiling of BTV isolate

The extracted RNA was used in electrophoresis through 1 % agarose gel. Ethidium bromide stain was used to stain the RNA segments. A total of 10 segments were observed in the extracted RNA of the isolate.

DISCUSSION

Earlier workers have observed 10 segments in Indian isolates of bluetongue virus (Mehrotra *et al*., 1995 and Roy, 1996). Similar observations in the present study indicated that the virus is indistinguishable than the bluetongue virus.

The bluetongue virus isolate used in the investigation was deposited in the virus repository at All India Network Programme on Bluetongue at Indian Veterinary Research Institute, Mukteshwar.

Conflict of Interest

None

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