LETTER TO THE EDITOR

GENOMIC DIVERSITY OF INDIAN ISOLATES OF BLUETONGUE VIRUS

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Bluetongue virus (BTV), the etiological agent of bluetongue belongs to the genus *Orbivirus,* the family *Reoviridae*. There are 24 BTV serotypes reported worldwide. BTV genome consists of 10 segments of double-stranded RNA (dsRNA) that can be separated by agarose or polyacrylamide gel electrophoresis (PAGE) (*1*). Differences in migration patterns have been observed among different serotypes and even among different isolates of the same serotype (*1–3*). Recently, the PAGE analysis of segmented dsRNA virus genome has attracted attention for its possible use in the detection of genetic diversity of BTV serotypes/ strains/isolates. Very little information is available about the genome diversity of Indian isolates of BTV.

In this study, the dsRNA genome profiles of six Indian isolates of BTV, namely BTV-18 Bhopal, BTV-18 Srinagar, BTV-23 Rahuri, BTV-23 Dehradun, BTV-23 Rishikesh, and BTV-23 Bangalore were compared by PAGE.

Total RNA was extracted with 750 µl of TRIzol^{R} reagent (Invitrogen) from the pellets originating from 1 ml aliquots of BTV cultures according to the manufacturer's instructions. Total RNA was dissolved in 200 µg of nuclease free water, mixed with 200 µl of 4 mol/l LiCl and kept at 4°C for 8 hrs to precipitate single-stranded cellular RNA (ssRNA). The latter was pelleted at 12,000 x g for 15 mins. The dsRNA in the supernatant was precipitated with an equal volume (400 µl) of isopropanol and dissolved in 50 µl of DEPC-treated water. The dsRNAs of the six BTV isolates were separated by non-denaturing PAGE in 10% discontinuous gels (*4, 5*) and stained with silver nitrate (*6*).

The figure shows that all the six isolates exhibited 10 classical dsRNA segments with identical migration pattern. However, three of them, namely BTV-23 Rishikesh (lane RII), BTV-23 Rahuri (lane Ra), and BTV-18 Srinagar (lane S). displayed additional segments, located between the segments 5 and 6, 6 and 7, 7 and 8, and below the segment 10. Three isolates, namely BTV-18 Bhopal (lane B), BTV-23 Dehradun (lane D), and BTV-23 Bangalore (lane Ban) had 10 classical segments only.

Vaccine strains of BTV-10 and BTV-13 have been shown to contain additional segments migrating between the segments 3 and 4 (*7*) Out of 32 bovine BTV isolates one was found to have a double band at the segment 7 (*2*). An additional band at the segment 10 was detected in an Australian BTV-21 isolate (*8*) and three additional bands (one between the segments 3 and 4 and two between the segments 6 and 7) were observed in a BTV-1 isolate (*3*).

The extra segment(s) observed in our study could be a concatemer(s) (*2, 7*). The virus with a concatemeric gene is known to interfere with the replication of wild-type parent virus with a monomeric gene (*8*). It is hypothesized that a mixed serotype BTV infection in animal population or continuous passaging of the virus in the cell cultures could lead to concatemer formation (*9, 10*).

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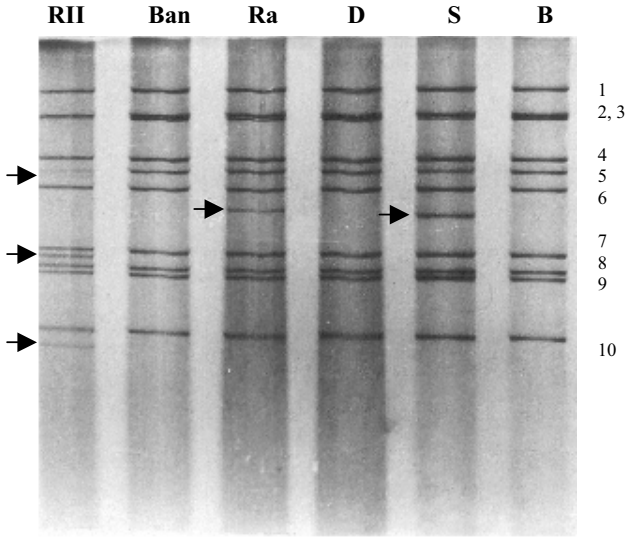
Abbreviations: BTV = Bluetongue virus; dsRNA = double-stranded RNA; ssRNA = single-stranded RNA: PAGE = polyacrylamide gel electrophoresis



The obtained results lead us to believe that all the six Indian isolates of BTV-18 and BTV-23 investigated in this study have diverged after evolving from a common gene pool. However, further studies based on plaque purification and gene sequencing are needed to unveil the mystery of the presence of additional gene segments in some BTV isolates.

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