

The Genome Sequence of Bluetongue Virus Type 2 from India: Evidence for Reassortment between Eastern and Western Topotype Field Strains

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Bluetongue virus type 2, isolated in India in 1982 (IND1982/01), was obtained from the Orbivirus Reference Collection at IAH Pirbright (http://www.reoviridae.org/dsRNA_virus_proteins/ReoID/btv-2.htm#IND1982/01). Full genome sequencing and phylogenetic analyses show that IND1982/01 is a reassortant virus containing genome segments derived from both eastern and western topotypes. These data will help to identify further reassortment events involving this or other virus lineages in the subcontinent.

Bluetongue virus (BTV) is the type species of the genus *Orbivirus* of the family *Reoviridae* (1, 11). The bluetongue virus is nonenveloped, with a three-layered, icosahedral protein capsid surrounding the 10 double-stranded RNA (dsRNA) genome segments (Seg-1 to Seg-10), and it encodes seven structural proteins (VP1 to VP7) and four distinct nonstructural proteins (NS1, NS2, NS3/3a, and NS4) (2, 4, 5, 13). The virus is transmitted by adult *Culicoides* “biting” midges (Diptera: Ceratopogonidae) and can infect a wide range of animal species, including ruminants, camelids, and some carnivores (9, 12). BTV is the etiological agent of bluetongue (BT), a hemorrhagic disease of ruminants (notably sheep, cattle, and some deer species) (10). However, the outcome of infection can vary from inapparent to fatal, depending on the virus strain, as well as the species, breed, age, and immune status of the host.

Severe BT outbreaks typically occur either when susceptible ruminants (particularly sheep) are introduced to areas where BT is endemic or when the virus spreads to areas containing susceptible/immunologically naive ruminants as well as vector-competent *Culicoides* species (as was recently seen in Europe) (7, 14).

Sequencing studies indicate that BTV strains in different geographic regions have evolved separately over long periods of time, acquiring multiple point mutations and developing characteristic regional variants, or topotypes, of each genome segment. Bluetongue is endemic in India, with isolation data or serological evidence for at least 21 of the 26 known serotypes (13). Full-genome sequence data have already been published for an isolate of BTV-2 from Taiwan (6), which belongs to the major eastern (e) topotype, as well as for four western (w) strains of BTV-2 (3). However, full-genome data are not yet available for BTV-2 from India.

BTV strain IND1982/02 was isolated in India in 1982 and is stored in the reference collection at IAH Pirbright (http://www.reoviridae.org/dsRNA_virus_proteins/ReoID/btv-2.htm#IND1982/01). Genomic dsRNA was extracted from IND1982/01 grown in BHK-21 cells and used to synthesize full-length cDNAs by reverse transcription (RT)-PCR (full-length amplification of cDNA; FLAC) as previously described (8). These PCR products were purified and sequenced on an ABI 3730 capillary DNA-sequencing instrument,

using phased primers to generate near-terminal sequences (8) and segment-specific walking primers.

Seg-1 to Seg-10 of IND1982/01 are 3,944, 2,943, 2,772, 1,981, 1,764, 1,635, 1,156, 1,125, 1,052, and 822 bp in length. They encode proteins with amino acid lengths as follows: VP1, 1,302; VP2, 962; VP3, 901; VP4, 644; NS1, 552; VP5, 526; VP7, 349; NS2, 354; VP6/NS4, 330/77; and NS3/NS3a, 229/216. Phylogenetic analyses showed that nine genome segments from IND1982/01 are unique, belonging to eastern lineages. However, Seg-5/NS1 exists as a monophyletic group within the major western BTV topotype, demonstrating for the first time the occurrence of reassortants (r) between eastern and western topotype field strains within the Indian subcontinent. These sequence data, for a reference strain of BTV-2r, will facilitate future investigations of molecular characteristics and phylogenetic relationships of other BTV strains in India.

Nucleotide sequence accession numbers. Nucleotide sequences for IND1982/01 have been deposited in GenBank under accession numbers [AJ585152](#), [AJ586675](#), and [AM900382](#) for Seg-2, Seg-6, and Seg-8 and JQ713557 to JQ713563 for Seg-1, Seg-3, Seg-4, Seg-5, Seg-7, Seg-9, and Seg-10.

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