

The Genome Sequence of Bluetongue Virus Type 10 from India: Evidence for Circulation of a Western Topotype Vaccine Strain

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Bluetongue virus is the type species of the genus Orbivirus in the family Reoviridae. We report the first complete genome sequence of an isolate (IND2004/01) of bluetongue virus serotype 10 (BTV-10) from Andhra Pradesh, India. This isolate, which is stored in the Orbivirus Reference Collection (ORC) at IAH Pirbright, shows >99% nucleotide identity in all 10 genome segments with a vaccine strain of BTV-10 from the United States.

B(BT), an arthropod-transmitted disease that can be fatal, particularly in sheep and certain wild ruminants (including whitetailed deer). The virus can infect most ruminants (including cattle and goats), as well as camelids and certain carnivores (12, 13). BTV is transmitted to its ruminant hosts primarily by vector-competent species of biting midges (*Culicoides* spp.). The incidence of BT is therefore dependent on the geographic distribution and seasonal activity of adults of these vector species (4).

BTV is one of 22 virus species within the genus *Orbivirus* in the family *Reoviridae* (2, 15), and to date 26 BTV serotypes have been described (9). The icosahedral BTV particle is nonenveloped, with three distinct capsid layers, including the subcore, outer core, and outer capsid, enclosing a 10-segmented double-stranded RNA (dsRNA) genome encoding seven structural proteins (VP1 to VP7) (9, 10, 14, 18) and four nonstructural proteins (NS1, NS2, NS3/3a, and NS4) (3, 5, 7, 15, 19, 20).

BTV strains show variations in their nucleotide sequences that reflect their origins from different geographic regions around the world (6, 8, 17), with a clear division of most genome segments into eastern and western groups/topotypes (8, 16). Geographical separation over long periods of time has allowed bluetongue viruses in different regions to acquire unique point mutations, some of which may make them particularly well suited to transmission and survival in their local ecosystems (8).

A sequence database for BTV strains from diverse geographical areas is needed to support molecular epidemiological studies to determine the origins and movements of different virus lineages. We have generated whole-genome sequence data, using full-length amplification of cDNA (FLAC) techniques (1, 11), for a BTV-10 strain (IND2004/01) isolated in 2004 from Andhra Pradesh in India (www.reoviridae.org/dsRNA_virus _proteins/ReoID/btv-untyped.htm#IND2004/01). Sequences of the 10 genome segments (Seg-1 to Seg-10) were obtained using generic primers designed for BTV termini (phased primers) (11), as well as by gene walking on an ABI 3730 DNA capillary sequencing instrument.

The complete genome of IND2004/01 is 19,184 bp. The sizes (in base pairs) of Seg-1 to Seg-10 are 3,944, 2,926, 2,772, 1,981, 1,769, 1,638, 1,156, 1,127, 1,049, and 822, respectively. They encode seven structural proteins, VP1 to VP7, with amino acid lengths as follows: VP1, 1,302; VP2, 956; VP3, 901; VP4, 644; VP5,

526; VP6, 329; and VP7, 349. The numbers of amino acids in the four nonstructural proteins are as follows: NS1, 552; NS2, 354; NS3/NS3a, 229/216; and NS4, 77.

Phylogenetic analyses of sequences show that all genome segments of IND2004/01 group within the major western topotype and most closely with a BTV-10 vaccine strain from the United States (>99% nucleotide identity in each genome segment). This very close relationship raises concerns over the introduction of western vaccine strains into India. The most likely mechanisms for such introductions appear to reflect human involvement, either via importation of infected animals or by the use of live attenuated BTV-10 vaccines from the United States within the subcontinent.

Nucleotide sequence accession numbers. The nucleotide sequences for IND2004/01 have been deposited in GenBank under accession numbers JQ740771 to JQ740780 for Seg-1 to Seg-10.

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