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GENOME ANNOUNCEMENT

The Genome Sequence of a Reassortant Bluetongue Virus Serotype 3 from India

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All 10 genome segments (Seg-1 to 10—a total of 19,188 bp) were sequenced from a strain of bluetongue virus serotype 3 (BTV-3) from India (strain IND2003/08). Sequence comparisons showed that nine of the genome segments from this virus group with other eastern topotype strains. Genome Seg-2 and Seg-6 group with eastern BTV-3 strains from Japan. However, Seg-5 (the NS1 gene) from IND2003/08 belongs to a western lineage, demonstrating that IND2003/08 is a reassortant between eastern and western topotype bluetongue viruses. This confirms that western BTV strains have been imported and are circulating within the subcontinent.

Bluetongue virus is the type species of the genus *Orbivirus*, within the family *Reoviridae*. The genome of bluetongue virus (BTV) is composed of 10 linear segments of double-stranded RNA (dsRNA), packaged within a three-layered protein capsid (2, 15, 21). The virus genome codes for 7 structural proteins (VP1 to VP7) (14, 18, 20) and 4 distinct nonstructural proteins (NS1, NS2, NS3/3a, and NS4) (2, 3, 5, 15). Although BTV can infect all ruminant species, severe disease (bluetongue [BT]) occurs primarily in naive sheep and some deer species (12). There are 26 BTV serotypes, identified by the specificity of interactions between the outer capsid (particularly protein VP2) and the neutralizing antibodies generated during infection of the host (8).

BTV is endemic between approximately 45 to 53°N and 35°S, including areas of North and South America, Africa, the Indian subcontinent, and Australasia (4, 13, 17, 22). BTV strains show variations in nucleotide sequence that reflect their geographic origins, with clear division of most genome segments into "eastern" and "western" groups/topotypes (7, 10, 16).

Severe BT outbreaks have been reported in India since 1964, with case fatality rates up to 31% in indigenous sheep breeds (17, 19). RNA was extracted from BHK-21 cells infected with an Indian isolate of BTV serotype 3 (BTV-3) from 2003 (IND2003/08) and then used for full-length amplification of cDNAs (FLAC) (1, 11). PCR products corresponding to the 10 BTV genome segments of IND2003/08 were sequenced using a 3730 DNA analyzer (Applied Biosystems). "Phased" primers generated near-terminal sequences (11), while "walking" segment-specific primers were used to determine the full nucleotide sequence. Epidemiological data for this BTV strain are available on the dsRNA web page (www.reoviridae.org/dsRNA_virus_proteins/ReoID/btv-3.htm#IND2003/08).

The complete genome of IND2003/08 was determined at 19,188 bp, including segments 1 (Seg-1) to 10 (Seg-10) (3,944, 2,934, 2,772, 1,981, 1,765, 1,637, 1,156, 1,125, 1,052, and 822 bp, respectively), encoding proteins VP1 (1,302 amino acids [aa]), VP2 (959 aa), VP3 (901 aa), VP4 (644 aa), VP5 (526 aa), VP6 (330 aa), and VP7 (349 aa) and NS1 (552 aa), NS2 (354 aa), NS3/NS3a (229 aa/216 aa), and NS4 (77 aa).

Phylogenetic analyses of IND2003/08 show that nine genome segments belong to an eastern lineage/topotype, with Seg-2 and Seg-6 (encoding outer capsid proteins VP2 and VP5) showing the highest identity levels (90% and 91%, respectively) to earlier Japanese (eastern) isolates (strain ON-6/B/98) of BTV-3. However, Seg-5/NS1 of IND2003/08 showed up to 99% identity with western topotype viruses (prototype 600565 strain), providing further evidence for the introduction of western BTV strains and reassortment between eastern and western field strains in India (6, 9, 23). Western topotype or reassortant BTV strains may be at least partly responsible for the increased virulence of bluetongue outbreaks seen in India, in indigenous sheep breeds. Seg-5 of IND2003/08 showed only 89% identity with a western BTV-10 vaccine strain detected in India (9) and is not therefore derived from this source.

These data represent the first report of a complete genome sequence for BTV-3 from India, supporting further molecular epidemiology studies of BTV in India and other geographic regions.

Nucleotide sequence accession numbers. The GenBank accession numbers for Seg-1 to Seg-10 of IND2003/08 are JQ771813 to JQ771822, respectively.

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