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GENOME ANNOUNCEMENT

Complete Genome Sequence Analysis of a Reference Strain of Bluetongue Virus Serotype 16

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The entire genome of the reference strain of bluetongue virus (BTV) serotype 16 (strain RSArrrr/16) was sequenced (a total of 23,518 base pairs). The virus was obtained from the Orbivirus Reference Collection (ORC) at IAH, Pirbright, United Kingdom. The virus strain, which was previously provided by the Onderstepoort Veterinary Research Institute in South Africa, was originally isolated from the Indian subcontinent (Hazara, West Pakistan) in 1960. Previous phylogenetic comparisons show that BTV RNA sequences cluster according to the geographic origins of the virus isolate/lineage, identifying distinct BTV topotypes. Sequence comparisons of segments Seg-1 to Seg-10 show that RSArrrr/16 belongs to the major eastern topotype of BTV (BTV-16e) and can be regarded as a reference strain of BTV-16e for phylogenetic and molecular epidemiology studies. All 10 genome segments of RSArrrr/16 group closely with the vaccine strain of BTV-16 (RSAvvvv/16) that was derived from it, as well as those recently published for a Chinese isolate of BTV-16 (>99% nucleotide identity), suggesting a very recent common ancestry for all three viruses.

Bluetongue (BT) is an economically important arthropod-borne disease, caused by the bluetongue virus (BTV), which can infect most ruminants (including sheep, goats, cattle, and deer). *Bluetongue virus* is the type species of the genus *Orbivirus* (family *Reoviridae*). The BTV genome, which codes for 7 structural and 4 nonstructural proteins, is composed of 10 linear segments of double-stranded RNA (dsRNA) that are packaged within an icosahedral, ~90-nm-diameter, triple-layered protein capsid (1, 2, 4, 11, 12, 14, 15).

Multiple BTV strains have recently emerged in Europe, emphasizing the importance of accurate strain identification (through molecular typing and epidemiology studies) to support the development and deployment of appropriate vaccines.

BTV-16 was first isolated in 1960 in Pakistan (5, 13). We report the complete genome sequence of the BTV-16 reference strain (RSArrrr/16) (which was supplied to the Orbivirus Reference Collection [ORC] at IAH, Pirbright, by the Onderstepoort Veterinary Institute [OVI], South Africa). The genome of RSArrrr/16 was converted to cDNA by full-length amplification of cDNAs (FLAC) (10). Corresponding cDNAs for each segment were purified and sequenced on a 3730 capillary sequencer (Applied Biosystems), using "phased primers" to generate near-terminal sequences (10) and "walking-segment-specific primers" to determine the full nucleotide sequence. The sizes (in base pairs) of Seg-1 to Seg-10 from RSArrrr/16 are 3,944, 2,935, 2,772, 1,981, 1,763, 1,637, 1,156, 1,127, 1,054, and 822, respectively, encoding structural proteins VP1 to VP7 (with open reading frame [ORFs] sizes of 1,302 aa for VP1, 959 for VP2, 901 for VP3, 644 for VP4, 526 for VP5, 330 for VP6, and 349 for VP7) and four nonstructural proteins, NS1 to NS4 (length of 552 aa for NS1, 354 for NS2, 229/216 for NS3/NS3a, and 77 for NS4).

Previously, two BTV-16 field strains from China and Australia have been fully sequenced (3, 16). All genome segments of the BTV-16 reference strain (RSArrrr/16) showed >99% sequence identity to the BTV-16 vaccine strain (RSAvvvv/16) that was derived from it and to the Chinese BTV-16 (strain BN96/16) isolated from a sheep in Yunnan province during 1996 (16). These data indicate that these three virus strains are all derived from a common source. In contrast, the genome segments of RSArrrr/16 show lower levels of identity (90% to 95%) with the BTV-16 from Australia (strain DPP96), indicating that it represents a distinct virus strain/lineage, although still within the major eastern topotype.

Multiple BTV serotypes are circulating in the Indian subcontinent (13), including both eastern and western topotypes (6–9), inevitably providing multiple opportunities for genome segment reassortment. Full genome sequence data and identification of RSArrrr/16 as an eastern-topotype reference strain (RSArrrr/16e) will help in the identification of novel reassortant viruses that are generated in the field. These data also indicate the value of full-genome sequencing during characterization of novel BTV isolates.

Nucleotide sequence accession numbers. Nucleotide sequences for RSArrrr/16 have been deposited in GenBank under accession numbers AJ585149 (Seg-2), DQ186796 (Seg-3), AM773707 (Seg-5/NS1 gene), and AJ586719 (Seg-6) and JX129381 to JX129386 for Seg-1, Seg-4, and Seg-7 to Seg-10, respectively.

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