

# Full Genome Sequence of a Western Reference Strain of Bluetongue Virus Serotype 16 from Nigeria

Peter P. C. Mertens,<sup>a</sup> Narender S. Maan,<sup>a,b</sup> Manjunatha N. Belaganahalli,<sup>a</sup> Karam Pal Singh,<sup>c</sup> Kyriaki Nomikou,<sup>a</sup> Sushila Maan<sup>a,b</sup>

Vector-borne Diseases Programme, the Pirbright Institute, Woking, Surrey, United Kingdom<sup>a</sup>; College of Veterinary Sciences, LLR University of Veterinary and Animal Sciences, Hisar, Haryana, India<sup>b</sup>; Pathology Laboratory, Centre for Animal Disease Research and Diagnosis, Indian Veterinary Research Institute, Izatnagar, India<sup>c</sup>

**The genome of NIG1982/10, a Nigerian bluetongue virus serotype 16 (BTV-16) strain, was sequenced (19,193 bp). Comparisons to BTV strains from other areas of the world show that all 10 genome segments of NIG1982/10 are derived from a western lineage (w), indicating that it represents a suitable reference strain of BTV-16w.**

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Address correspondence to Peter P. C. Mertens, peter.mertens@pirbright.ac.uk, or Sushila Maan, sushilamaan105@gmail.com.

Bluetongue virus (BTV) is a double-stranded RNA virus (genus *Orbivirus*, family *Reoviridae*) (1, 2) that infects domesticated and wild ruminants. The virus is transmitted between mammalian hosts by vector-competent *Culicoides* species, and it can be transmitted vertically in ruminants or by ingestion of infected tissues. Bluetongue (BT) represents a significant threat to livestock industries worldwide. The recent emergence of 10 BTV serotypes (since 1998) and massive outbreaks in Europe caused by BTV serotype 8, western lineage (BTV-8w) (2006 to 2009), illustrate the threat posed by emerging arboviral diseases (3).

The BTV capsid is icosahedral and nonenveloped, composed of three concentric protein layers (4). The 10 linear double-stranded RNA (dsRNA) genome segments (Seg-1 to Seg-10) encode 7 structural proteins (VP1 to VP7) and at least 4 nonstructural proteins (NS1, NS2, NS3/3a, and NS4) (5, 6). VP2 and VP5 (encoded by Seg-2 and Seg-6, respectively) are the most variable BTV proteins. They form the outer capsid, representing a target for neutralizing antibodies (particularly VP2), and determine virus serotype (26 known serotypes) (7, 8).

We report the complete genome sequencing of a 1982 Nigerian BTV-16 isolate (NIG1982/10) provided by the Onderstepoort Veterinary Research Institute, South Africa ([http://www.reoviridae.org/dsRNA\\_virus\\_proteins/ReoID/virus-nos-by-country.htm](http://www.reoviridae.org/dsRNA_virus_proteins/ReoID/virus-nos-by-country.htm)). The NIG1982/10 genome was converted to cDNA by full-length amplification of cDNAs (FLAC) and sequenced as described elsewhere (9).

The NIG1982/10 genome comprises 19,193 bp, with Seg-1 to Seg-10 being 3,944, 2,935, 2,772, 1,981, 1,773, 1,637, 1,156, 1,125, 1,048, and 822 bp, respectively. The structural proteins are encoded by Seg-1 (VP1), 1,302 amino acids (aa); Seg-2 (VP2), 959 aa; Seg-3 (VP3), 901 aa; Seg-4 (VP4), 644 aa; Seg-6 (VP5), 526 aa; Seg-7 (VP7), 349 aa; and Seg-9 (VP6), 329 aa. The nonstructural proteins are encoded by Seg-5 (NS1), 552 aa; Seg-8 (NS2), 354 aa; Seg-9 (NS4), 77 aa; and Seg-10 (NS3/NS3a), 229/216 aa.

Four eastern BTV-16 strains (Indian, Chinese, Australian, and a South African reference strain) have been sequenced (10–13). All 10 genome segments of the reference, vaccine, and Chinese strains (RSArxxx/16, RSAvvvv/16, and BN96/16) (13) are derived

from a very recent common ancestor, showing >99% sequence identity. A BTV-16 strain from Australia (strain DPP96) represents a distinct virus lineage within the major eastern topotype (e). The Indian strain of BTV-16 is a reassortant, showing high identity levels (92 to 97%) to RSArxxx/16 in most genome segments but containing a western topotype (w) Seg-5 (89% nucleotide [nt] identity).

All 10 genome segments of NIG1982/10 are derived from a western lineage, with only 77% to 84% nt identity to the eastern topotype reference strain RSArxxx/16 from Pakistan and 76.4% to 83% identity to the Australian BTV-16e strain (DPP96). Seg-2 (VP2) and Seg-6 (VP5) of NIG1982/10 show up to 95% nt (97% aa) and 97% nt (100% aa) identities, respectively, to other BTV-16w strains, and the conserved genome segments (Seg-1, 3, 4, 5, 7, 8, and 9) of NIG1982/10 show >85.4% identity to other western topotype BTVs. The topotype of Seg-10 from NIG1982/10 is less clear-cut, with ≥81.8% and 81.2% to 84.5% nt identities to BTVw and BTVe strains, respectively.

We conclude that NIG1982/10 is suitable as a reference strain of BTV-16w for serological, phylogenetic, and molecular epidemiology studies. The use of full-genome sequencing for the characterization of novel BTV isolates helps to identify virus topotype, as well as cross-topotype reassortant viruses generated in the field.

**Nucleotide sequence accession numbers.** The nucleotide sequences for NIG1982/10 are deposited in GenBank under accession no. [AJ585150](https://www.ncbi.nlm.nih.gov/nuclot/AJ585150) (Seg-2), [DQ186819](https://www.ncbi.nlm.nih.gov/nuclot/DQ186819) (Seg-3), [AJ586694](https://www.ncbi.nlm.nih.gov/nuclot/AJ586694) (Seg-6), and [KC853052](https://www.ncbi.nlm.nih.gov/nuclot/KC853052) to [KC853058](https://www.ncbi.nlm.nih.gov/nuclot/KC853058) for Seg-1, Seg-4, Seg-5/NS1, and Seg-7 to Seg-10, respectively.

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## REFERENCES

1. Attoui H, Maan S, Anthony SJ, Mertens PPC. 2009. Bluetongue virus, other orbiviruses and other reoviruses: their relationships and taxonomy, p 23–52. *In* Mellor PS, Baylis M, Mertens PPC (ed), *Bluetongue monograph*, 1st ed. Elsevier/Academic Press, London, United Kingdom.
2. Mertens PPC, Maan S, Samuel A, Attoui H. 2005. Orbiviruses, *Reoviridae*, p 466–483. *In* Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA (ed), *Virus taxonomy*. Eighth Report of the International Committee on Taxonomy of Viruses. Elsevier/Academic Press, London, United Kingdom.
3. Zientara S, Sánchez-Vizcaino JM. 2013. Control of bluetongue in Europe. *Vet. Microbiol.* 165:33–37.
4. Maan S, Maan NS, Nomikou K, Veronesi E, Bachanek-Bankowska K, Belaganahalli MN, Attoui H, Mertens PP. 2011. Complete genome characterisation of a novel 26th bluetongue virus serotype from Kuwait. *PLoS One* 6:e26147. doi:10.1371/journal.pone.0026147.
5. Belhouchet M, Mohd Jaafar F, Firth AE, Grimes JM, Mertens PPC, Attoui H. 2011. Detection of a fourth orbivirus non-structural protein. *PLoS One* 6:e25697. doi:10.1371/journal.pone.0025697.
6. Ratnier M, Caporale M, Golder M, Franzoni G, Allan K, Nunes SF, Armezzani A, Bayoumy A, Rixon F, Shaw A, Palmarini M. 2011. Identification and characterization of a novel non-structural protein of bluetongue virus. *PLoS Pathog.* 7:e1002477. doi:10.1371/journal.ppat.1002477.
7. Maan S, Maan NS, Samuel AR, Rao S, Attoui H, Mertens PP. 2007. Analysis and phylogenetic comparisons of full-length VP2 genes of the 24 bluetongue virus serotypes. *J. Gen. Virol.* 88:621–630.
8. Maan NS, Maan S, Belaganahalli MN, Ostlund EN, Johnson DJ, Nomikou K, Mertens PP. 2012. Identification and differentiation of the twenty six bluetongue virus serotypes by RT-PCR amplification of the serotype-specific genome segment 2. *PLoS One* 7:e32601. doi:10.1371/journal.pone.0032601.
9. Maan S, Rao S, Maan NS, Anthony SJ, Attoui H, Samuel AR, Mertens PP. 2007. Rapid cDNA synthesis and sequencing techniques for the genetic study of bluetongue and other dsRNA viruses. *J. Virol. Methods* 143:132–139.
10. Maan S, Maan NS, Singh KP, Belaganahalli MN, Guimera M, Pullinger G, Nomikou K, Mertens PP. 2012. Complete genome sequence analysis of a reference strain of bluetongue virus serotype 16. *J. Virol.* 86:10255–10256.
11. Minakshi P, Singh R, Ranjan K, Kumar P, Joshi CG, Reddy YK, Prasad G. 2012. Complete genome sequence of bluetongue virus serotype 16 of goat origin from India. *J. Virol.* 86:8337–8338.
12. Boyle DB, Bulach DM, Amos-Ritchie R, Adams MM, Walker PJ, Weir R. 2012. Genomic sequences of Australian bluetongue virus prototype serotypes reveal global relationships and possible routes of entry into Australia. *J. Virol.* 86:6724–6731.
13. Yang T, Liu N, Xu Q, Sun E, Qin Y, Zhao J, Wu D. 2011. Complete genomic sequence of bluetongue virus serotype 16 from China. *J. Virol.* 85:13472. doi:10.1128/JVI.06402-11.