

Short Communications

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## Whole Genome Sequence Analysis of Bluetongue Virus Serotype 12 from Northern India

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### ABSTRACT

#### Keywords

Bluetongue virus, BTV-12, Complete genome, Sequence, India.

#### Article Info

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All ten genome segments of a field strain (isolate ID: IND2016/118) of Bluetongue virus serotype 12 (BTV-12), isolated from affected sheep from village Pataudi, Gurugram, Haryana, northern India were sequenced. The total genome size was 19,186 bp. Sequence comparisons of complete genome except Seg-2, Seg-5, Seg-6, Seg-7 and Seg-10, showed that IND2016/118 belonged to the major eastern topotype of BTV.

### Introduction

Domestic as well as wild ruminants particularly sheep, white tailed deer and pronghorn health are severely affected by bluetongue disease. Bluetongue is an economically important disease that has an impact on international trade. The etiological agent, bluetongue virus (BTV) is the type species of the genus *Orbivirus*, family *Reoviridae*. BTV is non-enveloped with icosahedral symmetry encasing ten linear double-stranded RNA (dsRNA) genome segments (Seg-1 to Seg-10, with respect to size in decreasing order). The virion consists of three concentric protein shells *viz.* the outer

capsid formed by VP2 and VP5, VP7 forms the core and VP3 forms subcore. The viral genome encodes 7 structural (VP1 to VP7) and 5 non-structural (NS1, NS2, NS3/ NS3a, NS4 and NS5 [S-10 ORF-2]) proteins (Pullenger *et al.*, 2016, Maan *et al.*, 2015, Maan *et al.*, 2016). Apart from horizontal and vertical transmission, the virus is mainly transmitted biologically by *Culicoides* sp. biting midges (Maan *et al.*, 2011; Mellor *et al.*, 2000). Till date, a total of twenty-seven serotypes of BTV have been isolated and sequenced, although there are evidences of two additional putative serotypes (Maan *et*

*al.*, 2017). The dsRNA of BTV is highly prone to undergo point mutations as well as antigenic shifts. Therefore, the diverse BTV strains also have characteristic regional variants called topotypes with respect to each genome segment. This complexity in the BTV makes sequence analysis of whole genome of the virus crucial for molecular epidemiology studies.

Till date, 15 BTV serotypes (BTV-1, BTV-2, BTV-3, BTV-4, BTV-5, BTV-6, BTV-9, BTV-10, BTV-12, BTV-16, BTV-17, BTV-18, BTV-21, BTV-23 and BTV-24) have been isolated in India since 2001 (Krishnajyothi *et al.*, 2016; Rao *et al.*, 2016; Biswas *et al.*, 2010; Gollapalli *et al.*, 2012; Dadawala *et al.*, 2012; Rao *et al.*, 2012; Himadri *et al.*, 2016). BTV-12 has been isolated and sequenced earlier from Andhra Pradesh, one of the southern states of India in 2011 (Rao *et al.*, 2015). BTV-12 has been previously reported from South Africa, Kenya, Japan and Taiwan.

Here we present the whole-genome sequence analysis of an Indian isolate (IND2016/118) of BTV-12 that was isolated from blood of a clinically affected sheep from Pataudi Tehsil of Gurugram district of Haryana (Latitude: 28.4595°N; Longitude: 77.0266° E). The virus was initially adapted in KC cells (derived from *Culicoides sonorensis*) and then grown in bulk in BHK-21 cells. The viral dsRNA genome was purified using TRIzol reagent (Life Technologies), and amplified following FLAC (Maan *et al.*, 2007). These PCR products after purification were sequenced on an ABI capillary sequencer 3130 using segment-specific primers. Sizes of encoded proteins by Seg-1 to Seg-10 of IND2016/118 were 1302 aa, 950 aa, 901 aa, 644 aa, 552 aa, 528 aa, 349 aa, 354 aa, 292 aa and 229 aa, respectively. Phylogenetic analysis showed that IND2016/118 contains genome segments derived mainly from eastern lineages, as segments -1, -3, -4, -8,

and -9 have grouped within the eastern topotype along with viruses from Australia, China, and Far East. Analysis of Seg-2, -5, -6, -7 and -10- showed grouping within the western topotype cluster i.e., 84.4-97.8% nt identity with BTV-12 isolate 75005 and BTV-12 reference strain from South Africa. The phenomenon of reassortment has repeatedly been seen in isolates collected post-1982, especially for Seg-5 (NS1).

It has been postulated that, the western NS1 contributes to enhanced transmission of the virus. Conclusively, analyses of serotype determining segments (Seg-2 and Seg-6) have grouped IND2016/118 within serotype 12, with high level of nucleotide sequence identity (>99%) to the previous BTV-12 isolates within the western topotype, confirming its serotype. Moreover, detailed analysis of clustering of Seg-2, Seg-5, Seg-6, Seg-7 and Seg-10 of western topotype in Indo-Asian BTV isolates is required as these may have significant role in molecular epidemiology of BTV.

### **Nucleotide sequence accession number**

The complete genome sequence of BTV isolate IND2014/118 was deposited in GenBank under the accession numbers MF615237 to MF615246.

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### **References**

Pullinger GD, Guimera Busquets M, Nomikou K, Boyce M, Attoui H, Mertens PP. Identification of the Genome Segments of Bluetongue Virus Serotype 26 (Isolate

- KUW2010/02) that Restrict Replication in a *Culicoides sonorensis* Cell Line (KC Cells). *PLoS One*. 2016;11(2):e0149709.
- Maan S, Maan NS, Belaganahalli MN, Rao PP, Singh KP, Hemadri D, *et al.*, Full-Genome Sequencing as a Basis for Molecular Epidemiology Studies of Bluetongue Virus in India. *PLoS One*. 2015;10(6):e0131257.
- Maan S, Maan NS, Batra K, Kumar A, Gupta A, Rao PP, *et al.*, Reverse transcription loop-mediated isothermal amplification assays for rapid identification of eastern and western strains of bluetongue virus in India. *J Virol Methods*. 2016;234:65-74.
- Maan S, Maan NS, Nomikou K, Batten C, Antony F, Belaganahalli MN, *et al.*, Novel bluetongue virus serotype from kuwait. *Emerg Infect Dis*. 2011;17(5):886-9.
- Mellor PS, Boorman J, Baylis M. *Culicoides* biting midges: their role as arbovirus vectors. *Annu Rev Entomol*. 2000;45:307-40.
- Maan S, Kumar A, Gupta AK, Dalal A, Chaudhary D, Gupta TK, *et al.*, Concurrent infection of Bluetongue and Peste-des-petits-ruminants virus in small ruminants in Haryana State of India. *Transbound Emerg Dis*. 2017;00 1–5. doi:10.1111/tbed.12610.
- Krishnajyothi Y, Maan S, Kandimalla K, Maan NS, Tutika RB, Reddy YV, *et al.*, Isolation of Bluetongue Virus 24 from India - An Exotic Serotype to Australasia. *Transbound Emerg Dis*. 2016;63(4):360-4.
- Rao PP, Hegde NR, Reddy YN, Krishnajyothi Y, Reddy YV, Susmitha B, *et al.*, Epidemiology of Bluetongue in India. *Transbound Emerg Dis*. 2016;63(2):e151-64.
- Biswas SK, Chand K, De A, Pandey LK, Mohapatra JK, Prasad G, *et al.*, Isolation of bluetongue virus serotype 1 (BTV-1) from goats and its phylogenetic relationship to other BTV-1 isolates worldwide based on full-length sequence of genome segment-2. *Arch Virol*. 2010;155(12):2041-6.
- Gollapalli SR, Mallavarapu S, Uma M, Rao PP, Susmitha B, Prasad PU, *et al.*, Sequences of genes encoding type-specific and group-specific antigens of an Indian isolate of bluetongue virus serotype 10 (BTV-10) and implications for their origin. *Transbound Emerg Dis*. 2012;59(2):165-72.
- Dadawala AI, Biswas SK, Rehman W, Chand K, De A, Mathapati BS, *et al.*, Isolation of bluetongue virus serotype 1 from *Culicoides* vector captured in livestock farms and sequence analysis of the viral genome segment-2. *Transbound Emerg Dis*. 2012;59(4):361-8.
- Rao PP, Reddy YN, Hegde NR. Complete genome sequence of bluetongue virus serotype 9: implications for serotyping. *J Virol*. 2012;86(15):8333.
- Hemadri D, Maan S, Chanda MM, Rao PP, Putty K, Krishnajyothi Y, *et al.*, Dual Infection with Bluetongue Virus Serotypes and First-Time Isolation of Serotype 5 in India. *Transbound Emerg Dis*. 2016;00:1-6. doi:10.1111/tbed.12589.
- Rao PP, Reddy YV, Hegde NR. Isolation and Complete Genome Sequencing of Bluetongue Virus Serotype 12 from India. *Transbound Emerg Dis*. 2015;62(5):e52-9. doi: 10.1111/tbed.12199.
- Maan S, Rao S, Maan NS, Anthony SJ, Attoui H, Samuel AR, *et al.*, Rapid cDNA synthesis and sequencing techniques for the genetic study of bluetongue and other dsRNA viruses. *J Virol Methods*. 2007;143(2):132-9.

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