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Isolation of bluetongue virus-1 from cattle in India and phylogenetic analysis of the complete coding sequence of the segment-2 gene

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Abstract. The isolation of BTV-1 serotype from cattle in India and its phylogenetic relationship based on VP2 gene has been reported. Virus (JBP42/12/Ind) is isolated in BHK-21 cell line from blood sample tested positive for BTV antigen in sandwich ELISA from asymptomatic cattle. Full length VP2 gene of cattle isolate was amplified, cloned and sequenced. On BLAST analysis virus isolate was identified as BTV-1 serotype. Phylogenetic tree based on complete VP2 coding region segregated Indian isolates, Australian isolates and African/European isolates in three distinct clusters. Segregation of Indian BTV-1 isolates at close proximity to the monophyletic cluster of Australian BTV-1 isolates indicates the present isolate as "eastern topotype' of BTV. Multiple alignments of VP2 gene nucleotide sequences suggest that, Indian BTV-1 isolate is more closely related to Australian BTV-1 isolates; where 14.1% to 14.4% and 6.8% to 7.4% divergence was observed at nucleotide and deduced amino acid sequence level respectively.

Bluetongue (BT) is an arthropod borne disease of domestic and wild ruminants caused by Bluetongue virus (BTV) (Spreull, 1905; Mertens *et al.*, 2005). The cattle usually exhibit subclinical form of the disease with prolonged viraemia. However, in the epidemics caused by BTV-8 in Western and Central Europe, even cattle showed clinical disease (Darpel *et al.*, 2007). At present, worldwide 27 distinct BTV serotypes of BTV have been recognized (Zientara *et al.*, 2014). BTV is endemic in India and at least 13 different serotypes (BTV-1-4, 6, 9, 10, 12 16-18, 21 and 23) of BTV were isolated (Chand *et al.*, 2015). The segment-2 (VP2) of BTV genome is highly variable and sequence analysis of VP2 gene provides important information related to serotype of the virus, virus evolution and its relatedness to other viruses (Huismans *et al.*, 1987). Sequencing of VP2 gene provides information about the serotype, topotype and evolutionary background of BTV isolates. The present study was taken up with the isolation, genetic characterization and phylogenetic relationship of Indian BTV cattle isolates with other isolates reported worldwide.

The randomly collected blood samples from asymptomatic cattle were tested by sandwich ELISA (s-ELISA) (Chand *et al.*, 2009). The sample (JBP42/12/Ind) positive for BTV antigen was isolated in BHK-21 cells and dsRNA extracted and cDNA synthesized following standard protocol. Full length VP2 gene was amplified in different overlapping fragments of 893 bp, 878 bp, 845 bp and 1184

bp amplicon size using four pairs of primers (Biswas *et al.*, 2010). The PCR was carried out employing the standard protocol with cycling condition: 35 cycles of denaturation at 94°C for 45 second, annealing at 57°C for 45 second and extension at 72°C for 1 min. The amplified product was visualized in 1% agarose gel and cloned in pGEM®-T Easy vector system-I (Promega, Madison, USA). The positive clones were sequenced using vector specific primers on commercial basis. Identity of the gene was initially checked by Basic Local Alignment Search Tool (BLAST, http://blast.ncbi.nlm.nih.gov/). BLAST analysis confirmed that all contiguous sequence was matched with published VP2 gene sequences of BTV-1 serotype available in the database. The full length VP2 gene was 2940 nucleotides long having an open reading frame (ORF) of 2886 nucleotides (18 to 2903) which codes for VP2 protein (961 amino acids). The full length VP2 gene sequence of Indian BTV-1 cattle isolate has been submitted to GenBank (KP735614).

Multiple alignments of full length nucleotide sequence and deduced amino acid sequence of BTV-1 Indian cattle isolate was done with isolates of BTV-1 from India and different geographical region. The BTV-1 cattle isolates is more closely related to Australian BTV-1 isolates where 14.1% to 14.4% and 6.8% to 7.4% divergence was observed at nucleotide and deduced amino acid sequence level respectively. Close relatedness of Indian and Australian BTV (Pritchard and Gould, 1995; Biswas *et al.*, 2015) signifies that the present isolate belongs to eastern topotype of BTV. African isolates were found to be distantly related with Indian BTV-1 isolates where 31.9% divergence was observed. Multiple alignments of deduced amino acid sequences of VP2 protein suggest close relationship of Indian isolates with Australian BTV-1and possess threonine (Thr/T) in place of cysteine at amino acid position 645 unlike African and European origin.

The phylogenetic analysis of the nucleotide (nt) sequences of present isolate and nucleotide sequences of respective genes available in the public database (NCBI/ GenBank) segregated Indian isolates, Australian isolates and African isolates in to three distinct clusters (Fig. 1) and corroborated with others (Maan *et al.*, 2004; Biswas *et al.*, 2015).

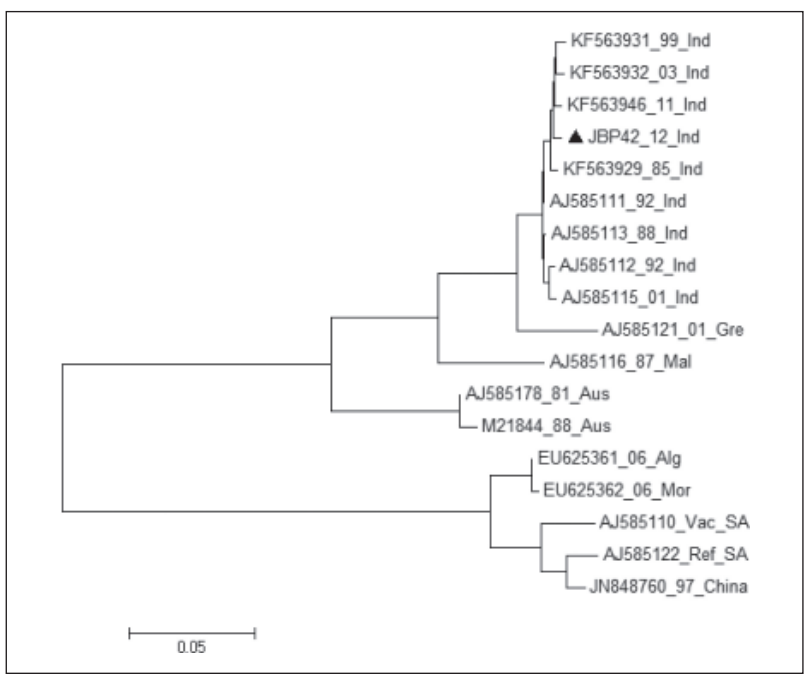

Figure 1. Maximum likelihood tree depicting phylogenetic relationship among BTV-1 cattle isolates at full length VP2 gene nucleotide sequence.

Among Indian BTV isolates, BTV-1 is one of the oldest bluetongue virus isolated from India (Jain *et al.*, 1986). In present study, the BTV-1 was isolated from asymptomatic cattle in BHK-21 cells.

The present study on phylogenetic showed close proximity to the monophyletic cluster of Australian BTV-1 isolates signifies the viruses as eastern topotype of BTV. The isolation and characterization of BTV from cattle would help in identifying the ancestor, migration route, evolution and global distribution of virus and its suitability to use as vaccine candidate.

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