SEGMENT 10 SEQUENCE BASED MOLECULAR CHARACTERIZATION OF BLUETONGUE VIRUS OF INDIAN ORIGIN

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ABSTRACT

Bluetongue disease (BT) is an infectious but non-contagious viral disease of wild and domestic ruminants. The complete genome of BTV isolate K31-08/ABT/HSR was sequenced using Ion-Torrent PGM system. The sequence data were *denovo* assembled and contig sequences were prepared with reference to known sequences from GenBank. The segment 10 based analysis segregates BTV in five distinct topotypes. The segment 10 of K31-08/ABT/HSR isolate showed maximum identity of >99/99%, nucleotide/amino acid with BTV 16 isolates from India and placed under eastern topotype viruses from India and several other countries. The clustering of BTV isolates from different geographical regions into same group indicated the spatial spread of the segment 10 through introduction of new genes via trade or illegal live vaccine or reassortment. It also indicates the common origin of segment 10 irrespective of BTV serotype. The effect of reassortment and genetic drift on BTV can be predicted using complete genome sequencing technique.

KEY WORDS: Bluetongue virus, serotype, topotype, segment 10, RT-PCR

INTRODUCTION

Bluetongue (BT) is an infectious, non-contagious viral disease of domestic and wild ruminants. BT is caused by bluetongue virus (BTV) which belongs to genus *Orbivirus* and family *Reoviridae*. BTV is transmitted by certain species of biting midges (*Culicoides* spp.). The BT is seen in ruminants, camelids and occasionally in carnivores (Ruiz-Fons et al., 2008; Meyer et al., 2009). However, the clinical signs of BT are predominantly seen in native population of sheep or white-tailed deer (Darpel et al., 2007). Cattle and goats usually show sub clinical form of disease and are treated as reservoir hosts (Chaignat et al., 2009).

The diameter of BTV particle is 70 nm and has three concentric protein layers, which surrounds 10 linear dsRNA genome segments. The genome segments encode virus specific seven structural (VP1 to VP7) and four non-structural proteins (NS1, NS2, NS3/NS3a, NS4) (Belhouchet et al., 2011; Janowicz et al., 2015). The serotype specific outer capsid protein VP2 and VP5 are encoded by segment 2 and segment 6 respectively. Segment 10 encodes non-structural protein NS3/3a in infected host cell, which is essential for virus egression from host cell (Janowicz et al., 2015). In several BTV isolates one more open reading frame called ORF2 have been detected which encodes a putative protein of 50-59 amino acids in length. The ORF2 inhibits the gene expression, but not the RNA translation (Stewart et al., 2015). Till date, there are twenty seven distinct BTV serotypes have been reported globally (Hofmann et al., 2008; Jenckel et al., 2015). In India, since first BT outbreak was reported in sheep and goat in 1961 in Maharashtra, till date 22 distinct BTV serotypes have been reported from several outbreaks in different states (Prasad et al., 2009; Susmitha et al., 2012).

BTV isolates can be divided into distinct phylogenetic groups which indicate their geographical origin i.e. eastern or western topotypes (Maan et al., 2008). The segmented nature of BTV genome

facilitates the reassortment of BTV segments especially when mammalian host or *Culicoides* vector is simultaneously infected by two or more different serotypes (Batten et al., 2008). This is a major event which allows the evolution of newer isolates and serotypes of BTV. The segment 10 of BTV may also undergo reassortment and lead to evolution of newer virus with altered pathogenicity and serological characteristic.

MATERIALS AND METHODS

Virus isolation and nucleic acid extraction

The previously characterized BTV16 isolate (K31-08/ABT/HSR) was used for molecular characterization of segment 10. The virus isolate was originally isolated from sheep blood in 2008 from Andhra Pradesh state. The blood sample was taken at the height of hyperthermia (104°C) from sheep. The blood sample was processed to inoculate in 11 day old hen embryo. After appearance of embryopathic effect, subsequent passage was given in BHK-21 cells for ten passages. The BHK-21 cells were pelleted down using table top centrifuge at 5000 x g for 10 minute. The viral dsRNA was extracted using TRIzol reagent (Invitrogen, USA) and ssRNA was selectively removed using 2M LiCl and 7.5 M Ammonium acetate (Maan et al., 2007b).

BTV cDNA Preparation

The purified viral dsRNA was ligated to an anchor primer (Maan et al., 2007a) and ligated product was purified using commercially available MicroElute RNA Cleanup kit (Omega, USA). The full-length cDNA copies of all ten BTV genome segments were synthesized using 'Transcriptor High Fidelity cDNA Synthesis Kit' (Roche, USA) and cDNA copies were subjected to full length PCR amplification (Maan et al., 2007a). The library was prepared from full length cDNA using Ion Xpress™ Plus Fragment Library Kit (Ion Torrent, USA).

High-throughput sequencing and sequence data analysis

The BTV libraries were sequenced for complete genome sequencing using 316 chip with 400 base sequencing chemistry on Ion Torrent Personal Genome Machine (PGM) (Ion Torrent, USA). The sequence data *de novo* assembly and contig preparation was done using CLC genomic workbench 4.6. The contig sequences were further analyzed using BLASTN 2.3.1+ of NCBI (Zhang et al., 2000). Suitable reference sequences for BTV16 (Minakshi et al., 2012) was selected from NCBI database and genome sequences of K31-08/ABT/HSR isolate were identified. A combination of *de novo* assembled and raw read mapped sequences were used to generate final consensus sequences of all the ten genome segments. The segment 10 of K31-08/ABT/HSR was further analyzed using bioinformatics tools. The coding regions of segment 10 nucleotides and its deduced amino acid sequences and similar sequences of different serotypes from several regions of the world were used for alignment and percent identity matrix calculation using Bioedit v 7.2.1 programme (Hall, 1999). The phylogenetic analysis of segment 10 nucleotide sequences were calculated using p-distance determination algorithm of MEGA 5.2 programme (Tamura et al., 2011).

RESULTS AND DISCUSSION

Several BTV isolates of eastern as well as western topotypes and their reassortant strains were isolated from India (Minakshi et al., 2012). These topotypes might be entered to India through trade of livestock and their products. Moreover, illegal import of live attenuated vaccine from western countries have also played major role in occurrence of western BTV genome segments in India (Rao et al., 2012). The complete genome sequence based study of a reassortant strain of BTV16 of goat origin from India has been done recently (Minakshi et al., 2012).

The cDNA of K31-08/ABT/HSR isolate was sequenced on Ion torrent PGM platform with 20X coverage. The nucleotide sequence of segment 10 was deposited to GenBank database with

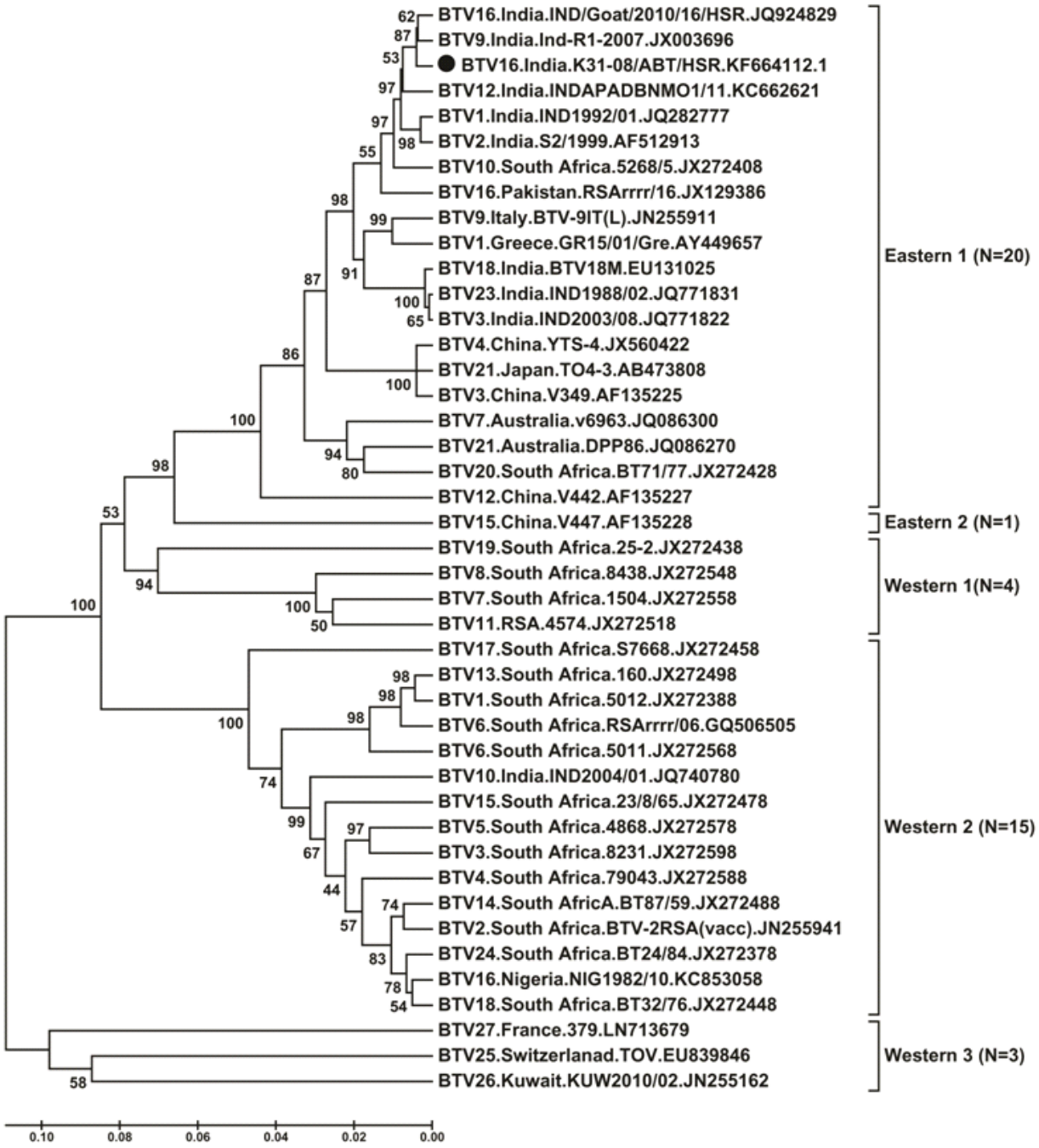

Figure 1: Phylogenetic analysis of segment 10 of Indian BTV isolate K31-08/ABT/HSR with global BTV isolates. Tree was constructed using neighbour joining method with 1000 bootstrap values in Mega5 software programme (Tamura et al., 2011). ●%= Isolate selected in this study.

accession number KF664112. The complete coding region of segment 10 of K31-08/ABT/HSR isolate was used for further analysis with similar BTV sequences from different parts of the world. Segment-10 encodes two related non-structural proteins NS3 and NS3a. The NS3 and NS3a of K31-08/ABT/HSR were consisting of 229 and 216 amino acid respectively. On comparison of

segment-10 across all the 27 BTV serotypes, it could divide BTV into eastern and western topotypes (Figure 1). The eastern and western viruses could again be subdivided into two eastern and three western topotypes (Steyn and Venter, 2016). The K31-08/ABT/HSR isolate was grouped in eastern 1 group along with viruses from Asia, Australia, South Africa, Greece and Italy.

The closest identity of K31-08/ABT/HSR isolate was observed with BTV16 (IND/Goat/2010/16/HSR, 99.2/99.5% nt/aa) and BTV9 (Ind-R1-2007, 99.1/100.0% nt/aa) from India. It also showed a significant identity (96-98.8/98.2-100 nt/aa) with several eastern BTV topotypes from India. The eastern 2 group was consisted of single virus BTV15 (isolate V447) from China. The nt/aa identity of this isolate with western BTV isolates from South Africa, India and Nigeria was recorded as 82.1-86.5/94.7-98.2%. Thus, phylogenetic analysis and sequence identity study showed that segment 10 of isolate in study is of eastern origin.

Segment 10 encoded NS3/3a proteins are involved in virus release from infected insect cells However, it is established that NS3/NS3a is not essential for virus replication in host cell (van Gennip et al., 2014). Moreover, BTV having mutated genome segment 10 can be used for differentiation of infected from vaccinated animals. The inactivated (mutated) BT vaccine having mutation in segment 10 will not show false positive PCR results post vaccination. However, animals actively infected with BTV can be positively diagnosed with Segment-10 pan BTV PCR test (DIVA-test) (van Rijn et al., 2013).

Along with VP7 it may also influence the dissemination of virion particle within the individual insect. It is assumed that genomic variation in segment 7 and 10 may influence the BTV transmission by vector species in animal population in different regions of the world (Maan et al., 2008). Therefore, the genomic variations in segment 7 and 10 of BTV16 may reflect the adaptation and transmission of BTV by vectors in India. However, further study is required to confirm the effect of variations in segment 10 in transmission efficiency of BTV16.

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