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Genomic diversity among eastern and western topotypes of bluetongue virus serotype 16 based on whole genome sequence analysis

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Abstract

Bluetongue (BT) is a noncontagious, vector-borne disease of domestic and wild ruminants. The causative agent bluetongue virus (BTV) is a double stranded RNA virus belonging to genus *Orbivirus* within family *Reoviridae.* Eradication of BTV from endemic regions like India is not an easy task due to the widely distributed *Culicoides* spp. midge vectors, the ubiquitous distribution of vertebrate hosts and existence of a large number of serotypes of the virus (at least 26 till date). The complete genomes (19,193 base-pairs) of several strains of bluetongue virus serotype 16 (BTV-16) originated from Australia, China, Indian subcontinent, Mediterranean basin, Middle East, Africa (Nigeria) and Europe, were compared. These analysis showed that all ten genome segments of a Nigerian strain are derived from a western lineage, showing only 77% - 84% nt identity with the eastern topotype reference strain 'RSArrrr/16' (and its derivative 'RSAvvvv/16', a vaccine strain) that was originally isolated in Pakistan, 76.4% - 83% with eastern BTV-16 strain from Australia (DPP96) and 77% - 89% with a reassortant strain from India. These detailed comparisons involving global strains showed that there is a very high degree of variation (up to 24%) between BTV-16 strains from eastern and western geographical regions. These data confirm the value of whole genome sequencing for characterization of novel BTV isolates and has helped to identify representative suitable 'reference-strain' of eastern topotype (BTV-16e), western topotype (BTV-16w), as well as 'cross-topotype' reassortant strain (BTV-16r) that are generated in the field for further serological, phylogenetic and molecular epidemiology studies.

Key words: *Bluetongue virus*, BTV-16, eastern, *Orbivirus*, reference strain, western, topotype, whole genome sequencing (WGS).

Bluetongue virus (BTV) is a double stranded RNA virus (genus *Orbivirus*; family *Reoviridae*) [1,2], which can infect both domestic and wild ruminants. Although BTV can acquire a membrane envelope during budding from infected cells, it is usually regarded as non-enveloped, consisting of a three layered icosahedral protein capsid [3]. The ten linear dsRNA genome segments of BTV (Seg-1 to Seg-10), encode a total of 7 structural proteins (VP1-VP7) and at least 4 non-structural proteins (NS1, NS2, NS3/3a and NS4) [4,5]. VP2 and VP5 (encoded by Seg-2 and Seg-6) are the most variable of the viral proteins and form the outermost layer of the BTV capsid. They also represent a target for neutralising antibodies (in particular VP2) and determine the serotype of BTV [6,7] .

The virus is transmitted between its mammalian hosts via the bites of vector-competent *Culicoides* species, although it can also be transmitted vertically in ruminants, or by ingestion of infected tissues, resulting in financial burden and trade restrictions. Therefore, bluetongue (BT) represents a significant worldwide threat to livestock industries.

The recent emergence of ten BTV serotypes in Europe, eight new serotypes in the USA, two new serotypes in Australia and isolation of seven different serotypes in India in last two decades [8,9], illustrate the increased risks posed by BT and other emerging arboviral diseases worldwide [10].

High incidence of reassortment due to segmented genome, existence of multiple serotypes, geographical restriction in the distribution of many of the serotypes, use of live attenuated vaccines in several parts of the world and the lack of complete sequences of viruses isolated from several parts of the globe have complicated our understanding of the origin, movement and distribution of BTV.

The nucleotide sequences of BTV isolates reflect their geographic origins [11-13], and the majority of the BTV genome segments can clearly be divided into 'eastern' or 'western' groups / topotypes [6,14-16]. This indicates that these viruses have evolved, with little genetic exchange between regions, over a very long period of time, allowing them to acquire multiple point mutations and clear regional differences.

Outbreaks caused by BTV-16 occurred in Greece during 1999-2000, and in the Turkish province of Izmir during 2000 [15-17]. During 2002 to 2006 further outbreaks occurred in the Mediterranean region. Sequence analysis of Seg-2, indicates that all of the Mediterranean / European isolates of BTV-16 belong to an eastern group of viruses and are closely related to the South African BTV-16 vaccine (<0.7% variation), suggesting a recent common ancestry [16]. The BTV-16 vaccine strain can cause severe disease in some

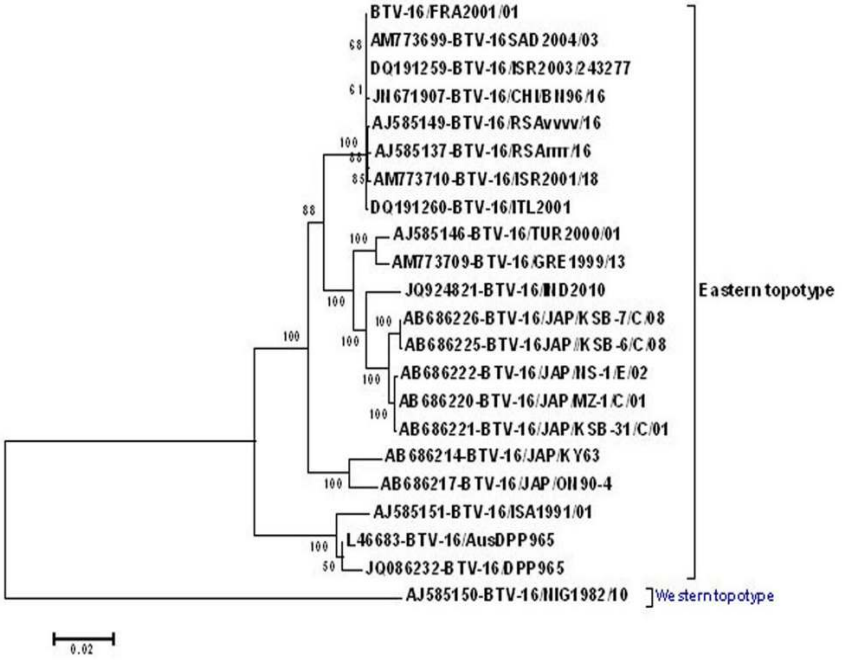

Figure-1. Unrooted neighbour-joining phylogenetic tree using segment 2 sequences of BTV-16. The tree was constructed using a p-distance algorithm and pairwise deletion parameters. Texas show GenBank accession numbers and geographic origin of strains that were compared. Bootstrap values (%) are represented at each tree node. Node support was assessed with 500 bootstrap pseudo-replicates. BTV-16 strains from different geographical regions make two major eastern and western topotypic groups.

European breeds of sheep that are sufficient to allow infection of feeding *Culicoides* and therefore onward transmission of the virus [18]. The vaccine strain was derived from the reference strain that was originally isolated from Hazara in West Pakistan during 1960 [19]. This close relationship suggests that the live, attenuated vaccine may be involved in the origins of all of the European incursions of BTV-16. The live vaccine strain of BTV-16 was used as part of an annual vaccination campaign in Israel and could represent a source for the European viruses [20]. The outbreak in Sardinia during 2004 was caused by the reassortant BTV-16 vaccine used in Italy during 2004 [21,22] and was not caused by the strain from Greece and Turkey. In November 2008, there were reports of further outbreaks due to BTV-16 in Greek island of Lesvos returning after ~7 years and in both Israel and Oman. Since 2010, there were several isolation of BTV-16 from India [23].

Until now, four eastern BTV-16 strains, from India, China, Australia and the South African reference strain have been fully sequenced [23-26]. Previous analyses [24] show that the BTV-16 reference strain (RSArrrr/16), the BTV-16 vaccine strain (RSAvvvv/16) and a Chinese BTV-16 (strain number BN96/16) [26], have >99% sequence identity in all ten genome segments, indicating that they are collectively derived from 'a very recent common ancestor'. In contrast, BTV-16 from Australia (strain DPP96), represents a distinct virus lineage, although still within the major-eastern-topotype (Fig.1).The Indian strain of BTV-16 is a reassortant, showing high levels of nt identity (92-97%) in the majority of its genome segments to the RSArrrr/16, but containing Seg-5 derived from a western topotype (with 89% nt identity).

Our recent full genome sequence data on a western topotype of BTV-16 from Nigeria (NIG1982/10), made it possible to carry out a detailed analysis of its relationships to BTV strains from other areas of the world [27]. All ten genome segments of NIG1982/10 are derived from a western lineage, showing only 77% -84% nucleotide identity with the eastern topotype reference strain 'RSArrrr/16' that was originally isolated in Pakistan, 76.4% - 83% with eastern BTV-16 strain from Australia (DPP96) and 77%-89% with reassortant strain from India. We had concluded that Nigerian strain (NIG1982/10) represented a suitable 'reference-strain' of BTV-16w, for further serological, phylogenetic and molecular epidemiology studies [27]. These data showed that there is a very high degree of variation (up to 24%) between BTV-16 strains from eastern and western geographical origins.

These data confirm the value of whole genome sequencing (WGS) for characterization of novel BTV isolates and will help to identify other eastern - western BTV isolates, as well as 'cross-topotype' reassortant-viruses that are generated in the field.

Authors' contributions

SM and NSM: Substantial contribution in conception and design of experiments, acquisition and analysis of data, drafted and revised the manuscript. AG, KB and AK: acquisition of data. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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