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Mini Review

Diversity among Topotypes of Bluetongue Virus Serotype 9 as Revealed by Whole Genome Sequence Analysis

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ABSTRACT

To understand the diversity and molecular epidemiology of bluetongue virus serotype 9 (BTV–9), the complete genomes (19,177 base pairs) of several strains of BTV–9 originated from Europe, Greek islands, Mediterranean basin, Middle East, South Africa, Australia and Indian subcontinent were compared. These comparisons showed that all ten genome segments of a south African reference strain (RSArrrr/09) and its derivative vaccine strain (RSAvvvv/09) belong to western lineage, showing only 68% – 69% nucleotide (nt) identity with the eastern topotypic field strains including eastern strain from Australia (DP0837), Europe and Mediterranean region as well as reassortant strains from India. These detailed comparisons involving global strains showed that there is a very high degree of variation (up to 32%) between BTV–9 strains from eastern and western geographical regions. Full genome sequencing and availability of data are therefore highly recommended in order to quickly and accurately identify the emergence of viruses with novel genome segment constellation. These studies can also help to identify representative suitable 'reference–strain' of eastern topotype (BTV–9e), western topotype (BTV–9w), as well as 'cross–topotype' reassortant strains (BTV–9r) of BTV–9 which are circulating in the field.

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INTRODUCTION

Bluetongue (BT) is an arboviral disease of domestic and wild ruminants which is characterized by inflammation of the nasal and muzzle area, ulcers around the teeth and on the tongue, rapid weight loss, depression, diarrhea, and inflammation in the coronary band and laminar corium of the hoof resulting in lameness. The causative agent bluetongue virus (BTV) is a double stranded RNA virus belonging to genus *Orbivirus* within family *Reoviridae* and is transmitted biologically between its vertebrate hosts (ruminants) by certain species of *Culicoides* biting midge (Diptera: Ceratopogonidae) (Mertens et al., 2005; Attoui et al., 2009).

Twenty six serotypes of BTV are now recognized globally (Hofmann et al., 2008; Maan et al., 2011a). BTV possesses a segmented genome comprising 10 segments of dsRNA which encode 11 proteins. Segmented viruses have the ability to 'reassort' genomic segments with related members of the same species (Shaw et al., 2013). The process of reassortment can cause fundamental shifts in the phenotypic characteristics of a virus. Reassortment event between pathogenic and non–pathogenic strains of a virus can lead to an increase in the pathogenicity of a previously avirulent strain (and vice versa) (Shelton et al., 2012). This phenomenon is of great importance with regard to the development of live attenuated vaccines for segmented viruses. The possibility exists for live attenuated vaccine and field viruses to reassert leading to viruses with a novel phenotype.

The size of the 10 genome segments of BTV–9 typically ranges from 822 to 3,944 bp. The lengths of these proteins (in amino acid residues (aa)) for BTV–9 are 1,302 (VP1), 955 (VP2), 901 (VP3), 644 (VP4), 526 (VP5), 330 (VP6), 552 (NS1), 354 (NS2), 229/ 216 (NS3/NS3A), and 77 (NS4), respectively (Grubman et al., 1983; Mertens et al., 1984; Belhouchet et al., 2011; Ratinier et al., 2011). These segments encode for seven structural and four nonstructural proteins observed during infection and replication. The virus core comprises the dsRNA genome segments associated with transcriptase complex of the virus which is made up of VP1 (RNA dependent RNA polymerase), VP4 (capping enzyme) and VP6 (the viral helicase), enclosed within consecutive layers of VP3 and VP7 (Grimes et al., 1998; Roy and Noad, 2006). The core is surrounded by an outer capsid layer comprising the variable proteins VP2 and VP5. NS1 is heavily expressed during infection and forms abundant cytoplasmic tubules which may be associated with cytopathogenicity (Owens et al., 2004). NS2 is an RNA–interacting phosphoprotein and is the major constituent of viral inclusion bodies (VIBs) where viral transcription and morphogenesis take place (Brookes et al., 1993). NS3/NS3A is a glycoprotein which is profoundly involved in virus egress and cell exit (Celma and Roy, 2009). NS4 is the most recently discovered BTV protein, which confers a replication advantage to cells pre–treated with interferon and has nucleolar localization (Belhouchet et al., 2011; Ratinier et al., 2011). Segments 2 and 5, which code for VP2 and VP5, determine the serotype of BTV (Maan et al., 2011b). Based on sequence

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differences, global isolates of BTV–9 can be grouped into eastern and western topotypes (Maan et al., 2009; Rao et al., 2012b).

More recently the global distribution of BTV has altered significantly with the introduction of novel serotypes into new areas, particularly in the northern hemisphere (Gibbs and Greiner, 1994; Saegerman et al., 2008; Tabachnick, 2010; Maclachlan, 2011; Maclachlan and Mayo, 2013). Thus, BT is regarded as a globally emerging disease (Purse et al., 2008; Maclachlan and Guthrie, 2010; Weaver and Reisen, 2010). Outbreaks of BT can be economically devastating to livestock production and the presence of BTV in a country can adversely impact the trade and movement of livestock (MacLachlan and Osburn, 2006; Velthuis et al., 2010; Rao et al., 2012). Predicting the course and geographic spread of an infectious disease is critical for its control. Control of BTV infection is difficult due to widely distributed *Culicoides* spp. midge vectors, the presence of vertebrate hosts and existence of a large number of serotypes of the virus. Prevention of the disease is characteristically dependent on vaccination of susceptible livestock or animal movement restrictions (Maclachlan and Mayo, 2013). Seg–2 was thus the original focus of sequence analyses for molecular epidemiology studies of BTV throughout the Mediterranean basin, northern Europe and elsewhere (Breard et al., 2003; Dahiya et al., 2004; Maan et al., 2004). However, whole genome sequencing studies have increasingly revealed evidence of reassortment between multiple strains of BTV in the field (Batten et al., 2008; Maan et al., 2008).

Whole genome sequencing (WGS) analysis studies that can thoroughly characterize the BTV type(s) circulating in a region and/or those involved in an outbreak, are necessary for the design and implementation of appropriate control strategies. Cross–reactive vaccines against multiple BTV strains/types would be of particular value in regions that are under the threat of incursions by multiple BTV serotypes. This article reviews the global distribution and overall genomic diversity as revealed by WGS analysis in the eastern and western topotypes of BTV–9.

Global Distribution of BTV –9

BTV–9 was first isolated in Africa (Dungu et al., 2004), and later in Australia, South East Asia, and India (Pritchard et al., 2004; Prasad et al., 2009). Outbreaks caused by BTV–9 have been reported from the Mediterranean basin and Europe since 1998 (Saegerman et al., 2008). The first outbreaks of BT in Mediterranean basin since the 1980s were caused by BTV–9 and occurred on four Greek islands (Rhodes, Leros, Kos and Samos) during 1998. This was first report of BTV–9 in Europe, although there was earlier serological evidence for its presence in Turkey (Taylor and Mellor, 1994). Sequence analysis of Seg–2 demonstrated that this BTV–9 strain belongs to an eastern group of viruses, a finding that is consistent with its arrival in eastern Europe via Turkey, distinguishing it from the South African BTV–9 vaccine strains that belong to a western group (Maan et al., 2009). Using these sequence data, it has been possible to design primers that distinguish the European vaccine and field strains of BTV–9, by simply identifying their different topotypes (Mertens et al., 2007).

BTV–9, from the same eastern lineage, was identified as the cause of outbreaks in mainland Greece during 1999, south–eastern Bulgaria and European Turkey. During 2000, further outbreaks caused by BTV–9 were reported in north–western and central Greece, then in 2001 from Serbia, Montenegro, Kosovo, Macedonia, Bulgaria, Croatia, mainland Italy and Sicily. In 2002, BTV–9 was identified again in Bosnia, Bulgaria, Montenegro, Yugoslavia and Albania, and there was an unconfirmed report of BT in Kosovo (Calistri et al., 2004). BTV–9 was a major serotype which caused outbreaks from 2002 to 2006 in the state of Andhra Pradesh in India (Rao et al., 2012b).

Genomic Diversity in BTV–9 Strains

Sequence comparisons of Seg–2 and Seg–6 showed that the isolates analyzed from the BTV–9 outbreaks in Europe are almost identical and can be grouped together as a single lineage (<12.9% nt sequence variation in Seg–2). All of these viruses belong to the same eastern geographic group as BTV–9 from Australia, India and Indonesia. BTV–9 had previously been reported in Anatolian Turkey, Syria, Jordan and Israel (Taylor and Mellor, 1994). Sequencing studies have shown >99% nt identity in Seg–2 between the BTV–9 field isolates from Italy 2001 and the Greek strains of BTV–9 from 1999, indicating that this serotype initially arrived in Italy from an easterly direction (Savini et al., 2004). Most of the European isolates of BTV–9 that have been characterized fully are distinct 'eastern' strains different from the South African reference and vaccine strains which belong to a distinct 'western' topotype of BTV–9 (Maan et al., 2009; Caporale et al., 2011). Three Indian isolate of BTV–9 from a southern state (Andhra Pradesh) of India (isolated in 2006 and 2007) have been fully characterized (Rao et al., 2012a; Rao et al., 2012b; Rao et al., 2013). These data have shown that these are reassortant strains, which have nine genome segments derived from eastern (e) lineage whereas Seg–5/NS1 gene from western (w) lineage strains. The co–circulation of a diverse array of BTV strains within the same population of animals and midges provides multiple opportunities for reassortment, resulting in the possibility of novel viruses with unknown or altered serological and/or pathogenic characteristics. Reassortment of an exotic virus having western Seg–5/NS1 gene with an endemic eastern strain of BTV–9 appears to favour the subsequent persistence of this gene from the exotic strain, which can be of key epidemiological significance in India.

Until now, seven eastern BTV–9 strains, from India, Australia and Italy along with two western strains, the South African reference and its derived vaccine strain, have been fully sequenced (Yang et al., 2011; Maan et al., 2012; Boyle et al., 2012). Previous analyses (Maan et al., 2012) show that the BTV–9 reference strain (RSArrrr/09), the BTV–9 vaccine strain (RSAvvvv/09) and a Sicilian BTV–9 (ITL2003/01) (Maan et al., 2009), which was recovered from an animal that died within a week after vaccination with the live BTV–9 vaccine, have >99% sequence identity in all ten genome segments, indicating that they are collectively derived from 'a very recent common ancestor'. In contrast, BTV–9 from Australia (strain DP0837), represents a distinct virus lineage, although still grouping within the major eastern topotypic cluster (Figure 1). The Indian strains of BTV–9 are reassortants, showing high levels of nt identity in the majority of their genome segments to the European and Mediterranean strains but containing Seg–5 derived from a western topotype (with 98% nt identity South African BTV–3 reference virus) (Caporale et al., 2011; Rao et al., 2012b). Whole genome sequence comparisons also revealed that all ten genome segments of a south African reference strain (RSArrrr/09) and its derivative vaccine strain (RSAvvvv/09) shared only 68% – 69% nt identity in Seg–2 with the eastern topotypic field strains from Australia (DP0837) and reassortant strains from India. The Seg–6/VP5 of RSArrrr/09 and RSAvvvv/09 grouped within nucleotype C, different from eastern BTV–9 isolates including Indian reassortant strains which grouped within nucleotype B with nt/aa identity of 70 and 79%, respectively. These detailed comparisons involving global strains showed that there is a high degree of variation (up to 32%) between BTV–9 strains from eastern and western geographical regions.

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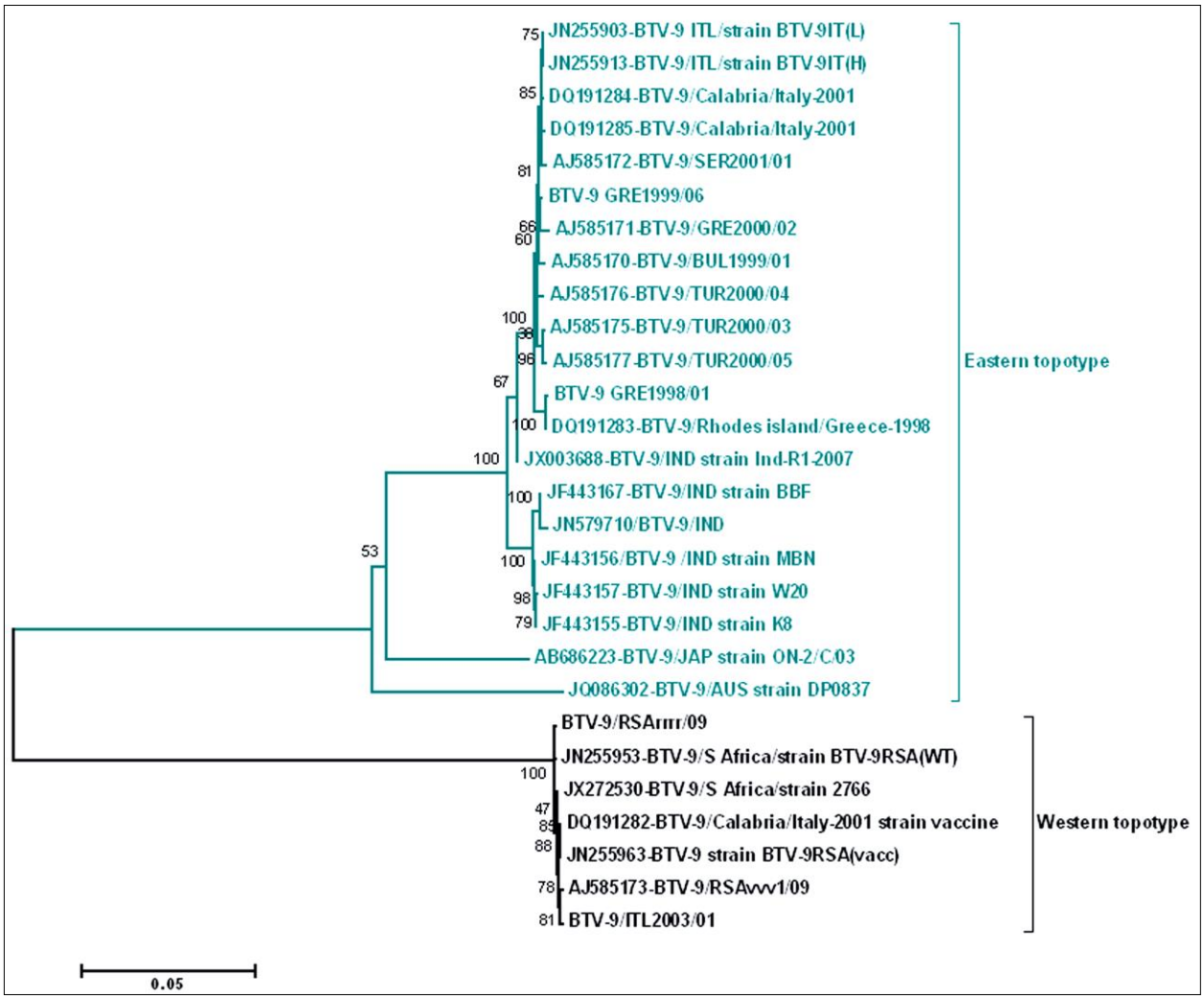

Figure 1: Unrooted neighbour–joining phylogenetic tree using segment 2 sequences of BTV–9. The tree was constructed using a p–distance algorithm and pairwise deletion parameters. Texas shows Gen Bank accession numbers and geographic origin of strains that were compared. Bootstrap values (%) are represented at each tree node. Node support was assessed with 500 bootstrap pseudo–replicates. BTV–9 strains from different geographical regions make two major eastern and western topotypic groups.

CONCLUDING REMARKS

Results reviewed in this document contribute to the opinion that the epidemiological situation of BTV infections (including that of BTV–9) in Europe and Mediterranean basin illustrates the risk to the entire world of emerging diseases that were previously confined to specific geographic areas. Climate change and increased trade could be contributing factors for this changed scenario. From these detailed comparisons of BTV–9 strains it can be concluded that south African reference strain (RSArrrr/09), Australian strain (DP0837) and Indian strain (BEF) represented a suitable representative 'reference–strain' of BTV–9w, BTV–9e and BTV–9r for further serological, phylogenetic and molecular epidemiology studies (Mertens et al., 2013). Whole–genome sequencing and availability of these data facilitates quick and accurate identification of the emergence of viruses with novel genome segment constellation, which should be taken into account in BT control.

Author's Contributions

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

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COMPETING INTERESTS

The authors declared that they have no competing interests.

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