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Molecular epidemiology of bluetongue virus serotype 1 circulating in Italy and its connection with northern Africa

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ABSTRACT

Western BTV-1 emerged in the Mediterranean basin in 2006 and it has since been isolated in southern and northern European countries. Six BTV-1 strains isolated from infected sheep in Italy between 2006 and 2013 and a BTV-1 strain isolated from an infected sheep in Tunisia in 2011 were fully sequenced. The seven strains were shown to be nearly identical in each gene segment. The Seg-2 sequences of the BTV-1 strains group according to the year of isolation reflecting the time of BTV incursions in Italy. Combined results suggest that BTV-1 strains isolated in Sardinia, Sicily and mainland Italy in 2012 and 2013 have a direct northern African origin. The Italian strains originated from a strain closely related to a BTV-1 strain isolated in Tunisia in 2011. Better surveillance programs with northern and sub-Saharan African countries should be implemented making the control of spread of BTV easier and effective.

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1. Introduction

Bluetongue virus (BTV) is the prototype species of the genus *Orbivirus*, within the family *Reoviridae* (Mertens et al., 2005). BTV is the etiological agent of the bluetongue (BT), and includes 26 serotypes (Mertens et al., 2005; Chaignat et al., 2009; Maan et al., 2011). Its genome constellation is composed of ten segments (Seg-1 to Seg-10) of linear double stranded RNA of nearly 19200 base pairs which encode for 7 structural proteins and 5 non-structural proteins (Ratinier et al., 2011). VP2 and VP5 (encoded by Seg-2 and Seg-6, respectively) are the most variable BTV proteins. They form the outer capsid, representing a target for neutralizing antibodies (particularly VP2), and determine virus serotype. In the last fifteen years, Europe has been continuously targeted by several incursions of BTV which entered Europe through two main corridors: western via north Africa and eastern via Turkey and Greece. Bluetongue first appeared in Europe in 1998 and since then, different BTV incursions including bluetongue virus (BTV) serotypes 1, 2, 4, 6, 8, 9, 11 and 16 have happened so far and BTV-1 represents the last occurring serotype. Based upon the analysis of sequences available on public databases, BTV-1 strains circulating worldwide have been shown to cluster mainly in two groups, western and eastern. Eastern group (EG) comprises BTV-1 strains isolated in the Australasian countries whereas in the western group (WG) are comprised BTV-1 strains isolated in Europe since 2006 (Cetre-Sossah et al., 2011) and in South Africa including the isolates RSAvvvv/01 and RSArrrr/01 (Fig. 1). Unfortunately, very few data are available for BTV-1 strains circulating in the Americas. WG BTV-1 appeared for the first time in Algeria in 2006 (Cetre-Sossah et al., 2011) and since, it spread all over the south Mediterranean basin and, surprisingly, northward reaching France and threatening the UK. Following the incursions of 2006 and 2010, BTV-1 re-occurred in Sardinia together with a BTV-4 reassortant strain in the autumn of 2012 (Lorusso et al., 2013a) and re-appeared alone in the following year (Lorusso et al., 2014). Unlike the year 2012, the infection in 2013 spread all over the Sardinian Island invading Corsica (Sailleau et al., 2014), Sicily and mainland Italy (Fig. 2). In this study the whole genome sequences of seven BTV-1 strains including BTV-1 SAD2013, BTV-1 LAZ2013, BTV-1 SIC2013, BTV-1 SAD2012, BTV-1 SAD2010 and BTV-1 SAD2006 isolated from BTV infected sheep in Sardinia, Lazio and Sicily regions between the years 2006 and 2013 and BTV-1 TUN2011 which was isolated from an infected sheep from Tunisia in 2011, are reported. BTV-1 SAD2012 and BTV-1 TUN2011 were sequenced by the Illumina platform whereas the remaining strains were sequenced by the Ion Torrent platform. The seven strains

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were sequenced in order to obtain insights into the molecular evolution of BTV-1 in Italy from 2006 until present.

2. Materials and methods

2.1. Isolates

The history of BTV-1 isolates is summarized in Table 1. Isolation and dsRNA purification were performed as previously described (Lorusso et al., 2013a; Maan et al., 2007). Sequencing libraries for BTV-1 SAD2012 and BTV TUN2011 for the Illumina platform were generated and sequenced at the BaseClear BV (Leiden, The Netherlands) as previously described (Lorusso et al., 2013b). Conversely, the remaining six Italian BTV-1 strains were sequenced at the OIE Reference Laboratory for Bluetongue in Teramo, through a recently assessed fast and innovative Next Generation Sequencing protocol based on the Ion Torrent technology (Lorusso et al., 2014).

2.2. Illumina

The sequencing run from the Illumina platform was analyzed with the Illumina CASAVA pipeline (v1.8.3), with demultiplexing based on sample-specific barcodes, obtaining between 260 and 372 Mb of sequence data for BTV-1 SAD2012 and BTV-1 TUN2011, respectively. The raw sequencing data produced was processed removing the sequence reads which were of too low quality (only "passing filter" reads were selected) and discarding reads containing adaptor sequences or PhiX control with an in-house filtering protocol. The quality of the sequences was enhanced by trimming off low-quality bases using the "Trim sequences" option of the CLC Genomics Workbench, version 6.0.2. The quality-filtered sequence reads were subsequently aligned against the reference sequences using the "Map reads to reference" option of the CLC Genomics Workbench version 6.0.2 with default settings. The 10 segments of BTV-1 SAD2012 and BTV-1 TUN2011 were successfully amplified for their entire length. Sequences were deposited in GenBank under the accession numbers KJ577094–KJ577103 and KJ577104–KJ577113 for BTV-1 SAD2012 and BTV-1 TUN2011, respectively.

2.3. Ion Torrent

The whole genomes of BTV-1 SAD2013, BTV-1 LAZ2013, BTV-1 SIC2013, BTV-1 SAD2010 and BTV-1 SAD2006 were sequenced starting from purification of viral dsRNA from cell culture (Lorusso et al., 2014). Raw data was quality assessed and trimmed by using FASTQC package [http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc] and ad-hoc python scripts. Genome assembly was performed by MIRA 4.0 software (Chevreux et al., 1999) followed by visual inspection of the assembly files. Sequences were deposited under the accession numbers: KJ019205–KJ019214, BTV-1 SAD2013; KJ577124–KJ577133, BTV-1 LAZ2013; KJ577114–KJ577123, BTV-1 SAD2010; KJ661729–KJ661738, BTV-1 SIC2013, KJ736001–KJ736010, BTV-1 SAD2006.

2.4. Phylogeny and dN/dS analysis

Complete genomes of the seven BTV-1 strains of this study were compared between them and with previously published BTV-1 whole genome sequences of the WG and EG groups. The available

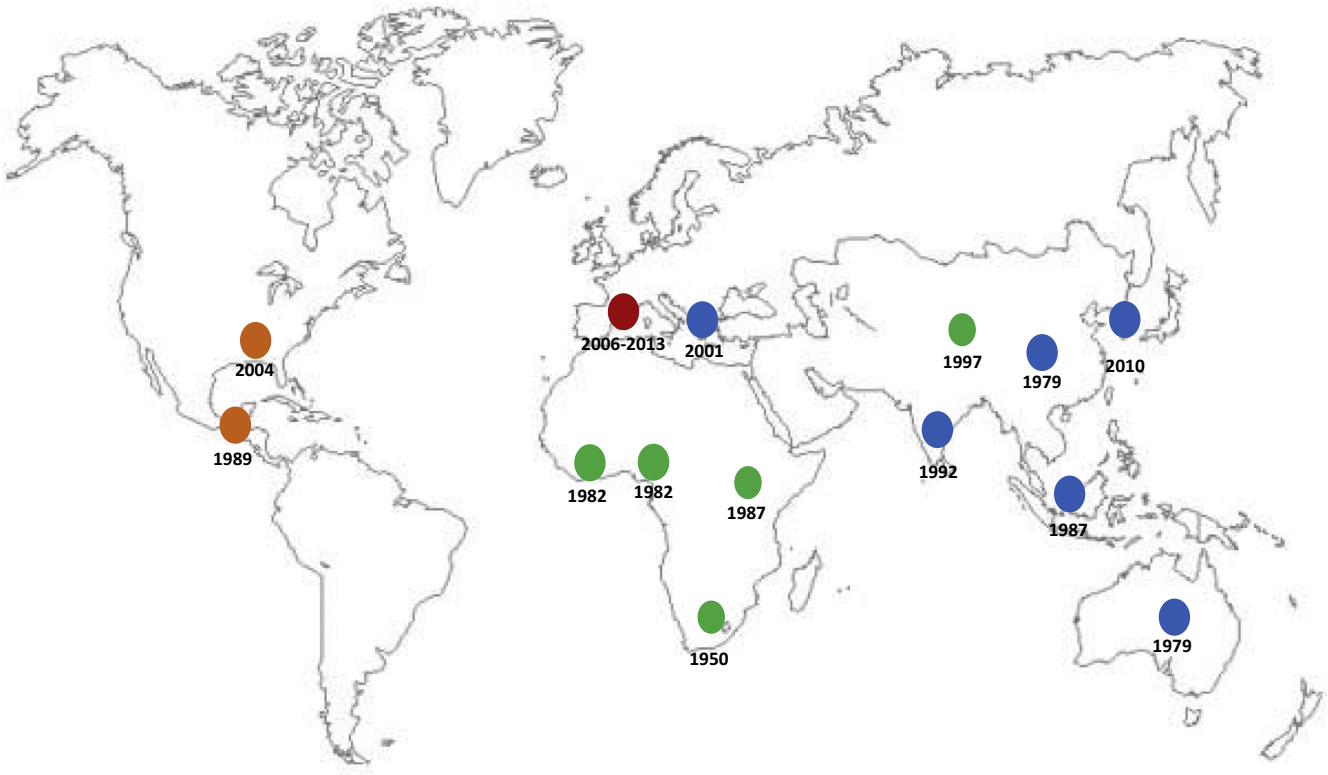

Fig. 1. Global geographical map showing BTV-1 distribution. Green dots, South African lineage; brown dots, Mediterranean basin lineage; blue dots, eastern group BTV-1; orange dots, north American BTV-1 with only scarce available information. The year below each dot indicates the year of isolation of BTV strains used for phylogenetic analysis depicted in Fig. 3. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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