Polish Journal of Veterinary Sciences Vol. 16, No. 3 (2013), 611–616

DOI 10.2478/pjvs-2013-0086

Review

The evolution of bluetongue virus: genetic and phenotypic diversity of field strains

W. Niedbalski

Department of Foot and Mouth Disease, National Veterinary Research Institute, 98-220 Zduńska Wola, Wodna 7, Poland

Abstract

Bluetongue virus (BTV), the aetiological agent of bluetongue (BT), is a small (about 70 nm in diameter) icosahedral virus with a genome composed of ten linear segments of double-stranded RNA (dsRNA), which is packaged within an icosahedral nucleocapsid composed of seven structural proteins. The BTV genome evolves rapidly via genetic drift, reassortment of genome segments (genetic shift) and intragenic recombination. This evolution, and random fixation of quasispecies variants during transmission of BTV between susceptible animals and vectors appear to be the main mechanism leading to the observed genetic diversity amongst BTV field strains. The individual BTV gene segments evolve independently of one another by genetic drift in a host-specific fashion, generating quasispecies populations in both ruminant and insect hosts. Reassortment of BTV genes is responsible for genetic shift among strains of BTV, and has been demonstrated after infection of either the ruminant host or insect vector with different strains or serotypes of BTV. Intragenetic recombination, whereby mosaic genes are generated from the "splicing" together of homologous genes from different ancestral viral strains, has been demonstrated for BTV. The genetic variation of BTV is likely responsible for differences in the virulence and other phenotypic properties of individual field strains of the virus.

Key words: bluetongue virus, evolution, genetic drift, genetic shift

Introduction

Bluetongue (BT) is an arthropod-borne viral disease of ruminants and camelids (Vervoerd and Erasmus 2004), caused by an arbovirus and transmitted by haematophagous midges of the genus *Culicoides* (Mellor et al. 2000). BT has a significant economic impact, mainly due to the disease effect on animals (morbidity, mortality, reproductive failure, reduction in milk yields and weight gain) and, most of all, to the disruption of international trade of animals and animal products (Saegerman et al. 2008). The aetiological agent of BT, bluetongue virus (BTV), belongs to the family *Reoviridae* and the genus *Orbivirus* (Mertens et al. 2005). Twenty six immunologically distinct serotypes of BTV have been identified worldwide to date (Schwartz-Cornil et al. 2008, Chaignat et al. 2009, Mann et al. 2011). The occurrence of BTV closely matches the distribution of *Culicoides* midges and climate conditions that support a large population of these insects. Therefore, BTV is endemic in many tropical, sub-tropical and temperate regions of the

Correspondence to: W. Niedbalski, e-mail: wieslaw.niedbalski@piwzp.pl, tel.: +48 43 825 31 24

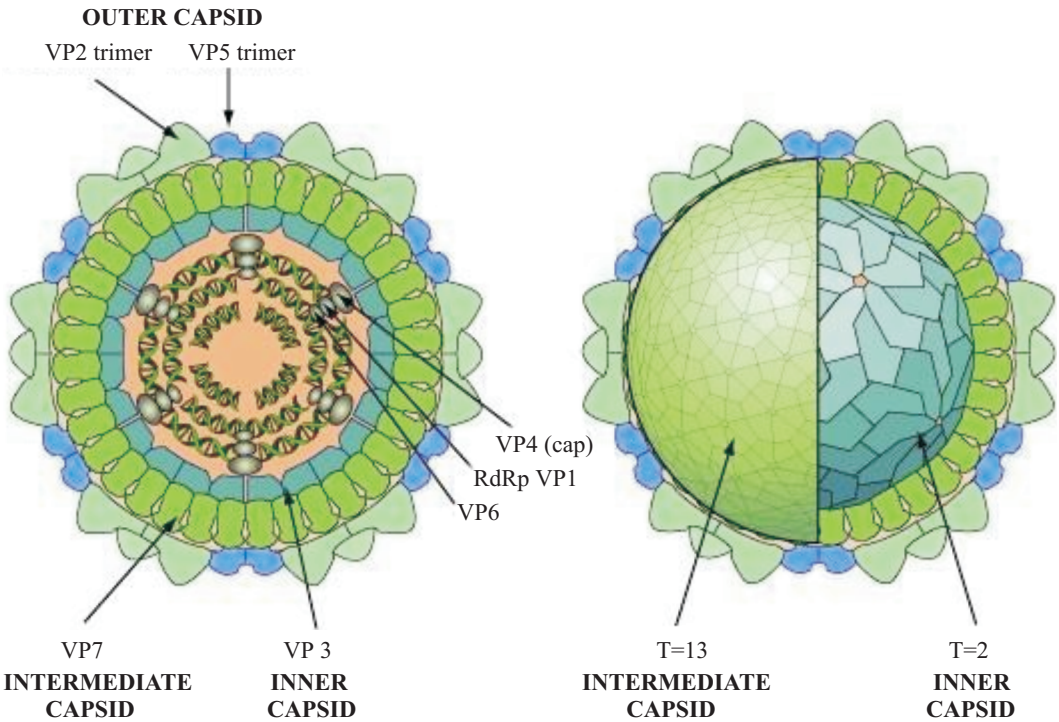

Fig. 1. Bluetongue virus morphology.
Source: (http://viralzone.expasy.org/all_by_protein/106.html)

world, between latitudes 40ºS and 53ºN, during times of the year that are optimal for vector activity (Mellor et al. 2000).

Serotype heterogeneity of BTV and strain variation within each serotype occurs as a result of a high rate of mutation of viral RNA during replication and recombination/reassortment between genetic material of relative strains during co-infection. This article reviews the structure and evolution of BTV driven by genetic drift, reassortment (genetic shift) and intragenic recombination.

The structure of BTV

BTV is a small (about 70 nm in diameter) icosahedral virus with a genome of approximately 19 200 base pairs, composed of ten linear segments of double-stranded RNA (dsRNA), which is packaged within an icosahedral nucleocapsid composed of seven structural proteins (Roy and Noad 2006). BTV particles are architecturally complex in structure (Fig. 1). The inner capsid, forming the core, is made up of 60 dimers of the major protein VP3 (coded for by segment 3) and three minor (VP1, VP4 and VP6) enzymatic viral proteins (RNA-dependent RNA polymerase, capping enzyme and helicase, respectively) which are relatively conserved among members of the BTV serogroup. The middle shell is composed of 780 copies of VP7 (coded for by segment 7) which are arranged as 260 trimers. The "core" proteins (VP1, VP3, VP4, VP6 and VP7) are thought to be relatively conserved, and are antigenically cross-reactive between different strains and serotypes of BTV. However, cross-hybridisation and sequencing studies have shown that these genome segments can vary in a manner that reflects the geographic origin of the virus strain (topotype) (Mertens et al. 1987, Pritchard et al. 2004). The outer capsid is composed of two major structural proteins, 60 VP2 trimers (coded for by segment 2) and 120 VP5 trimers (coded for by segment 6) (Roy 2005). Genome segments 2 and 6 show high levels of sequence variation which correlate with virus serotype (Mann et al. 2007, Mertens et al. 2007). The BTV outer capsid proteins VP2 and VP5 determine the specificity of interactions between the virus particle and the neutralizing antibodies generated during infection of the mammalian host (Mertens et al. 1989). Additionally, the viral subcore consists of the nonstructural proteins NS1, NS2, NS3, and NS3A, which participate in the control of BTV replication, maturation, and export from the infected cell (Roy and Noad 2006). Genome segment 10 (encoding NS3/NS3A of BTV) is more variable than the majority of the genome segments encoding the other non-structural or core proteins. It has been suggested

that variation in segment 10 might relate to transmission of the virus by different insect vector populations and species (Balasuriya et al. 2008).

The evolution of BTV

During replication of viruses, in the process of producing copies of viral nucleic acid, mistakes occur which are known as mutations. Viruses containing RNA, such as BTV, generate a higher rate of mutations than viruses containing DNA because there is no effective proof-reading mechanism in the replication strategies employed by RNA viruses and, as a result, mutants or quasispecies are generated during infection (Domingo and Holland 1997). Mutation by nucleotide substitution is considered to be one of the important evolutionary mechanisms because it is a major source of new mutant BTV RNA. The analysis of segments 2, 3, 6 and 10 of time stratified nucleotide sequences of a worldwide panel of BTV isolates has shown that the evolutionary rate in these segments varies from between 0.52 and 6.0×10^{-4} nucleotide substitutions per site/per year (Carpi et al. 2010). The evolution of BTV segment 3 suggested that very strong purifying selection reduces the rate of substitution at the first and second codon positions (Nomikou et al. 2009). Previous estimates of the nucleotide substitution rate in BTV have ranged from 2.2×10^{-3} to 4.2×10^{-4} subs/site/year, which are similar to those observed in other RNA viruses (Kowalik and Li 1991).

The BTV genome evolves rapidly via genetic drift (Bonneau et al. 2001), reassortment of genome segments, producing antigenic shift (Oberst et al. 1987, Stott et al. 1987, Shaw et al. 2013), and through intragenic recombination (He et al. 2010). These evolutionary processes result in the generation of quasispecies populations in the host of vector, from which variants with optimal fitness may be selected under different environmental conditions. This evolution and random fixation of quasispecies variants during the transmission of BTV between susceptible animals and vector appear to be the main mechanism leading to the observed genetic diversity amongst BTV field strains (Bonneau et al. 2001, Balasuriya et al. 2008). The sequential transmission of plaque purified BTV-10 between sheep, cattle and *Culicoides sonorensis* midges and the genetic characterization of viral genes (VP2 and NS3 encoding genome segments) indicate that whereas the BTV-10 consensus sequence remained largely conserved, viral replication in each host also resulted in the establishment of a unique set of minor quasispecies variants in both ruminant and insect hosts. Thus, the authors conclude that genetic drift and founder effect contribute to diversification of individual gene segments of field strains of BTV (Bonneau et al. 2001). Furthermore, negative selection of individual BTV genes occurs over time following the incursion of novel viruses into new regions, creating genetically distinct region-specific clusters (topotypes) of certain virus genes (Bonneau et al. 1999, Balasuriya et al. 2008).

Reassortment of viral genome segments clearly plays a major role in the generation of genetic heterogeneity. Reassortment of BTV genes is responsible for genetic shift among different strains of the virus, and has been demonstrated after infection of either the ruminant host or insect vector with different strains or serotypes of BTV (Samal et al. 1987b). The segmented nature of the BTV genome allows the virus to freely exchange its genome segments between different co-infecting strains in concurrently infected host cells. The opportunity for BTV to re-sort in nature is emphasized through the observation that concurrent infection with more than one strain of ruminants in the field have frequently been demonstrated (Brenner at al. 2010). Evidence for the reassortment of viral genes in BTV field isolates has also been obtained though hybridization (De Mattos et al. 1991) and molecular sequencing studies (White et al. 2006, Maan et al. 2012). The occurrence of genome segment reassortment between two antigenically related BTV serotypes (BTV-11 and BTV-17) was demonstrated in cattle. A minimum of six genome segments participated in reassortment, with 16 unique reassortant constellations being identified (Stott et al. 1987). The epizootic consequences of genome segment reassortment are significant. The viruses isolated from a given animal in the field will reflect only those which were best suited for preferential replication. Animals infected with two or more viruses may not express parental genotypes in significant titers even though these genotypes contributed extensively to the genomic complement of high-titer progeny reassortants (Stott et al. 1987). Studies on the kinetics of BTV genetic reassortment have shown that this process occurs at varying frequencies in different host systems, e. g. the fraction of reassortment progeny clones recovered from viraemic sheep was approximately 5% (Samal et al. 1987b) while in the bovine it was much higher (89%) (Stott et al. 1987). From a mixed invertebrate host (*Culicoides* midges) a high fraction of reassortments (7-78% of clones recovered per infected midge) has also been recovered (Samal et al 1987a), and these results suggest that the insects are a highly permissive host for the reassortment of the virus in nature. This is especially significant when one considers that *Culicoides* are infected with the virus for the duration of their adult life span and that adult

female biting midges may take multiple blood meals (Mellor et al. 2000). The high frequency (54%) of reassortment of the study on the BTV genome has also been shown in infected African green monkey kidney (Vero) cells (Raming et al. 1989). The recent study of the process of BTV reassortment between BTV-1 and BTV-8 has shown that reassortment in BTV is very flexible, and there is no fundamental barrier to the reassortment of any genome segments (Shaw et al. 2013).

Typically one of the parental viral strains dominated the yield of recovered virions with the other parental strains only being represented by its contribution of genome segments to the reassortant progeny. Multiple unique reassortant strains could further be isolated from each of the host systems, with some strains dominating the yield of the recovered reassortant progeny later during infection. This suggests that these viruses had either reassorted their genome segments earlier during the infection cycle, and had therefore replicated to a higher titre, or that the viruses had acquired genome segments that conferred a selective replication advantage over the other reassortant progeny. Indeed, reassortment appears to occur non-randomly for some genome segments (El Hussein et al. 1989, Ramig et al. 1989). BTV genetic reassortment may be facilitated by the selection of viral RNA by non-structural protein 2 (NS2) during the packaging of the viral genome segments into sub-core particles. The NS2 rapidly forms as a matrix around individual transcribing viral cores in the cytoplasm of infected cells (Gould and Hyatt 1994).

Intragenic recombination, whereby mosaic genes are generated from the "splicing" together of homologous genes from different ancestral viral strains, has been demonstrated for BTV. Analysis of complete BTV gene sequences representing all of the genome segments of the virus has indicated that up to 1.6% of the analyzed sequences represented unique recombination events (He et al. 2010). Recombination has occurred in all of the viral genome segments except segments 5 and 6, with recombinants having undergone either single, double or multiple cross-over events. It was found that several BTV strains isolated at different time points and from different geographical locations contained genome segments which appeared to be descended from common mosaic ancestors, indicating that recombinant genes had become fixed amongst dominant strains in the field. This study shows that acquisition of the recombinant genes may have resulted in an increased evolutionary fitness of the viruses in the field and can play a potential role in generating the genetic diversity of BTV and exert its influence on the change in BTV epidemiology (He et al. 2010). The authors suggest that the recombination can be facilitated by the regions within the viral genome that are prone to the formation of RNA secondary structures. RNA structure predictions indicated that the regions around the breaking points of all mosaic sequences demonstrated the potential for RNA secondary structures to form (He et al. 2010).

The circulation of BTV in different regions of the world has led to the evolution of distinct geographical strains or topotypes of the virus. On the basis on phylogenetic analysis of sequences of the majority of viral genome segments, BT viruses are divided into western and eastern lineages (Maan et al. 2010). BTV also demonstrates serious genetic variation amongst strains that circulate in the same geographical region. It was shown that the nucleotide sequence of the NS3/NS3A encoding gene of BTV isolates collected from pools of *Culicoides sonorensis* varied over a three month period from 97.54 to 100% nucleotide sequence identity (Bonneau et al. 2002). The genetic heterogeneity of BTV is likely responsible for differences in the virulence and other phenotypic properties of individual field strains of the virus. BTV serotype 4, isolated in South Africa, was highly virulent when inoculated experimentally into sheep (MacLachlan et al. 2008), whereas BTV-4 from South and Central America was less virulent and rarely produced clinical disease (Gibbs and Greiner 1994). The higher virulence of South African than Australian strains of BTV has also been reported (Kirkland 2004). The genetic heterogeneity are responsible for differences of phenotypic properties of BTV strains and these differences can complicate the design and use of nucleic acid based virus detection methods such as conventional RT-PCR or real-time RT-PCR assays.

References

Balasuriya UB, Nadler SA, Wilson WC, Pritchard LI, Smythe AB, Savini G, Monaco F, De Santis P, Zhang N, Tabachnick WJ, MacLachlan NJ (**2008**) The NS3 proteins of global strains of bluetongue virus evolve onto regional topotypes through negative (purifying) selection. Vet Microbiol 126: 91-100.

Bonneau KR, Mullens BA, MacLachlan NJ (**2001**) Occurrence of genetic drift and founder effect during quasispecies evolution of the VP2 and NS3/NS3A genes of bluetongue virus upon passage between sheep, cattle, and Culicoides sonorensis. J Virol 75: 8298-8305.

Bonneau KR, Topol JB, Gerry AC, Mullens BA, Velten RK, MacLachlan NJ (**2002**) Variation in the NS3/NS3A gene of bluetongue viruses contained in Culicoides sonorensis collected from a single site in southern California. Virus Res 84: 59-65.

Bonneau KR, Zhang N, Zhu J, Zhang F, Li Z, Zhang K, Xiao L, Xiang W, MacLachlan NJ (**1999**) Sequence comparison of the L2 and S10 genes of bluetongue viruses from the United States and the People's Republic of China. Virus Res 61: 153-160.

Brenner J, Oura C, Asis I, Maan S, Elad D, Maan N, Friedgut O, Nomikou K, Rotenberg D, Bumbarov V, Mertens P, Yadin H, Batten C (**2010**) Multiple serotypes of bluetongue virus in sheep and cattle, Israel. Emerg Infect Dis 16: 2003-2004.

Carpi G, Holmes EC, Kitchen A (**2010**) The evolutionary dynamics of bluetongue virus. J Mol Evol 70: 583-592.

Chaignat V, Worwa G, Scherrer N, Hilbe M, Ehrensperger F, Batten C, Cortyen M, Hofmann M, Thuer B (**2009**) Toggenburg Orbivirus, a new bluetongue virus: initial detection, first observation in field and experimental infection of goats and sheep. Vet Microbiol 138: 11-19.

De Mattos CC, de Mattos CA, Osburn BI, Ianconescu M, Kaufman R (**1991**) Evidence of genome segment 5 reassortment in bluetongue virus field isolates. Am J Vet Res 52: 1794-1798.

Domingo E, Holland JJ (**1997**) RNA virus mutations and fitness for survival. Annu Rev Microbiol 51: 151-178.

El Hussein A, Ramig RF, Holbrook FR, Beaty BJ (**1989**) Asynchronous mixed infection of *Culicoides variipennis* with bluetongue virus serotypes 10 and 17. J Gen Virol 70: 3355-3362.

Gibbs EP, Greiner EC (**1994**) The epidemiology of bluetongue. Comp Immunol Microbiol Infect Dis 17: 207-220.

Gould AR, Hyatt AD (**1994**) The orbivirus genus. Diversity, structure, replication and phylogenetic relationships. Comp Immunol Microbiol Infect Dis 17: 163-188.

He CQ, Ding NZ, He M, Li SN, Wang XM, He HB, Liu XF, Guo HS (**2010**) Intragenic recombination as a mechanism of genetic diversity in bluetongue virus. J Virol 84: 11487-11495.

Kirkland PD (**2004**) Bluetongue viruses, vectors and surveillance in Australia – the current situation and unique features. Vet Ital 40: 47-50

Kowalik TF, Li JK (**1991**) Bluetongue virus evolution: sequence analyses of the genomic S1 segments and major core protein VP7. Virology 181: 749-755.

Maan S, Maan NS, Samuel AR, Rao S, Attoui H, Mertens PP (**2007**) Analysis and phylogenetic comparisons of full-length VP2 genes of the 24 bluetongue virus serotypes. J Gen Virol 88: 621-630.

Maan S, Maan NS, Van Rijn PA, Van Gennip RG, Sanders A, Wright IM, Batten C, Hoffmann B, Eschbaumer M, Oura CA, Potgieter AC, Nomikou K, Mertens PP (**2010**) Full genome characterisation of bluetongue virus serotype 6 from the Netherlands 2008 and comparison to other field and vaccine strains. PLoS One 5: e10323.

Maan S, Maan NS, Nomikou K, Batten C, Antony F, Belaganahalli MN, Samy AM, Reda AA, Al-Rashid SA, El Batel M, Oura CA, Mertens PP (**2011**) Novel bluetongue virus serotype from Kuwait. Emerg Infect Dis 17: 886-889.

Maan NS, Maan S, Guimera M, Nomikou K, Morecroft E, Pullinger G, Belaganahalli MN, Mertens PP (**2012**) The genome sequence of a reassortant bluetongue virus serotype 3 from India. J Virol 86: 6375-6376.

MacLachlan NJ, Crafford JE, Vernau W, Gardner IA, Goddard A, Guthrie AJ, Venter EH (**2008**) Experimental reproduction of severe bluetongue in sheep. Vet Pathol 45: 310-315.

Mellor PS, Boorman J, Baylis M (**2000**) Culicoides biting midges: their role as arbovirus vectors. Annu Rev Entomol 45: 307-340.

Mertens PP, Pedley S, Cowley J, Burroughs JN, Corteyn AH, Jeggo MH, Jennings DM, Gorman BM (**1989**) Analysis of the roles of bluetongue virus outer capsid proteins VP2 and VP5 in determination of virus serotype. Virology 170: 561-565.

Mertens PP, Pedley S, Cowley J, Burroughs JN (**1987**) A comparison of six different bluetongue virus isolates by cross-hybridization of the dsRNA genome segments. Virology 161: 438-447.

Mertens PP, Maan S, Samuel A, Attoui H (**2005**) Orbivirus, Reoviridae. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA (eds) Virus Taxonomy: VIIIth Report of the ICTV. Elsevier/Academic Press, London, pp 466-483.

Mertens PP, Maan NS, Prasad G, Samuel AR, Shaw AE, Potgieter AC, Anthony SJ, Maan S (**2007**) Design of primers and use of RT-PCR assays for typing European bluetongue isolates: differentiation of field and vaccine strains. J Gen Virol 88: 2811-2823.

Nomikou K, Dovas CI, Maan S, Anthony SJ, Samuel AR, Papanastassopoulou M, Maan NS, Mangana O, Mertens PP (**2009**) Evolution and phylogenetic analysis of full-length VP3 genes of Eastern Mediterranean bluetongue virus isolates. PLoS One 4: e6437.

Oberst RD, Stott JL, Blanchard-Channell M, Osburn BI (**1987**) Genetic reassortment of bluetongue virus serotype 11 strains in the bovine. Vet Microbiol 15: 11-18.

Pritchard LI, Sendow I, Lunt R, Hassan SH, Kattenbelt J, Gould AR, Daniels PW, Eaton BT (**2004**) Genetic diversity of bluetongue viruses in south east Asia. Virus Res 101: 193-201.

Ramig RF, Garrison C, Chen D, Bell-Robinson D (**1989**) Analysis of reassortment and superinfection during mixed infection of Vero cells with bluetongue virus serotypes 10 and 17. J Gen Virol 70: 2595-2603.

Roy P (**2005**) Bluetongue virus proteins and particles and their role in virus entry, assembly, and release. Adv Virus Res 64: 69-123.

Roy P, Noad R (**2006**) Bluetongue virus assembly and morphogenesis. Curr Top Microbiol Immunol 309: 87-116.

Saegerman C, Berkvens D, Mellor PS (**2008**) Bluetongue epidemiology in the European Union. Emerg Infect Dis 14: 539-544.

Samal SK, EL-Hussein A, Holbrook FR, Beaty BJ, Ramig RF (**1987a**) Mixed infection of *Culicoides variipennis* with bluetongue virus serotypes 10 and 17: evidence for high frequency reassortment in the vector. J Gen Virol 68: 2319-2329.

Samal SK, Livingston CW Jr, McConnell S, Ramig RF (**1987b**) Analysis of mixed infection of sheep with bluetongue virus serotypes 10 and 17: evidence for genetic reassortment in the vertebrate host. J Virol 61: 1086-1091.

Schwartz-Cornil I, Mertens PP, Contreras V, Hemati B, Pascale F, Breard E, Mellor PS, MacLachlan J, Zientara S (**2008**) Bluetongue virus: virology, pathogenesis and immunity. Vet Res 39: 46.

Stott JL, Oberst RD, Channell MB, Osburn BI (**1987**) Genome segment reassortment between two serotypes of bluetongue virus in a natural host. J Virol 61: 2670-2674.

Shaw AE, Ratinier M, Nunes SF, Nomikou K, Caporale M, Golder M, Allan K, Hamers C, Hudelet P, Zientara S, Breard E, Mertens P, Palmarini M (**2013**) Reassortment between two serologically unrelated bluetongue virus strains is flexible and can involve and genome segment. J Virol 87: 543-557.

Verwoerd DW, Erasmus BJ (**2004**) Bluetongue. In: Tustin RC, Coetzer JA (eds) Infectious disease of livestock. Oxford University Press, Cape Town, pp 1201-1220.

White DM, Blair CD, Beaty BJ (**2006**) Molecular epidemiology of bluetongue virus in northern Colorado. Virus Res 118: 39-45.