Jagadeesh Bayry *Editor*

Emerging and Re-emerging Infectious Diseases of Livestock

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Preface

Emerging and reemerging infectious diseases caused by virus, bacteria, fungi and parasites are causing significant morbidity and mortality not only in humans but also in various livestock including cattle, horses, birds, pigs, sheep, camels and others. In addition, these diseases are instigating significant economy and trade losses and disruption of global travel. Many of these diseases, including influenza, Middle East respiratory syndrome and Hanta, are of public health importance. The reasons for alarmingly raising prevalence of emerging infectious diseases are multifactorial such as deforestation and increased contact with wild animals and birds, climate changes, increase in global travel and altered life cycle of vectors.

In veterinary science, an appropriate referencing book on emerging and reemerging infectious diseases is lacking. Therefore, Springer has recently taken initiatives to start a book programme in this field. This book of *Emerging and Re-emerging Infectious Diseases of Livestock* focuses on various aspects of emerging and reemerging infectious diseases such as details on etiological agent, host range, epidemiology, pathogenesis, diagnosis, therapy and preventive measures including vaccines. The Chaps. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 and 16 mainly present emerging viral diseases of livestock. Chapter 17 provides details on rickettsial disease. Chapters 18 and 19 describe parasitic and mycotic diseases, while Chap. 20 outlines emerging infectious diseases of camelids.

I hope that this book will serve as good reference for veterinary scientists, field veterinarians, general public and policy makers. I am also confident that this book will inspire new investigations on pathogenesis, diagnosis, therapies and preventive measures for these infectious diseases and might prove useful in the event of emergence of new infectious diseases. I am indebted to all the contributors for writing excellent and detailed chapters on individual diseases, to my family and to Silvia Herold, editor of Biomedicine/Life Sciences, Springer, for her assistance and support.

Paris, France — Jagadeesh Bayry

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Part I

Emerging Viral Diseases of Livestock

Bluetongue: Aetiology, Epidemiology, Pathogenesis, Diagnosis and Control

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1.1 Bluetongue Virus (BTV) and Its Biology

1.1.1 BTV Structure and Proteins

Bluetongue virus (BTV) is the type species of genus *Orbivirus*, subfamily *Sedoreovirinae*, family *Reoviridae*. The virus particle contains seven distinct proteins, comprising three concentric capsid layers that encase the ten linear segments of the dsRNA genome. The innermost 'sub-core' layer is composed of viral protein 3 [VP3(T2)], which encloses the ribonucleoprotein 'transcriptase complexes'

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(TC), each of which comprises of an individual genome segment closely associated with the viral RNA polymerase VP1(Pol), the RNA capping enzyme and transmethylase VP4(CaP) and the viral helicase VP6(Hel) (Mertens and Diprose 2004). The outer surface of the sub-core provides a base for the attachment of the 'outer core' layer, composed of VP7(T13), which provides added strength and rigidity to the sub-core layer. The outer core is surrounded by an 'outer capsid' composed of VP2 (outer capsid protein-1) [VP2(OC1)] and VP5 (outer capsid protein-2) [VP5(OC2)]. Each virus particle encapsidates one copy of each of the ten dsRNA segments (identified as Seg-1 to Seg-10 in order of decreasing molecular weight) (Sung and Roy 2014).

Besides the typical fully intact non-enveloped particles, BTV can exist as other structural variants. The virus can bud out of infected cells to produce membrane-enveloped virus particles (MEVP). Protease treatment of BTV particles cleaves VP2(OC1), although the cleavage products are still associated with the surface of the resulting 'infectious subviral particles' (ISVP) (Mertens et al. 2008). In addition, 'core particles' lacking the outer capsid proteins can also be observed.

Each of BTV's seven 'structural' proteins as well as two nonstructural (NS) proteins [tubule protein NS1(TuP) and viral inclusion body matrix protein NS2(ViP)] is encoded by different genome segments (Roy 2005). However, VP6 (Hel) and NS4 are both translated from different reading frames of Seg-9 (Belhouchet et al. 2011; Ratinier et al. 2011), while NS3 and NS3a are produced from alternate initiation sites within Seg-10 (Wu et al. 1992). Seg-10 has also recently been shown to encode the putative protein NS5 from an alternate reading frame (Stewart et al. 2015). The structure of the BTV particle is shown in Fig. 1.1, and characteristics of various proteins encoded by the different genome segments of BTV are shown in Table 1.1.

1.1.2 BTV Entry, Transcription, Genome Replication, Assembly, Egress and Release

The BTV infectious particle (MEVP, 'intact' virus particle, ISVP, or core) can enter host cells by clathrin-mediated endocytosis, micropinocytosis or via other as yet undetermined mechanisms (Hassan and Roy 1999; Hassan et al. 2001; Gold et al. 2010). Attachment of intact virus particles, or ISVP, to mammalian cells occurs through the binding of VP2(OC1) to an as yet unknown sialoglycoprotein and/or possibly to other receptors or co-receptors (Zhang et al. 2010). The BTV core particles that have lost the outer capsid proteins have a surface composed entirely of the VP7(T13) and have reduced infectivity for mammalian cells (e.g. baby hamster kidney (BHK)-21 fibroblast cells) but are highly infectious to adult *Culicoides* midges or *Culicoides* cell lines (KC cells). The BTV core particle interacts with unknown receptors on the cell surface and can be neutralized by antibodies to VP7(T13). The core particles can bind to glycosaminoglycans, and VP7(T13) contains a surface-exposed conserved Asp-Gly-Glu (RGD) motif, suggesting the involvement of integrins in attachment to cells (Xu et al. 1997).

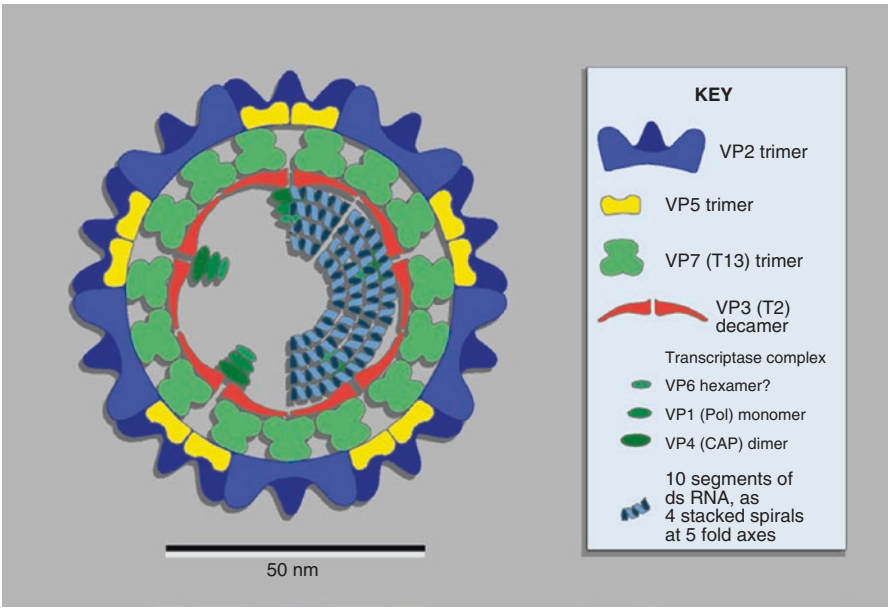

Fig. 1.1 Relative positions and organization of the major structural proteins and genomic RNAs of BTV particle. The virus particle has a triple-layered structure from inside to outside of *VP3*(*T2*), *VP7*(*T13*) and *VP2*(*OC1*)/*VP5*(*OC2*) which enclose the transcription complex of *VP1*(*Pol*)/*VP4*(*CaP*)/*VP6*(*Hel*) which closely associates with the ten segmented dsRNA genome. Further descriptions can be found in the text

During endocytosis of the BTV particle, the low pH of endosomes triggers conformational changes in the outer capsid proteins, facilitating uncoating of the virus core, and is essential for the intact virion to infect mammalian cells. Uncoating is followed by penetration of the core particle through the endosomal membrane, which is mediated by the acid-dependent fusion activity activated by conformational changes in VP5(OC2). Removal of the outer capsid proteins from the core particle activates the TCs, initiating viral mRNA synthesis as soon as the core is released into the cytoplasm, without further disassembly (Mertens et al. 2004; Noad et al. 2009). Since BTV cores can infect *Culicoides* cells in the absence of either VP2(OC1) or VP5(OC2), there must be alternative mechanism(s) to translocate the core particle across the cell membrane in these cells.

Viral RNA synthesis is mediated by the TCs. The polymerase VP1(Pol) transcribes from the negative strand of the dsRNA genome to produce positive sense ssRNA (transcripts). Its function is aided by the VP6(Hel), which separates the dsRNA into its component strands as well as helping to release the nascent transcript from the parental negative strand. Evidence suggests that each of the genome segments is associated with a single TC, allowing all ten mRNAs to be transcribed simultaneously and for the reinitiation of transcription of each segment as soon as the previous round is completed. Consequently, more mRNA copies are generated for the smaller genome segments. The rate of transcription can be enhanced by NS1,

Table 1.1 BTV genome segments, the encoded proteins and their characteristics and functions

Genome segment	Segment size (bp)	Protein(s) encoded	Protein length (aa)	Protein molecular weight (kDa)	Estimated subunits per virion	Location in the virion	Characteristics/functions
Seg-1	3954	VP1(Pol)	1302	150.6	10–12	Transcriptase complex (TC) within the sub-core	RNA-dependent RNA polymerase (Pol; transcriptase and replicase)
Seg-2	2926	VP2(OC1)	956	111.1	180 (60 trimers)	Outer shell	Cell attachment, entry
							Haemagglutination
							Virus neutralization antigen, determines serotype
Seg-3	2770	VP3(T2)	901	103.3	120 (12 decamers)	Sub-core capsid shell	*T*=2 icosahedral symmetry
							Scaffold for addition of core surface layer, outer coat proteins
							Localizes TCs on internal surface of the capsid, at 5-fold axes
Seg-4	1981	VP4(CaP)	644	76.4	5–24	TC within the sub-core	Capping enzyme (CaP) – nucleoside phosphohydrolase, guanylyltransferase, transmethylase
							Covalently binds GMP from GTP
Seg-5	1769	NS1(TuP)	552	64.4	NA	Nonstructural	Abundant in infected cell cytoplasm
							Forms tubules (TuP), with unknown function
							Aids virus release from insect cells
							Viral protein translation enhancer
							Co-localizes with the centrosome – may play a role in disruption and blocking of cell division in mammalian cells

Seg-6	1638	VP5(OC2)	526	59.1	360	Outer capsids	Has coiled coil domain – induces membrane permeabilization during initiation of infection and can cause syncytia formation
							Affects specificity of virus neutralization
Seg-7	1156	VP7(T13)	349	38.5	780 (260 trimers)	Core surface	Core structural protein
							T=13 icosahedral symmetry
							Receptor binding for *Culicoides* cells
							Group-/virus-species-specific antigen
Seg-8	1124	NS2(ViP)	357	41.0	NA	Nonstructural	Forms inclusion bodies (ViP)
							Aids in early morphogenesis
							Binds mRNA
							Phosphorylated by protein kinase 1
							Blocks spindle formation and cell division
Seg-9	1046	VP6(Hel)	329	35.7	37 / 72	Inner core	Helicase (Hel)
							Binds ss/dsRNA
							ATPase
		NS4	78	17	NA	Nonstructural	Imparts viral fitness to IFN response

(continued)

Table 1.1 (continued)

Genome segment	Segment size (bp)	Protein(s) encoded	Protein length (aa)	Protein molecular weight (kDa)	Estimated subunits per virion	Location in the virion	Characteristics/functions
Seg-10	822	NS3	229	25.6	NA	Nonstructural	Glycoprotein
							Binds cellular exocytotic components
							Aids virus release
							Contains coiled coil domain
							Localizes to cell membrane late in infection – interacts with lipid droplets in infected cell
		NS3A	216	24.0	NA	Nonstructural	Glycoprotein
							Binds cellular exocytotic components
							Viroporin – aids virus release from insect cells
		NS5	50–59	7.6	NA	Nonstructural	Localizes to nucleus Interferon antagonist?

Information available at http://www.reoviridae.org/dsRNA_virus_proteins/BTV.htm
NA not applicable

although it is not required for the function of the TCs (Patel and Roy 2014). The polymerase is fully conservative and consequently both strands of the dsRNA genome segments are retained within the BTV core, while the newly synthesized mRNA strands are exported out of the core via pores through the VP3(T2) and VP7(T13) layers. The viral transcripts are capped and methylated (addition of m^7GpppG at the 5′ end) by VP4(CaP), a requirement for efficient translation, but they are not poly-adenylated (Patel and Roy 2014).

The assembly of virus particles takes place in the host-cell cytoplasm, within viral inclusion bodies (VIBs). The NS proteins play essential roles in virus replication, assembly and egress. NS1(TuP) forms characteristic 'tubules' (Patel and Roy 2014), although its contribution to BTV assembly is unknown. NS2(ViP) forms VIBs at perinuclear locations; associates intimately with VP1(Pol), VP3(T2), VP4(CaP) and VP6(Hel) within the VIB; and facilitates their assembly in the vicinity of one another (Patel and Roy 2014). Preassembled VP1(Pol)-VP4(CaP)-VP6(Hel)-dsRNA complexes are rapidly recruited by VIB-resident VP3(T2) structures which are immediately stabilized by VP7(T13) (Patel and Roy 2014). Packaging of all ten dsRNA segments is essential for virus replication (Feenstra et al. 2014b), and the process requires the RNA cap structure, the secondary structure at the 3′ non-coding region, conformation formed by the interaction of the 5′ and 3′ termini of the RNA segments as well as specific interactions between short complimentary oligonucleotide regions on different viral mRNAs (Boyce and McCrae 2015). NS2 also co-localizes with the centrosomes and the condensed chromosomes during mitosis and appears to block attachment of the spindle fibres, leading to cell cycle arrest in mammalian cells (Shaw et al. 2013a). The incorporation of ssRNAs into nascent progeny subviral particles proceeds from small to large segments (Sung and Roy 2014), and NS2(ViP) is required for the recruitment of ssRNA (Patel and Roy 2014). At a late phase of assembly of nascent virus particles, VP1(Pol) synthesizes the negative sense RNA strand, reforming the dsRNA genome segments, a process that is thought to accompany the importation of the positive strand ssRNAs into the sub-core.

The final assembly of the outer capsid layer occurs at the periphery of the VIB, as progeny core particles are released into the cell cytoplasm. The sub-core proteins, VP1(Pol), VP4(CaP), VP6(Hel), VP3(T2), along with NS2(ViP) and the ten ssRNAs are sufficient to produce infectious particles, but the inclusion of VP7(T13) and NS1 greatly enhances infectivity (Patel and Roy 2014). Assembly of the outer capsid requires membrane microdomains (lipid rafts) of exocytic vesicles where VP2(OC1), VP5(OC2) and NS3 co-localize (Patel and Roy 2014). The virion co-opts cellular secretory machinery via VP5(OC2) and VP2(OC1), as well as NS3/NS3a, in order to be transported from the VIB to the cell membrane in mammalian cells. NS3 redirects virions from the periphery of the VIBs to a secretory process, leading to the release of BTV particles from insect cells, with little apparent damage to the cell membrane. This, along with the ability of NS3 to control the extent of BTV replication, may allow persistent infection of insect, but not mammalian cells (Patel and Roy 2014). The NS4 protein does not take part in virus replication or assembly per se, but is involved in counteracting host cell's antiviral functions (see

below). The presence of NS4 in the cell membrane late in infection suggests that it may be present in the envelope of MEVP budding out of infected mammalian cells. The role of NS5 has not yet been determined, although it appears to be an interferon antagonist. The possibility of expression of additional NS proteins has not been excluded.

The assembled particles are released through direct membrane penetration, cell lysis or budding in mammalian cells and by budding or pore formation in insect cells, the divergent pathways being regulated by NS3/NS3a, and possibly also by NS1(TuP) (Patel and Roy 2014). The MEVP that are released from BTV-infected mammalian cells are thought to be unstable and have not been fully characterized. It is uncertain if they are infectious on their own or whether they play any significant role in virus infectivity and dissemination. The ISVP, on the other hand, can enter mammalian cells as efficiently as intact virion, but are >100-fold more infectious for *Culicoides* cells or adult *Culicoides* insects, suggesting that they play an important role in initiation of infection in the insect vector (Darpel et al. 2011; Drolet et al. 2015).

Although all seven structural proteins, along with the genome segments, are part of the fully intact and infectious BTV particle, removal of the outer capsid proteins to release the virus core does not completely abrogate infectivity (Mertens et al. 2008). Non-infectious virus-like particles (VLP), lacking the genome and the associated TC proteins, can be generated by co-expression of just VP2(OC1), VP3(T2), VP5(OC2) and VP7(T13) (Hewat et al. 1994).

1.2 Epidemiology of Bluetongue

1.2.1 BTV Serotypes, Topotypes and Nucleotypes

Twenty-seven distinct serotypes of BTV have been recognized based on the ability of antibodies generated during infection of the mammalian host to neutralize only the homologous virus type (Hofmann et al. 2008; Maan et al. 2011; Zientara et al. 2014). There are also reports of two other putative serotypes (Nomikou et al. 2015a; Wright et al. 2012). Variations in the sequence of Seg-2 and of its translated protein VP2(OC1) correlate with BTV serotype. Although the sequence of Seg-6 and its product VP5(OC2) can also show a partial correlation with serotype, large variations are sometimes detected in different isolates of the same serotype, and isolates of different serotypes may contain Seg-6/VP5(OC2) with almost identical sequences (Maan et al. 2009). Identifying BTV serotypes, and analysing their appearance and reappearance, provides important epidemiological data and is essential for the design and implementation of effective control and prevention strategies for BT (particularly vaccine matching).

Genome segments can be exchanged between different serotypes, and all segments can reassort independently (Shaw et al. 2013b), potentially giving rise to a complex mosaic of viruses. Many different BTV serotypes exist in multiple but separated geographic regions, but show sequence variations in different segments

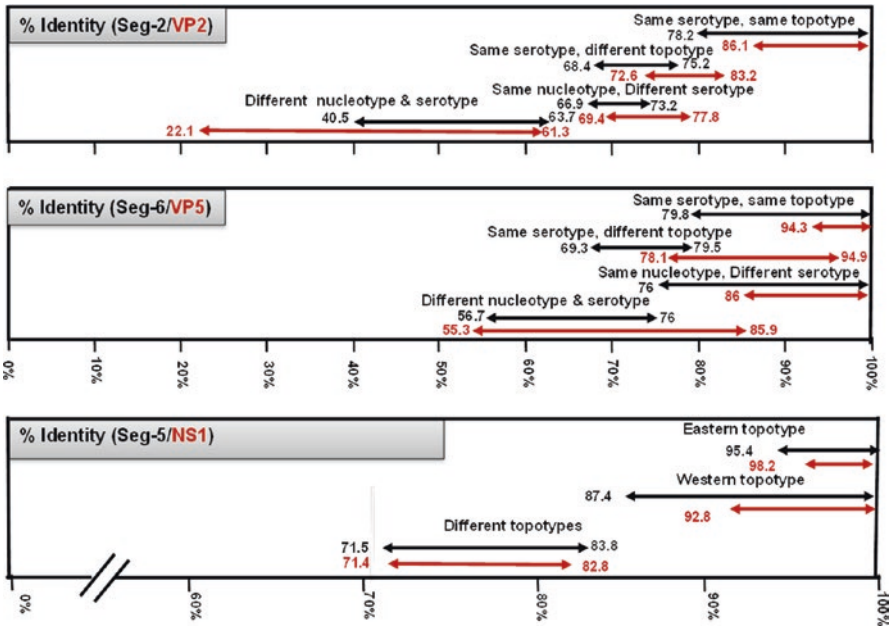

Fig. 1.2 Sequence identities in Seg-2/VP2(OC1), Seg-6/VP5(OC2) and Seg-5/NS1(TuP) within and between BTV serotypes and topotypes. The levels of sequence identity detected within the major eastern and western topotypes are given for Seg-2, Seg-5 and Seg-6 (*in black*) and proteins VP2(OC1), VP5 (OC2) and NS1(ViP) (*in red*)

that reflect their geographic separation (topotypes) (Maan et al. 2009, 2010). The genome segments of most BTV isolates can be grouped into either the major 'eastern' or 'western' topotypes (Maan et al. 2009, 2010). The major eastern group includes isolates from Australasia, the Middle East and the Mediterranean, while the western group includes viruses from Africa, the Mediterranean and the Americas. In addition, there is evidence for several further diverse groups including the recently discovered BTV-25 (SWI2008/01), BTV-26 (KUW2010/02) and BTV-27. The nucleotide/amino acid identity levels are shown in Fig. 1.2.

The eastern topotypes for Seg-2 and Seg-6 (the serotype-determining segments) of BTV-1, BTV-2, BTV-3, BTV-4, BTV-9 and BTV-16 have 25–30 % nucleotide sequence variation with their western topotype counterparts. Similar levels of sequence variation have also been observed for all non-serotype-determining segments. However, multiple clusters have been identified for both eastern and western topotypes of Seg-7 and Seg-10, suggesting that factors other than simple geographic separation and sequence drift have shaped the evolution of these two segments. These additional groups may reflect different insect populations/species that act as vectors within the separated eastern and western geographic regions, since both VP7(T13) and NS3 (encoded by Seg-7 and Seg-10, respectively) are believed to play important roles in the infection of, and viral exit from, insect cells (Tan et al. 2001; Celma and Roy 2009).

Within each major topotype, lower levels of variation (subgroups) can also be detected in the different genome segments, indicating further separation into more closely related local topotypes (Pritchard et al. 2004). For example, within the western topotype, a separate American subgroup has been observed for most of the segments. The level of divergence between the eastern and western topotype viruses indicates that they have been evolving in geographically separated regions for a long time. The levels of divergence between the American and African viruses indicate that they separated more recently than the eastern and western (African) viruses.

The presence of the same BTV serotypes within different regions (topotypes) suggests that these serotypes initially emerged from a common ancestor, before spreading out to different regions, gradually acquiring point mutations through successive rounds of virus replication in the vertebrate and invertebrate hosts, as well as adaptation to local ecosystems (and vector populations) over long periods of separation. The processes of random mutation, genome segment reassortment and selection may have contributed to the divergence of BTV into topotypes in most of the BTV genome segments. However, new strains of BTV, representing exotic topotypes or serotypes, have occasionally entered naïve or endemic areas via natural or anthropogenic routes, potentially changing the genome segment 'mix' and local genotype, and are frequently associated with severe clinical disease outbreaks (Nomikou et al. 2015b).

By considering the major topotypes separately, it is possible to set levels of sequence variation that may be used as a guide to identify different BTV serotypes. Overall, BTV strains within the same serotype can show up to 31.6 % nucleotide (nt) and 27.4 % amino acid (aa) variation in Seg-2 and VP2(OC1) (Maan et al. 2009, 2010). Viruses belonging to different serotypes can show up to 26.8 % nt and 22.2 % aa identity, making the delineation of clear limits for identification of distinct serotypes difficult. However, the maximum level of variation in Seg-2/VP2(OC1) for isolates of the same serotype and the same major topotype drops to a 21.8 % nt and 13.9 % aa sequences, respectively, 5.0 % and 8.3 % lower than the minimum levels of nucleotide and amino acid variation detected between different BTV serotypes. These levels, based on Seg-2 sequences, can therefore be used as a guide for the identification of existing serotypes, regardless of their geographic origin. If a novel isolate falls into the 'gap' [(between 26.8 % and 21.8 % nt and 22.2 % and13.9 % aa variation in Seg-2/VP2(OC1) compared to known BTV isolates], it could represent either an entirely novel serotype that is related to but distinct from other established serotypes or an isolate of an existing serotype but belonging to a different and distinct 'major' topotype (Maan et al., in preparation). In either case, additional serological analyses would be required. Such 'new viruses' would then become 'reference strains' for the new serotype or the topotype. It is therefore important to accurately identify and curate reference strains. A collection of well-documented BTV isolates, as well as sequence data and other available information, can be accessed at http://www.reoviridae.org/dsRNA_virus_proteins/ReoID/virus-nos-by-country.htm.

There is a clear correlation between the low levels of serological cross-reactivity observed between different BTV serotypes and nucleotide sequence similarities in Seg-2 and Seg-6. These clusters of related virus serotypes are said to belong to the

Table 1.2 Seg-2- and Seg-6-based nucleotypes and their relationship to serotypes

Seg-2 nucleotype	Serotype	Seg-6 nucleotype
A	BTV-4, BTV-10, BTV-11, BTV-17, BTV-20 and BTV-24	A
B	BTV-3, BTV-13 and BTV-16	B
C	BTV-6, BTV-14 and BTV-21	B
E	BTV-5, BTV-9 (eastern)	B
	BTV-9 (western)	C
H	BTV-1	C
I	BTV-2	C
D	BTV-23	C
	BTV-8, BTV-18	G
F	BTV-7 and BTV-19	D
G	BTV-12 and BTV-22	E
J	BTV-15	F
K	BTV-25 and BTV-27	H
L	BTV-26	I

same nucleotype (for Seg-2 and Seg-6 separately). Nucleotypes may represent a node of divergence among different serotypes during evolution. The distribution of various serotypes among the different Seg-2 and Seg-6 nucleotypes is shown in Table 1.2. As expected, serotypes from different Seg-2 nucleotypes are more distinct. However, Seg-6 from different serotypes within a particular 'Seg-6 nucleotype' can show very high levels of identity, approaching 100 % (Fig. 1.2), suggesting that they contain an essentially similar Seg-6/VP5(OC2) as a result of genome segment reassortment, but this does not result in an identical serotype, because of the more dominant variation in Seg-2/VP2(OC1).

1.2.2 The Incidence and Prevalence of Disease and BTV Serotypes

Bluetongue (BT) was regarded as an African disease until 1943, when an outbreak was recorded in Cyprus. Since then, the disease has been reported from the Americas, Australia, Europe, the Indian subcontinent and parts of the Far East (Walton 2004). The increasing distribution and devastation of outbreaks suggest that major and worldwide changes are still ongoing in the epidemiology of BT. Table 1.3 summarizes the BTV serotypes reported from the various regions.

1.2.2.1 Sub-Saharan Africa

Although BT is endemic in sub-Saharan Africa, most of the information concerning BT from this region is from South Africa, where BTV circulation is year-round in the north and seasonal in the south. South Africa presents the highest diversity of BTV serotypes with only BTV-20 and BTV-21 of the 24 'classical' serotypes not

Table 1.3 BTV serotype distribution in different geographic regions

Episystem	Western topotype	Eastern topotype
Sub-Saharan Africa	1–19, 22, 24, (1 putative new serotype)	
Americas	1–6, 8–14, 17–19, 22, 24	
Australasia	2, 5, 7, 10, 12, 24	1–4, 9, 15, 16, 20–21, 23
Middle East, Mediterranean and Europe	1, 2, 4, 5, 6, 8, 10, 11, 12, 14, 15–24, 25, 26, 27, (1 putative new serotype)	1, 9, 16,

reported from there. A putative new serotype has also been reported from the region (Wright et al. 2012). Besides the circulation of multiple BTV serotypes, more than one serotype is also commonly detected during an outbreak. In general, three to five serotypes dominate every season in South Africa, possibly reflecting dynamic changes in herd immunity to specific serotypes (Coetzee et al. 2012).

1.2.2.2 The Mediterranean, Middle East and Europe

North Africa, west Asia and Southern European countries surrounding the Mediterranean form an endemic area for BT. This region is separated from the rest of Africa by the Sahara and the rest of Asia by the Arabian Desert and other west Asian arid areas. BTV-2, BTV-4, BTV-6, BTV-10 and BTV-16 are considered endemic in Israel, along with active circulation in 2008 of BTV-5, BTV-8 and BTV-24 and, more recently, BTV-12 and BTV-15 (Brenner et al. 2010; Shimshony 2004). In Lebanon, BTV-1, BTV-4, BTV-6, BTV-8, BTV-16 and BTV-24 were identified in 2011 (El Hage et al. 2013). In other west Asian countries, BT has been reported from Iraq, Iran, Jordan, Oman, Saudi Arabia, Syria, Turkey and Yemen; the newer serotype, BTV-26, has been isolated from Kuwait (Maan et al. 2011). In North Africa, BTV-1, BTV-4, BTV-10 and BTV-12 have been reported from Egypt (eubtnet.izs.it/btnet/inFocus/pdf/bluetoungeDisease_mod_1.pps), while BTV-1, BTV-2 and BTV-4 have been reported from Algeria, Libya, Morocco and Tunisia since 2002 (WAHIS 2015).

In southern Europe, BTV-3 was first reported in Cyprus in 1924, followed by BTV-10 in Spain and Portugal during 1956–1960 and BTV-4 again in Cyprus in 1969. Since 1998, 11 different serotypes (BTV-1, BTV-2, BTV-4, BTV-6, BTV-8, BTV-9, BTV-11, BTV-14, BTV-16, BTV-25 and BTV-27) have been reported in Europe (Wilson and Mellor 2009), including strains of BTV-6, BTV-11 and BTV-14 with very high identity levels to modified live virus (MLV) vaccines (http://ec.europa.eu/food/animal/diseases/controlmeasures/bluetongue_en.htm#serotypes). BTV-25 and BTV-27 have also been isolated from this region (Hofmann et al. 2008; Zientara et al. 2014). Regular incursions of BTV-1 and BTV-4 into southern Europe have been observed between 2006 and 2015. During 2006, BTV was detected for the first time in northern Europe, beyond the traditional BTV boundary. This outbreak, caused by BTV-8 (Maan et al. 2008), spread across most parts of Europe and was successfully controlled through the use of inactivated vaccines, but it reappeared in France during 2015 after a gap of 5 years (Sailleau et al. 2015).

The incursions of BT into Europe have occurred mainly via three routes: (i) from Morocco to Spain through the Straits of Gibraltar, (ii) from Tunisia to Italy through Sicily or Sardinia, and (iii) from Turkey to Greece and Bulgaria through the Aegean islands or the land borders between these countries (Gomez-Trejedor 2004; Wilson and Mellor 2009). However, BTV-8, which is thought to have originated from sub-Saharan Africa (Maan et al. 2008), arrived directly into northern Europe via an unknown route. It is also unclear how vaccine strains of BTV-6, BTV-11 and BTV-14 arrived in Europe, although it could be due to illegal use of MLV vaccines. Genome analysis of viruses that have circulated in West Asia, North Africa and Europe since 1998 indicates that some of BTV-1, BTV-9 and BTV-16 may have originated from an 'eastern' ecosystem; however, the origin of BTV-16 is less certain due to the use of MLV vaccines in southern Europe and the Middle East (Mellor and Wittmann 2002; Gomez-Trejedor 2004; Savini et al. 2008). Strains of BTV-25, BTV-26 and BTV-27 show high levels of diversity compared to BTV-1 to BTV-24, and these may represent ancient lineages of BTV that have existed in Europe, the Middle East and the Mediterranean for a long period.

1.2.2.3 Australasia

In Australasia, the incidence of BT extends from northern China, Mongolia, Kazakhstan and the Asian part of southern Russia, through to northern Australia (Kirkland 2004; Koltsov et al. 2014). Tropical southern and Southeast Asia forms a major endemic area, with several serotypes in circulation (Boyle et al. 2012). Phylogenetic analyses of Seg-2 and Seg-6 sequences of viruses circulating in Australasia indicate that BTV-1, BTV-2, BTV-3, BTV-4, BTV-9 and BTV-16 belong to the eastern topotype (Boyle et al. 2012; Yang et al. 2012; Maan et al. 2012b, 2015b; Rao et al. 2012), indicating the spread of these serotypes from Southeast Asia to Australia, while BTV-20, BTV-21 and BTV-23 are unique to this region (Maan et al. 2009; Boyle et al. 2012; Susmitha et al. 2012). Western topotype strains of BTV-5, BTV-7, BTV-12 and BTV-24 have also recently entered the area (Lee et al. 2011; Boyle et al. 2012; Rao et al. 2015a; Yang et al. 2012, 2015; WAHIS 2015; Krishnajyothi et al., 2016; Yang et al. 2016; unpublished data) and have become established in more than one country, although western topotype MLV-like strains of BTV-2 and BTV-10 (Gollapalli et al. 2012; Maan et al. 2012c) did not become widely established.

1.2.2.4 The Americas

Serological evidence exists for BTV circulation in most parts of the USA and Central and South America, except the southern parts of the Pampas and Patagonia, and most of Canada and Alaska (Tabachnick 2004; Legisa et al. 2014). The serotypes reported in South America include (a) BTV-1, BTV-2, BTV-6, BTV-10, BTV-12, BTV-13, BTV-14, BTV-17 and BTV-24 from French Guyana and BTV-4 from Argentina by virus isolation (Legisa et al. 2014), and (b) BTV-4, BTV-6, BTV-14, BTV-17, BTV-18 and BTV-20 from Brazil; BTV-12, BTV-14 and BTV-17 from Colombia; BTV-6, BTV-14 and BTV-17 from Suriname and BTV-14 and BTV-17 from Guyana by serology (Wilson et al. 2009b). BTV-4 and BTV-12 are reported to

be isolated from animals imported from Brazil and are under quarantine in the USA (Groocock and Campbell 1982).

In the USA and Mexico, BTV-2, BTV-10, BTV-11, BTV-13 and BTV-17 are considered endemic; several other serotypes (BTV-1, BTV-3, BTV-5, BTV-6, BTV-9, BTV-12, BTV-14, BTV-19, BTV-22 and BTV-24) have also been isolated from the USA (Johnson et al. 2007). Occasional incursions of BTV from the USA into the Okanagan Valley, British Columbia, Canada (Clavijo et al. 2000b; Dulac et al. 1989) have been observed, and the virus has been recently reported for the first time in southwestern Ontario (http://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?reportid=18593). Limited studies of the isolates from this region indicate genetic relatedness among the isolates and suggest to and fro movement of viruses from the Caribbean Basin, Central America and North America (Wilson et al. 2000; MacLachlan et al. 2007; Balasuriya et al. 2008; Legisa et al. 2013; Viarouge et al. 2014).

1.2.3 BTV Transmission and Movement

Traditionally, BT has been restricted to areas between the latitudes 40°S and 53°N, coinciding with the distribution of the transmission-competent *Culicoides* vector species. Both natural and anthropogenic factors that contribute to movement of vertebrate and invertebrate hosts of BTV can increase the spread and transmission of the virus. Adult *Culicoides* can fly 2–5 km in a few days, leading to the local spread of BTV. However, long-distance spread can also occur as a result of dispersal of the insects by wind, particularly over water (Eagles et al. 2014). Anthropogenic activities including the use of incompletely attenuated/inactivated vaccines, import of infected ruminants, semen and embryos, and transportation of infected midges via planes, ships and containers may also contribute to the long-distance and even transcontinental spread of BT.

Once introduced, the survival and transmission of BTV in a new area depends on the availability and density of susceptible vertebrate hosts, their collective herd immunity as well as the transmission competency, seasonal activity and the density of adult *Culicoides* populations. Once the virus has been introduced, domestic ruminant population densities are often sufficient to sustain BTV. Consequently, the presence and activity of competent *Culicoides* is thought to be a limiting factor for BTV transmission and spread (Mellor and Boorman 1995).

The geographical range of *Culicoides* is dictated by climatic conditions. Models predicting significant correlations between climatic zones and geographical range of different *Culicoides* species (Brugger and Rubel 2013; Guichard et al. 2014) have indicated their spread to and within temperate areas and towards the poles, associated with global warming. This therefore represents an increased risk for the incidence and spread of BT in these regions. On the other hand, BTV infection of animals at temperate latitudes is distinctly seasonal (typically July to November in the northern hemisphere). However, BTV can survive from one "vector season" to the next during the winter months (overwintering) when cold temperatures restrict

the numbers, activity and reproduction of adult *Culicoides*, effectively preventing vector transmission. The mechanisms involved in overwintering are poorly understood, although vertical transmission of BTV to the ruminant foetus, resulting in release of the virus at birth and horizontal transmission to other naïve hosts, and persistent infection of adult vectors that manage to survive throughout the winter months have been suggested (Wilson et al. 2008; Darpel et al. 2009; Mayo et al. 2014). Viral RNA has been detected in midge larvae (White et al. 2005), suggesting that at least some component of the virus can be vertically transmitted in the insect vector. However, attempts to recover infectious virus have been unsuccessful. Transstadial and trans-ovarial passage of BTV in hard and soft ticks, respectively, suggests that they could also play a role in virus transmission and overwintering (Bouwknegt et al. 2010). Persistent infection of ovine T cells may provide another potential mechanism (Takamatsu et al. 2003).

Some BTV serotypes (e.g. BTV-1, BTV-8, BTV-26) can be transmitted horizontally in the absence of adult *Culicoides* (Menzies et al. 2008; van der Sluijs et al. 2011; Batten et al. 2014), and their transmission may therefore be independent of climatic conditions.

1.2.4 Vector Distribution and Bluetongue Epidemiology

Variations in the distribution, activity and virus transmission competence of adult *Culicoides* populations can determine if an area is endemic, disease-free or seasonally disease-free. Despite the existence of more than 2000 species of *Culicoides*, only a few are known to be competent vectors for BTV. Natural barriers such as oceans and deserts affect the free movement of insects and BTV between the different geographical areas where competent vector species are present.

The most widespread of the known BTV vector is *C. imicola*, with habitat extending from most parts of Africa, the Mediterranean, southern Europe, west and south Asia and east Asian countries, including Laos, Vietnam and China. Recently, *C. imicola* has been found in new areas of southern Europe, coinciding with the spread of BTV to these regions (Wilson and Mellor 2009; Carpenter et al. 2013). In endemic areas where *C. imicola* has not been detected, including the Americas, northern Europe, northern China, southeast Asia and Australia, other *Culicoides* species can act as vectors for BTV transmission (see Table 1.4) (Mellor et al. 2009).

1.2.5 BTV Episystems, Climatic Zones, 'Source' and 'Sink' Populations

Climatic and geographical barriers restrict the movement of both vectors and hosts between different ecosystems, limiting the spread of BTV strains and even individual BTV genome segments, leading to their independent evolution and genetic divergence over long periods of time (Gibbs and Greiner 1994; Nomikou et al. 2015b). It has been suggested that the distribution of different BTV serotypes in

Table 1.4 Vector species implicated in the transmission of BTV in different geographic regions

	Principle vector	Other known and potential species
Sub-Saharan Africa	*C. imicola*	*C. bolitinos*, *C. magnus*, *C. bedfordi*, *C. leucostictus*, *C. pycnostictus*, *C. gulbenkiani*, *C. milnei*, *C. tororoensis*
Mediterranean	*C. imicola*	
North Europe	*C. obsoletus*, *C. pulicaris*	
West Asia	*C. imicola*	
South Asia	*C. imicola*	*C. peregrinus*, *C. oxystoma*
East Asia	Unknown	*C. imicola, C. schultzei*, *C. gemellus*, *C. peregrinus*, *C. arakawae*, *C. circumscriptus, C. actoni, C. homotomus*
Southeast Asia	*C. fulvus*, *C. peregrinus*	*C. actoni*, *C. brevitarsis*, *C. fulvus*, *C. wadai, C. brevipalpis*, *C. peregrinus*, *C. oxystoma*, *C. nudipalpis*, *C. orientalis*
Australia	*C fulvus*, *C. wadai*, *C. actoni*, *C. brevitarsis*	*C. brevipalpis*, *C. peregrinus*
North America	*C. sonorensis*	*C. variipennis, C. stellifer, C. insignis*
Central America	*C. insignis*	*C. pusillus*
South America	*C. insignis*	*C. pusillus*

different geographical areas may be linked to the *Culicoides* species inhabiting those areas (episystems) (Tabachnick 2004). However, there is little unequivocal evidence to support this hypothesis. Indeed, recent incursions of exotic BTV serotypes and/or topotypes into the Americas, Europe and Australasia (Johnson et al. 2007; MacLachlan et al. 2007; Lee et al. 2011; Boyle et al. 2012; Legisa et al. 2014; Viarouge et al. 2014; Maan et al. 2015c; Nomikou et al. 2015b; Rao et al. 2016; Yang et al. 2015) demonstrate the abilities of different regional populations of *Culicoides* to transmit exotic BTV strains/genotypes.

Rather, the presence and spread of BTV into different climatic regions fits well into 'source' and 'sink' population dynamics. Tropical regions, with large vertebrate and invertebrate populations and conducive climate, support the circulation of multiple BTV serotypes throughout the year, acting as virus 'source' areas. In contrast, in temperate areas, BTV is often not sustained over long periods due to increasing herd immunity over time, and the massive reduction in adult vector populations during winters, favouring seasonal incursions caused by one or a few serotypes (Sellers 1980; Rao et al. 2016). Phylogenetic and evolutionary analyses show that the seasonal outbreaks in these virus 'sink areas', which may be interspersed with disease-free periods, reflect novel virus introductions from 'source areas' (Carpi et al. 2010; Nomikou et al. 2015b). The extent of source and sink areas depends on the availability of susceptible vertebrate host populations and competent vectors. South and Central America, North Africa and the Mediterranean and southeast Asia represent source areas, while most parts of North America, northern Europe and parts of Australia represent the associated sink areas,

respectively (Sellers and Maarouf 1989; Mellor and Wittmann 2002; Johnson et al. 2007; Purse et al. 2008; Daniels et al. 2009; Boyle et al. 2012). The 'sink' area is especially evident in northern Europe where different exotic serotypes have entered from neighbouring or distant endemic areas and have been sustained for short periods, followed by the re-emergence or entry of the same or different strains or serotypes (Nomikou et al. 2015b).

Combining source and sink systems with climatic zones, we propose the division of tropical areas into four episystems: sub-Saharan Africa, the Mediterranean, Australasia and the Americas. Each episystem contains its own source and sink areas overlapping with climatic zones (Fig. 1.3). Evidence exists for sharing and circulation of virus populations within these proposed episystems, and more specifically, each source and sink area within each episystem has closely related populations of circulating viruses (Wilson et al. 2000; Pritchard et al. 2004; Potgieter et al.

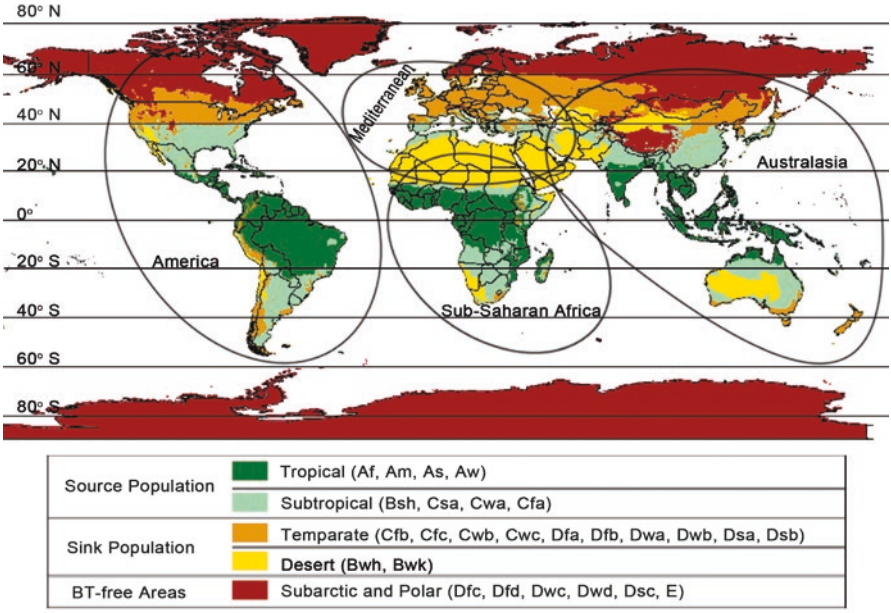

Fig. 1.3 Climatic zones and proposed BT episystems. Climatic zone map was prepared using FAO GeoNetwork (FAO 2013), as per Köppen's classification (Peel et al. 2007), and BTV sink and source population areas are mapped based on the conditions suitable for propagation of *Culicoides* and occurrence of BT.

Source areas include tropical (*dark green*) and subtropical areas including the Mediterranean (*light green*), which are most congenial for *Culicoides* propagation. Sink areas include hot and cold deserts (*yellow*) and temperate areas (*orange*), where only part of the year is suitable for the sustenance of *Culicoides* populations. Subarctic and polar regions (*red*) are considered as BTV-free zones, where harsh weather hampers the survival of *Culicoides*. Four distinct episystems (sub-Saharan Africa, the Mediterranean, the Americas and Australasia) are shown with each system having sink and source areas. The boundaries of both climatic zones and episystems are indicative and not definitive and may overlap