Bluetongue virus reassortment, an overlooked aspect of viral evolution with potentially serious implications

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Abstract

Bluetongue virus (BTV) is the prototype member of the *Orbivirus* genus in the family *Reoviridae* and is the aetiological agent of the arthropod transmitted disease, bluetongue (BT), which affects ruminant and camelid species. The disease is of significant global importance due to its economic impact and effects on animal welfare. Bluetongue virus, a segmented dsRNA virus, exists as a genetically and phenotypically heterogeneous entity in nature and has the ability to reassort its genome segments in vertebrate or vector cells which have concurrently been infected with more than one strain or serotype of the virus. Although the kinetics of BTV reassortment has been described in both *in vivo* and *in vitro* studies, relatively little is known about the consequences which the reassortment of different genome segments may have on the phenotypic properties of the virus. It has been speculated that the reassortment of genome segments between phenotypically distinct strains may result in the generation of novel reassortant viruses, which may display either enhanced virulence or transmission characteristics. The purpose of this review is to provide an overview of the mechanisms of viral evolution which underlie the generation of genetic and phenotypic differences among BTV field strains, to discuss the kinetics of BTV reassortment and to highlight documented examples of the effects of reassortment on the phenotype of the virus. Methods by which BTV reassortants may be generated *in vitro*, as well as possible approaches for evaluating the consequences of reassortment on the phenotypic properties of the virus are also discussed.

Keywords

Bluetongue virus, reassortment, genetic drift, genetic shift, recombination, phenotype, virulence, transmission

Introduction

Bluetongue virus (BTV) is the type species of the genus *Orbivirus* in the family *Reoviridae* and causes an infectious non-contagious, arthropod transmitted disease of ruminants called bluetongue (BT) (Mertens *et al.*, 2005). Twenty six serotypes of the virus have been identified (Maan *et al.*, 2011) which are transmitted primarily by biting midges which belong to the *Culicoides* genus (*Diptera: Ceratopogonidae*), the biological vectors of the virus (Mellor *et al.*, 2000). The distribution of BT is determined by the occurrence of vector-competent midge species and climatic conditions which support a large population of these insects. Bluetongue therefore occurs more commonly in tropical and sub-tropical regions between the latitudes of 40-50 °N and 35 °S and during times of the year which are optimal for vector activity (Mellor *et al.*, 2000). Bluetongue in ruminants is characterized by damage to endothelial cells lining small caliber blood vessels resulting in vascular thrombosis, ischaemic necrosis, haemorrhage and vascular leakage. Clinical signs of BT in sheep may include any combination of fever, depression, anorexia, nasal discharge, facial and pulmonary oedema, lameness due to coronitis, erosions of the mucosa of the buccal cavity, occasional cyanosis of the tongue and muscle degeneration (Maclachlan *et al.*, 2009). Bluetongue virus causes severe disease in certain breeds of sheep (especially European fine wool and mutton breeds), whereas cattle and goats

are usually sub-clinically affected (Barratt-Boyes & Maclachlan, 1995;Koumbati *et al.*, 1999). Even amongst highly susceptible breeds of sheep the clinical presentation can vary widely, ranging from sub-clinical to acute disease which can lead to the death of infected animals. This variation in the severity is influenced by the virulence of the infecting strain and by a number of ill-defined hosts, vector and environmental factors (i.e. breed, age, nutritional status, level of immunity, infectious dose exposure to ultraviolet radiation) (Maclachlan *et al.*, 2009).

Bluetongue virus has a segmented genome which consists of 10 linear strands of double stranded RNA (dsRNA) (Verwoerd *et al.*, 1970). The 10 viral genome segments each encode a distinct protein, seven of which are structural components (VP1-VP7), whereas three proteins are non-structural (NS1-NS3/A) and are only found in BTV infected cells (van Dijk & Huismans, 1988). The viral genome is enclosed within a triple layered protein capsid, consisting of a sub-core, core and outer capsid layer. The viral core is composed of VP3 which forms a scaffold for the assembly of the outer layers of the virion, the enzymatic proteins of the viral transcriptase complex (VP1, VP4 and VP6) and a core surface layer composed of VP7 (Mertens & Diprose, 2004), the latter which mediates the attachment and infection of insect cells (Xu *et al.*, 1997). Viral protein 1 (VP1) functions as the viral RNA dependant RNA polymerase (Boyce *et al.*, 2004), VP4 as the viral RNA capping and trans-methylation enzyme (Ramadevi *et al.*, 1998), while VP6 has been shown to possess helicase activity (Stauber *et al.*, 1997). The viral core is covered by an outer capsid composed of VP2 and VP5. Viral protein 2 (VP2) functions as the mammalian cell receptor ligand and also contains the majority of epitopes which determine virus serotype (Huismans & Erasmus, 1981). The viral fusion protein (VP5) facilitates the penetration of viral cores into the cytoplasm of mammalian cells during receptor-mediated endocytosis (Hassan *et*

al., 2001). Based on its conformational interaction with VP2 at the virion surface, VP5 also plays a minor role in determining serotype (Mertens *et al.*, 1989). Non-structural protein 1 (NS1) forms characteristic tubular structures in the cytoplasm of infected cells, the function of which has not been clearly determined (Urakawa & Roy, 1988), whereas NS2 is a major component of viral inclusion bodies (VIBs) which are sites of recruitment for viral single stranded RNA (ssRNA) transcripts and core assembly (Kar *et al.*, 2007). Non-structural protein 3 (NS3/A) facilitates the release of the virus from mammalian and insect cells either by forming pores in the cell membrane through which the virus is extruded (Han & Harty, 2004) or by bridging the outer capsid protein VP2 to the cellular export machinery resulting in viral budding (Beaton *et al.*, 2002;Wirblich *et al.*, 2006).

Emergence of reassortant BTVs in the field in Europe

The distribution of BT has recently changed dramatically in Europe, presumably due to the effects of global climate change and its influence on the distribution and vector competence of European *Culicoides* species (Purse *et al.*, 2005). Prior to 1998, BT was considered to be exotic in Europe, with outbreaks in southern Europe being caused by single serotypes which did not persist for more than a few vector seasons. This situation changed in 1998 when a series of outbreaks which were caused by several different strains belonging to five serotypes (1, 2, 4, 9 and 16) started in southern and central Europe (Mellor *et al.*, 2008). In 2006, a sixth serotype, BTV-8 was introduced via an unknown route into north-western Europe (Netherlands, Belgium Luxembourg, Germany and France). The introduction of BTV-8 marked the beginning of the most severe and economically damaging outbreak of BT on record. The outbreak in 2006 was

mild with relatively few animal holdings affected. However, the virus re-emerged in the vector season of 2007 with an increase in morbidity and mortality. During 2007 the disease managed to spread to additional European countries including Denmark, the United Kingdom, Switzerland, the Czech Republic, Spain, Portugal, Italy and Northern Ireland (Maan *et al.*, 2008).

The epidemiology of BT in north-western Europe has further been complicated by the introduction of several additional serotypes. In 2008, BTV-6 and BTV-11 of South African vaccine origin were detected in regions of the Netherlands, Belgium and Germany. These viruses were presumably introduced either through the illegal use of modified live vaccines (MLVs) in the field or through the importation of infected *Culicoides* or livestock into the region (De Clercq *et al.*, 2009;Maan *et al.*, 2010). Furthermore, a BTV-1 strain of Algerian origin has recently spread throughout Iberia and into regions of northern France (Cetre-Sossah *et al.*, 2011). Various combinations of MLV strains (3, 2, 4, 8-11, 16) of South Africa origin have furthermore been used in southern and central Europe as well as the Middle East in an attempt to control the disease. Several of these MLV strains have managed to persist in the field and have been isolated from both *Culicoides* as well as sentinel animals (Savini *et al.*, 2008;Veronesi *et al.*, 2005). The simultaneous introduction of several wild type and vaccine strains has resulted in an unprecedented mix of genetic diversity amongst BTVs circulating in the field in Europe. This has raised the possibility that these viruses may reassort with each other, potentially leading to the emergence of novel reassortant strains which may demonstrate unique biological properties (i.e. either enhanced virulence or an altered capacity to be transmitted by *Culicoides* vectors in the field) (Saegerman *et al.*, 2008).

The risk of BTV reassorting in the field in Europe was confirmed for the first time in 2002 when a double reassortant field isolate of BTV-16 was isolated in Italy. The reassortant contained a segment 2 (VP2) which was derived from a South African BTV-16 MLV strain and a segment 5 (NS1) which was derived from a South African BTV-2 MLV strain (Batten *et al.*, 2008). More recently the circulation of a BTV-6 MLV strain was detected for the first time in north-western Europe in the eastern Netherlands and later in adjacent parts of Germany in cattle which displayed mild non-specific clinical signs of BT. Whole genome sequencing confirmed that the majority of genome segments of this strain were closely related to the South African BTV-6 MLV strain. The analysis however also revealed that the virus had received its segment 10 (NS3/A) from a South African BTV-2 MLV strain. The emergence of wild type-vaccine reassortants as dominant strains Europe, suggest that these viruses have acquired unique adaptations, which allow them to compete effectively with other wild type strains in the field (Maan *et al.*, 2010). Although the nature of the reassortant events of these strains have been adequately described in the literature, the virulence markers of BTV and the exact effects which genome segment exchange may have on the phenotypic properties of parental BTV strains remains to be determined.

Genetic and phenotypic diversity of BTV

Bluetongue virus exists as a genetically and phenotypically heterogeneous entity in nature. This heterogeneity develops as a result of antigenic drift and shift as well as intragenic recombination (Bonneau *et al.*, 2001;He *et al.*, 2010). Cumulatively these processes give rise to

quasispecies populations in the infected vector or vertebrate host, which consist of a population of closely related variants which differ slightly from one or more population master consensus sequences. Quasispecies populations provide significant adaptive potential to BTV, as a range of mutants are present at any given time within an infected individual from which variants with optimal fitness may be selected for spread under different environmental conditions. Novel genotypes of BTV may also be fixed from a quasispecies population by founder effect. The random passage and amplification of particular variants (genetic bottleneck) in a quasispecies population during the transmission of the virus between its ruminant and invertebrate host, provides an additional mechanism which increases the genetic diversity of circulating field strains (Bonneau *et al.*, 2001).

The classification of BTV into serotypes depends on the interaction of neutralizing antibodies with a limited number of epitopes on the outer capsid of the virion. Serotype classification therefore gives an underestimation of the true underlying genetic diversity which exists within the BTV serogroup (White *et al.*, 2006). In endemic regions BTV circulates in temporally and geographically distinct episystems, which are defined by the occurrence of particular midge species. The isolated circulation of the virus in these episystems has over time led in the evolution of distinct geographical variants or topotypes of the virus (Gould & Hyatt, 1994). These topotypes may be distinguished from each other by phylogenetic analysis of nucleotide sequence data from the majority of viral genome segments. By using this approach BTVs can be divided into either eastern or western geographical lineages as well as additional regional subtypes (Carpi *et al.*, 2010;Maan *et al.*, 2010). Bluetongue viruses also demonstrate substantial genetic variation even amongst serotypes and strains which circulate in the same geographical

region. In one study for example it was demonstrated that the nucleotide sequence of the NS3/A gene of BTV field isolates which were collected from *Culicoides sonorenis* pools from a single dairy in California in the United States varied over a three month period from between 97.54 to 100% nucleotide sequence identity (Bonneau *et al.*, 2002).

Different geographical variants or subtypes of BTV may differ substantially from each other in regards to their phenotypic properties i.e. virulence and/or transmission potential. For example, it is known that differences exist in the virulence of BTV-4 from South Africa and the United States. BTV-4 from South Africa has been shown to be highly virulent when inoculated experimentally into Merino sheep (Maclachlan *et al.*, 2008), whereas BTV-4 from the United States appears to be less virulent and is rarely associated with clinical disease in the field (Maclachlan *et al.*, 2009). In addition it has long been known that certain serotypes in South Africa are more often isolated from *Culicoides* and that these serotypes have a high transmission potential, whereas other serotypes are more often isolated from clinically ill sheep and are thought to have a high pathogenic index (Dungu *et al.*, 2004). Differences have also been reported to exist in virulence between Australian and South African serotypes of BTV, with Australian serotypes generally being considered to be less virulent than serotypes from South Africa (Kirkland, 2004). A difference in virulence between BTV strains was also reflected during the recent outbreak of BTV-8 in north-western Europe. The BTV-8 strain caused disease not only in sheep, but also in cattle and goats (Dercksen *et al.*, 2007;Thiry *et al.*, 2006). The BTV-8 strain also demonstrated the unusual ability to cross the placenta of sheep and cattle at a high frequency, a property which had previously only generally been associated with the vaccination of ewes with MLV strains of the virus (Wouda *et al.*, 2008).

The exact genetic markers which influence the phenotypic properties (virulence and transmission potential) of BTV have not been clearly identified. It is probable that differences in viral phenotype are associated with the more variable genome segments (VP2, VP5, VP7 and NS3/A) of the BTV genome, especially when considering the function of these proteins in the viral replicative cycle. Cross hybridization studies have indicated that the outer capsid proteins (VP2 and VP5) responsible for the attachment and infection of mammalian cells undergo changes during attenuation of the virus in cell culture (Huismans & Howell, 1973), suggesting a role for these proteins in determining virulence. These studies have been confirmed in part by the demonstration that the attenuation of virulent field strains appears to be associated with amino acid changes in the VP2 protein (Gould & Eaton, 1990). Genetic variation in VP1, VP2, VP5 and NS2 has furthermore been associated with changes in virulence in experimentally infected mice (Caporale *et al.*, 2011;Carr *et al.*, 1994;Waldvogel *et al.*, 1986). Variation in the NS3/A protein may also potentially influence the virulence of BTV infections in the mammalian host. Changes in the protein which are associated with cytotoxicity in mammalian cells may for example influence the degree of virus release from infected cells and thus determine the ability of the virus to disseminate throughout the mammalian host (Huismans *et al.*, 2004). Indeed it has been demonstrated that changes in the NS3/A gene of the closely related African horse sickness virus (AHSV) is associated with differences in virulence (Meiring *et al.*, 2009).

The VP7 and NS3/A proteins play an important role in mediating the infection and dissemination of the virus in the insect vector. It has therefore been suggested that genetic variation in these proteins may relate to the transmission of the virus by different midge species or populations in different geographic locations (Maan *et al.*, 2010). The VP7 encoding

gene can be divided into several different clades through phylogenetic analysis, however only a weak correlation between these clades and particular *Culicoides* species or populations have been established (Wilson *et al.*, 2000). Similarly analysis of the amino acid sequence of the NS3/A gene of a global panel of BTV isolates failed to demonstrate positive selection which may be indicative of the co-evolution of this protein with particular *Culicoides* (Balasuriya *et al.*, 2008).

BTV genetic reassortment

Genetic reassortment has long been implicated as mechanism which can generate genetic diversity in RNA viruses with segmented genomes. In influenza viruses, the ability of the virus to reassort its genome segments is central to its ability to cross species barriers (Taubenberger & Cash, 2010). The ability of BTV to reassort its genome segments in the field is underscored by the observation that concurrent infections in ruminants in the field have frequently been demonstrated (Oberst *et al.*, 1985;Stott *et al.*, 1982). Phylogenetic analysis of sequence data from BTV field strains, have further indicated that genetic reassortment between different BTV strains and serotypes appear to occur quite commonly in nature (Mecham & Johnson, 2005;Pierce *et al.*, 1998). The recent determination of the "time to the most recent common ancestor" (TMRCA) for different viral genome segments of a globally representative panel of BTV field isolates, has further indicated that genetic reassortment of viral genome segments with radically different evolutionary histories has occurred in the past (Carpi *et al.*, 2010).

The consequences of BTV genetic reassortment on viral phenotype are difficult to predict due to the large number of reassortant strains which can potentially be generated. Indeed the

number of reassortants which can be generated increases dramatically with the number of circulating field strains. For two circulating strains, 2^{10} segment combinations are possible, which can lead to the generation of 1024 unique reassortant genotypes (Saegerman *et al.*, 2008). This situation is further complicated by the fact that the genetic markers and genome segments which influence BTV phenotypic properties have not been clearly identified.

Bluetongue virus reassortment is likely facilitated by the selection of viral ssRNA by viral inclusion bodies (NS2) during the assembly of viral core particles. The NS2 protein binds to ssRNA and is thought to play an important role in the selection and packaging of exactly one copy of each of the viral genome segments into progeny virions, possibly through the recognition of specific RNA secondary structures (Lymperopoulos *et al.*, 2006). The NS2 protein rapidly forms as a matrix around transcribing viral cores in the host cell cytoplasm and it has been suggested that the fusion of individual viral inclusion bodies originating from different co-infecting strains, creates a situation where genome segments from different viruses are brought into close association, which could then facilitate the occurrence of reassortment (Schwartz-Cornil *et al.*, 2008).

The reassortment of BTV has been investigated in *vivo* in sheep (Samal *et al.*, 1987b), bovine (Oberst *et al.*, 1987) and *Culicoides variipennis* (El Hussein *et al.*, 1989;Samal *et al.*, 1987a), as well as *in vitro* in cell culture (Ramig *et al.*, 1989). These studies have indicated that reassortment occurs at varying frequencies in the different host systems. In sheep, the fraction of reassortant progeny clones recovered from viraemic animals was approximately 5% (Samal *et al.*, 1987b), whereas in a similar study in bovine, the ratio of recovered reassortant progeny

was significantly higher (89%) (Stott *et al.*, 1987). The discrepancy between the fraction of reassortants between the two host species is not clear, although it has been speculated that an increased frequency of reassortment in cattle relative to sheep may reflect the prolonged viraemia in cattle, which may increase the opportunity for reassortment to occur (Stott *et al.*, 1987). A high fraction of reassortants have also been recovered from mixed infected *Culicoides* midges (7-78% of clones recovered per infected midge), suggesting that the insects are highly permissive hosts for reassortment of the virus in nature (Samal *et al.*, 1987a). 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 *Culicoides* may take multiple blood meals (Mellor *et al.*, 2000). The frequency of reassortment in infected African Green Monkey Kidney (Vero) cells has also been shown to be high, with 54% of recovered progeny virions being reassortants (Ramig *et al.*, 1989).

With regards to the type of reassortants which were isolated from the different host systems, typically one of the parental strains dominated the yield of recovered virions, with other parental strains only being represented by their contribution of genome segments to reassortant progeny. Multiple unique reassortant genotypes could further be isolated from each of the different host systems, with some reassortant genotypes dominating the yield of the recovered reassortant progeny later during infection. This observation suggested that these viruses had either reassorted their genome segment earlier during the infection cycle and therefore had replicated to a higher titre, or that these viruses may have acquired genome segments which conferred a selective advantage over the other reassortant genotypes. Indeed reassortment appears to occur non-randomly for some of the genome segments (segment 5, 7,

8, 9 and 10) (El Hussein *et al.*, 1989;Ramig *et al.*, 1989;Samal *et al.*, 1987a;Samal *et al.*, 1987b). The exact advantages which were conferred on the reassortant progeny by the selection for or against these genome segments are however unknown.

Finally the asynchronous infection of *Culicoides* as well as vertebrate cell culture with two or more serotypes has shown that cells which have been infected with a particular serotype become increasingly resistant against infection with a secondary serotype at increasing time intervals post infection, a phenomena which is called “viral exclusion”. Although the exact mechanism of viral exclusion has not been determined, it is thought to limit the frequency with which reassortment occurs in the vertebrate host and insect vector in nature to times when these hosts are infected simultaneously with more than one strain or serotype of the virus, or nearly so (El Hussein *et al.*, 1989;Ramig *et al.*, 1989).

Consequences of BTV reassortment

Published examples of the effects of reassortment on BTV phenotype are limited to only a few case studies. The most well-known example involves the study of naturally occurring VP5 reassortants of BTV-11 named UC-2 and UC-8 which were isolated from the field in the USA. Interestingly, UC-2 and UC-8 shared the same VP2 segments, but differed in regards to their VP5 segments. The UC2 strain derived its genome segment 5 from a BTV-11 MLV strain, while UC-8 derived its segment 5 from a BTV-10 MLV vaccine strain (Osburn, 1994). When inoculated into new-born mice, UC-2 was found to be virulent only when inoculated via the intra-cranial route, whereas UC-8 was virulent when inoculated via either the sub-cutaneous or intra-cranial route (Waldvogel *et al.*, 1987;Waldvogel *et al.*, 1986). The two viral strains also differed in

regards to their ability to cause disease in foetal calves, depending on the gestational age at which the foetus was infected. When foetuses were inoculated through the uterine wall at 120 days of gestation, both strains were able to cause neurological abnormalities (Waldvogel *et al.*, 1992b). In contrast, the inoculation of foetuses with UC-2 at 243 days of gestation led to the birth of healthy calves, while inoculation with UC-8 led to the premature birth of small and weak calves which displayed mild encephalitis (Waldvogel *et al.*, 1992a). The exact contribution of the particular version of VP5 in the enhanced neurovirulence of UC-8 is not known. It has been suggested that the enhanced neurovirulence of UC-8 relative to UC-2 may have been related to stearic interaction between the different versions of VP2 and VP5 at the virion surface, which could have affected viral binding as well as neutralization (Carr *et al.*, 1994).

Approaches for evaluating the effect of genome segment reassortment on viral phenotype

An investigation into the nature of the virulence markers as well as the effect of the reassortment of particular genome segments on BTV phenotype has until recently been hampered by the lack of a reverse genetics system which can allow for the introduction of defined mutations or reassortant events into the genome of the virus. Recently it was demonstrated that infectious virus was entirely recoverable by transfecting cells with *in vitro* transcribed ssRNA of each of the viral genome segments (Boyce *et al.*, 2008). This method

contains the advantage that genome segments may be modified by site directed mutagenesis in order to introduce defined mutations into any region of the viral genome, as well as that transcripts from different strains or serotypes can be combined during transfection to yield specific reassortant genotypes.

The development of a reverse genetics system through which specific BTV reassortant genotypes may be generated *in vitro*, has made it possible to evaluate the effect of genome segment reassortment and site directed mutagenesis on the phenotype of BTV. Several *in vivo* and *in vitro* approaches may prove useful for such a purpose. In order to evaluate differences in virulence between BTV strains, experimental transmission studies with BTV in ruminants have frequently been used. For example it is common practice to evaluate the degree of attenuation of MLV strains by comparing disease-specific parameters (temperature, length of viraemia and severity of oral lesions) in vaccinated and unvaccinated animals following a virus challenge (Savini *et al.*, 2008). Transmission studies in live animals have obvious disadvantages for ethical, animal welfare and economic reasons; however, BTV transmission studies in ruminants are also subject to several other disadvantages. The clinical signs of BT for example may be highly variable between individual animals, even if they are inoculated with the same strain and titre; they belong to the same breed and are kept under the same experimental conditions (Maclachlan *et al.*, 2009). Due to this variability, a comparison of the virulence between reassortants by using clinical reaction indexes or by comparing pathological features, may not give an accurate reflection of strain-specific differences. Furthermore these studies are expensive to conduct and require the use of a large number of animals as well as specialized vector-free facilities.

The use of small animal models provides a suitable alternative for comparing differences in BTV phenotype. It has been demonstrated by immunoperoxidase staining that 11-day old chicken embryos show a similar tissue tropism for the closely related African horse sickness virus as infected horses (Maartens, 2010). Similarly type 1 interferon receptor deficient (INFAR $^{(-/-)}$) mice are highly susceptible to BTV infection via both the oral and intravenous route, and demonstrate similar clinical and pathological features as the ruminant host (Calvo-Pinilla *et al.*, 2009). The use of *in vitro* methods to compare reassortant phenotypes also shows promise. The cultivation of lung micro-vascular and pulmonary artery endothelial cells from sheep and cattle has made it possible to compare some of the cellular and immunological features which are responsible for differences in the clinical presentation of BTV in these hosts. (Coen *et al.*, 1991;DeMaula *et al.*, 2001;DeMaula *et al.*, 2002a;DeMaula *et al.*, 2002b;Drew *et al.*, 2010b). Changes in trans-endothelial cell monolayer electrical resistance (TER) also provide a quantitative measurement of the degree of cell monolayer destruction following infection (Drew *et al.*, 2010a). It can be envisaged that these parameters may be compared in permissive cell lines which have been infected with different reassortant strains, in order to illustrate strain specific differences in BTV phenotype. Finally a comparison of the transmission potential between reassortant strains in *Culicoides* may be conducted through oral susceptibility studies. These studies are based on the oral infection of *Culicoides* by using blood meals which have been spiked with a defined titre of BTV, prior to a comparison of either total or disseminated infection rates as well as viral titre in infected midges after the extrinsic incubation period (period between blood feeding and the dissemination of the virus to the salivary glands) has been completed. Potential pitfalls of this strategy include the standardization of the virus titre

in the blood meal prior to blood feeding, the generally low oral susceptibility of *Culicoides* for BTV, as well as the potential varying oral susceptibility of different *Culicoides* populations based on their geographic origin (Venter & Paweska, 2007).

Concluding remarks

Genome segment reassortment between different strains of BTV likely confers the ability to the virus to rapidly adapt to changing environmental conditions. Although BTV reassortment occurs frequently under both natural and experimental conditions, the effects on virus phenotype remains relatively ill explored. With newer *in vivo* and *in vitro* infection models, as well as the recent advent of a reverse genetic system for BTV, the opportunity now exists to further investigate the effect of genome segment reassortment on the phenotype of the virus. These studies may assist to clarify the implication of reassortant virus emergence with regards to the epidemiology and control of BT and may also allow for the identification of the genetic markers which confer particular phenotypic properties to the virus.

Competing interests

The author(s) declare that they have no competing interests.

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