Antiviral Research 99 (2013) 79–90

Contents lists available at SciVerse ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Review

Potential strategies for control of bluetongue, a globally emerging, *Culicoides*-transmitted viral disease of ruminant livestock and wildlife

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ARTICLE INFO

Article history:
Received 28 March 2013
Revised 25 April 2013
Accepted 30 April 2013
Available online 9 May 2013

Keywords:
Bluetongue
Culicoides midges
Arbovirus
Orbivirus

ABSTRACT

Bluetongue (BT) is a non-zoonotic arboviral disease of certain wild and domestic species of cloven-hoofed ungulates. The causative agent, bluetongue virus (BTV), is spread through temperate and tropical regions of the world by biting *Culicoides* midges. Control of BTV infection is complicated by the plurality of virus serotypes and the ubiquity and opportunistic feeding behavior of its midge vector. The global distribution of BTV infection has recently altered, perhaps driven in part by climatic influences on midge species resident in different regions. The goal of this review is to evaluate realistic strategies that might be utilized to control or prevent future outbreaks of BT and other *Culicoides*-transmitted diseases. Importantly, optimal control of emerging, rapidly evolving arbovirus diseases such as BT will require integrated countermeasures that mitigate all aspects of the virus's transmission cycle. This will best be accomplished using preventative, rather than purely reactive strategies.

Contents

1. Introduction, history and pathogenesis

Bluetongue (BT) is a non-zoonotic arboviral disease of certain cloven-hoofed ungulates that was first recognized soon after European sheep were introduced to southern Africa over 200 years ago (Henning, 1956; Hutcheon, 1902; Spreull, 1905; Verwoerd, 2012). BT and closely related African horse sickness (AHS) were amongst the first animal diseases recognized to be caused by viruses and to be transmitted by insects (Table 1) (Du Toit, 1944; Spreull, 1905; Verwoerd, 2012). BT was only later recognized to occur outside of Africa, and notable epizootics occurred throughout the world during the 20th and 21st centuries (reviewed: Erasmus and Potgieter, 2009; Maclachlan, 2011; Mellor et al., 2008; Verwoerd and Erasmus, 2004; Wilson and Mellor, 2008; Walton, 2004; Tables 2 and 3). Although bluetongue virus (BTV) infection of animals is

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http://dx.doi.org/10.1016/j.antiviral.2013.04.021

Table 1
Characteristics of bluetongue virus, its arthropod vector, the disease it produces in animals, its pathogenesis, and related orbiviral diseases of animals.

Virus classification and structure	Bluetongue virus (BTV) is the prototype virus of the genus *Orbivirus*, family *Reoviridae*. Its genome consists of 10 segments of double-stranded RNA. The virions are unenveloped, have icosahederal symmetry, and a double shell particle with an outer capsid of viral proteins VP2 and VP5, and an inner core largely of VP3, VP7 and lesser amounts of VP1, VP4 and VP6. Five nonstructural proteins (NS1, NS 2, NS3, NS3A, NS4) are produced in virus-infected cells. There are currently 26 proposed BTV serotypes, with extensive genetic heterogeneity of field strains, regardless of serotype, which arises through genetic drift and shift
Arthropod vector	*Culicoides* midges are the biological vectors of BTV. Some 30 of >1000 species of midges that occur worldwide are considered vectors. Different midge species are vectors of different constellations of BTV serotypes in distinct global "episystems". Some midge species are voracious blood feeders that will feed on a variety of mammals, humans included
Geographic distribution	BTV infection occurs throughout temperate and tropical regions of the world coincident with the distribution of vector *Culicoides* midges; from approximately latitude 35° S to beyond 50° N but within this range there are groupings of different midge vectors and different constellations of BTV serotypes. The global range of *Culicoides* midges is greater than that of BTV. BT typically occurs at the upper and lower "incursional" areas of the virus' global range, and during the late summer and fall
Clinical syndrome in ruminant livestock and wildlife	Severe BT disease occurs most often in susceptible breeds of sheep, with high fever, hemorrhage and ulceration of the mucosal lining of the upper gastrointestinal tract, coronitis, and multicentric necrosis of both skeletal and cardiac muscle. Edema is characteristic, and pulmonary edema and pleural and pericardial effusion are typical of fatal cases. BT in wild ruminants can present as a peracute hemorrhagic diathesis. Many BTV infections are subclinical, especially in endemic areas and in cattle and wild African ungulates
Pathogenesis	Vascular injury is central to the pathogenesis of BT. BT is similar to other viral hemorrhagic fevers with cellular tropism for dendritic cells, endothelium, and mononuclear leukocytes, capillary leakage and thrombocytopenia, coagulopathy, and hemorrhagic diathesis. Virus is cell-associated during viremia and intimate association of virus with erythrocytes facilitates a prolonged, but not persistent infection of animals and infection of vector hematophagous midges
Other orbiviral diseases	African horse sickness, epizootic hemorrhagic disease of deer, equine encephalosis, Peruvian horse sickness and Elsye disease

frequently subclinical, severe BT disease can be dramatic, particularly in susceptible breeds of sheep and white-tailed deer, with high fever, hemorrhage and ulceration of the mucosal lining of the upper gastrointestinal tract, coronitis, and multicentric necrosis of both skeletal and cardiac muscle (Howerth et al., 1988; Maclachlan et al., 2008, 2009; Moulton, 1961; Spreull, 1905; Verwoerd and Erasmus, 2004). Edema as a consequence of capillary leakage is also characteristic, and pulmonary edema with accompanying pleural and pericardial effusion is typical of fatal cases.

Vascular injury is central to the pathogenesis of BT, thus BT shares many of the characteristic features of the better known viral hemorrhagic fevers such as Ebola, including cellular tropism for dendritic cells, endothelium, and mononuclear leukocytes; capillary leakage; and thrombocytopenia, coagulopathy, and hemorrhagic diathesis (Channappanavar et al., 2012; DeMaula et al., 2001, 2002; Drew et al., 2010a,b; Gowen and Holbrook, 2008; Hemati et al., 2009; Lee et al., 2011; Maclachlan et al., 2009). Although cattle are usually less affected by BTV infection than are sheep, some highly pathogenic virus strains can cause disease in all ruminant livestock, South American camelids, many non-African species of wild ruminants, and even certain carnivores (Darpel et al., 2007; Elbers et al., 2008; Maclachlan et al., 2009; Makoschey et al., 2009; Verwoerd and Erasmus, 2004). BTV infection of ruminants is characterized by a highly cell-associated viremia, and late in the course of infection the virus is associated principally with erythrocytes (Afshar, 1994; Barratt-Boyes and Maclachlan, 1994; Luedke et al., 1969; Maclachlan et al., 1990, 2009). This intimate binding of BTV to the erythrocyte cell membrane leads to prolonged but not persistent infection of animals by protecting the virus from immune clearance, and this interaction also provides an ingenious "Trojan Horse" mechanism for infection of the hematophagous *Culicoides* midges that serve as biological vectors of the virus (Brewer and Maclachlan, 1992, 1994; Maclachlan et al., 1994).

Outbreaks of severe BT can be economically devastating but, because BTV is transmitted by incompletely defined species of a relatively ubiquitous but poorly characterized genus of insect vector (Carpenter et al., 2008; Mellor et al., 2000), elimination of the infection in enzootic areas is difficult or impossible. *Culicoides* biting midges occur throughout most of the inhabited world where they transmit a wide variety (>50) of pathogens of animals and humans, including not only orbiviruses such as BTV, AHS virus and epizootic hemorrhagic disease (EHD) virus, but also rhabdoviruses, reoviruses, and pathogenic bunyaviruses such as Akabane and Schmallenberg viruses (Conraths et al., 2013; Mellor et al., 2000). Of relevance to the possible future emergence of new zoonotic diseases, some *Culicoides* midges have broad host feeding preferences that include humans as well as animals, and animal parasites can be transmitted to humans through the bites of *Culicoides* midges (Calvo et al., 2012; Lassen et al., 2012; Santiago-Alarcon et al., 2012). Thus, in an era of rapid change in global climate, travel, and social structures, strategies to mitigate outbreaks of BT and related animal diseases might someday guide the control of an as yet unknown, newly emergent zoonotic disease of humans that is also transmitted by *Culicoides* biting midges. The goal of this review, therefore, is to evaluate realistic strategies that might be utilized to control or prevent future outbreaks of BT and, potentially, other *Culicoides*-transmitted diseases.

2. Global distribution and recent changes

BTV infection occurs throughout temperate and tropical regions of the world coincident with the distribution of the species of hematophagous *Culicoides* midge that serve as biological vectors of the virus (reviewed: Gibbs and Greiner, 1994; Maclachlan, 2011; Tabachnick, 2004, 2010; Verwoerd and Erasmus, 2004). Although BTV has an extensive global distribution, it is to be stressed that occurrence of BT is more limited and typically occurs only at the northern and southern incursional limits of the virus's range and/or when fully susceptible animals are exposed to virulent strains of the virus. BT is uncommon in regions of the world where enzootic BTV infection occurs year-round, likely because of population immunity or as a result of prolonged co-evolution of the virus and its animal host, as presumably is the case of wild African ungulates that have evolved over the millennia in the continuous presence of the virus throughout much of the continent (Verwoerd, 2012).

The broad global range of BTV extends from approximately latitude 35° S to beyond 50° N. *Culicoides* midges occur even beyond this range, particularly in the northern hemisphere (Mellor et al., 2000). Within this extensive virus-enzootic global zone, different species of *Culicoides* midge transmit different constellations of BTV serotypes in relatively distinct "episystems" (Daniels et al.,

Table 2
Global outbreaks of bluetongue (BT) and isolation of BTV through the late 1980s.

18th Century, South Africa	Recognition of BT in sheep in late 18th century; initial published descriptions in late 19th/early 20th century; first descriptions of BTV and efforts at vaccination and vector control at turn of 20th century
1943, Cyprus	First published description of BT outside of Africa; up to 70% mortality in sheep in some flocks; similar reports from Palestine and Turkey; anecdotal reports describe regular earlier occurrence of BT on Cyprus
1949, Israel	First recognition led to use of live attenuated vaccines that remain in use
1952, United States	BT recognized in sheep in California; lead to development and use in sheep of live attenuated BTV serotype 10 vaccine; subsequent identification of BTV serotypes 2, 11, 13 and 17. Major subsequent economic impact on livestock trade (>$100 million/annum)
1956, Iberian Peninsula	Outbreak continued until 1960, single serotype (BTV-10). Estimated to have killed some 179,000 sheep; mortality rate up to 75%. Response of animal movement restrictions, culling, and use of live-attenuated vaccine
Pakistan, 1958	Initial outbreaks described in sheep
India, 1964	Initial outbreaks described in imported sheep breeds such as merino. BT now common in native sheep breeds along with subclinical infection of goats, cattle, buffalo, camels and wild ruminants
Japan, 1974	Detection of subclinical BTV infection of Japanese cattle, infection with multiple serotypes described sporadically since
Australia, 1977	Multiple BTV serotypes have been isolated from asymptomatic cattle in northern Australia; no reports of BT in sheep in field but virus strains are pathogenic for sheep; major economic impact on livestock trade.
1978, South and Central America, Caribbean Basin	Numerous virus serotypes recognized in these regions; generally asymptomatic infection; BT described in sheep in Brazil but rarely
Greece, 1979	Lesbos, BT in sheep
China, 1979	First recognition of BT; subsequent recognition of numerous serotypes
Indonesia, 1981; Malaysia, 1987	High morbidity and variable mortality of BT in imported sheep

Table 3
Outbreaks of bluetongue (BT) and isolation of BTV since the late 1990s.

1998; Mediterranean Europe	Outbreak in Greece, much of the Iberian Peninsula, Italy, the Balkan countries and Mediterranean islands; the causative BTVs invaded from North Africa and Asia and were spread by *C. imicola*, the principal Asian-African vector of BTV, and other indigenous midges. An estimated >1 million sheep succumbed, either from BT or control measures that included animal movement restrictions, culling, and vaccination
1998, Southeastern United States	Recognition of 10 additional BTV serotypes that likely spread from Caribbean Basin
2003, Taiwan	First reported isolation of BTV
2006–2008, Northern Europe	First appearance of BTV in the region; BTV-8 affected most of Europe and portions of England and Scandinavia. Outbreak controlled by animal movement restrictions and intensive vaccination. Extensive morbidity and mortality of ruminant livestock and non-African wild ungulates; estimated 200 million Euros costs just in the Netherlands, and >50,000 cases in France. Origin of virus unknown; unusual properties including high rate of vertical transmission in livestock
2007, Western United States	Limited outbreak of BTV-17 in sheep and wildlife; unknown economic impact but lead to animal movement restrictions between states
2008, Switzerland	Toggenberg orbivirus (putative 25th BTV serotype) identified in asymptomatically infected goats
2011, Kuwait	Putative 26th BTV serotype identified in sheep in Kuwait
2012, Mid-western United States	Outbreaks of severe epizootic hemorrhagic disease (EHD) and BT in cattle and deer; economic impact uncertain, but lead to multiple foreign animal disease investigations
2012, Russia and Baltic States	Detection of a live-attenuated vaccine strain of BTV-14; similar vaccine strains of BTV-6 and BTV-11 detected earlier in northern Europe. Route and method of introduction unknown

2004; Gibbs and Greiner, 1994; Maclachlan and Osburn, 2006; Tabachnick, 2004, 2010). For example, *Culicoides imicola* (*C. imicola*) is the principal vector of numerous BTV serotypes throughout extensive portions of Africa and Asia, whereas *Culicoides sonorensis* (*C. sonorensis*) is the major, perhaps exclusive vector of only BTV serotypes 10, 11, 13 and 17 throughout much of North America (Gibbs and Greiner, 1994; Gibbs et al., 2008; Tabachnick, 2004, 2010; Walton, 2004).

It is proposed that the boundaries of the global BTV episystems reflect variation in the vectorial capacity of populations of the different species of *Culicoides* midge that are resident within each episystem. Consistent with this premise, strains of BTV tend to genetically "topotype" to their episystem of origin, reflecting negative (purifying) selection of certain viral genes over time (Balasuriya et al., 2008; Bonneau et al., 1999; Gould and Pritchard, 1990; Nomikou et al., 2009). Uncertain, however, is the vector competence of many of the >1000 species of *Culicoides* midges that occur worldwide (Daniels et al., 2004; Meiswinkel et al., 2004; Mellor et al., 2000; Tabachnick, 2004, 2010). Furthermore, despite the apparent stability of the various global BTV episystems during much of the 20th century, there have been dramatic recent changes in the global distribution of BTV infection (Gibbs et al., 2008; Gould and Higgs, 2009; Maclachlan, 2011; Maclachlan and Guthrie, 2010; Tabachnick, 2010; Wilson and Mellor, 2008).

Recent changes in the global distribution of BTV have been especially profound in Europe where, prior to 1999, only transient and limited incursion of single BTV serotypes into Mediterranean portions of the continent had been documented (Gomez-Trejedor, 2004; Mellor et al., 2008; Rodriguez-Sanchez et al., 2008). Since the millennium, multiple BTV serotypes have invaded and spread throughout the northern rim of the Mediterranean Basin and the virus is now well established in parts of the region, most of central/southern Italy for example (Calistri et al., 2004; Rodriguez-Sanchez et al., 2008; Zientara and Sanchez-Vizcaino, in press; Fig. 1A and B). Climate change has been proposed as a potential driver of this recent northward expansion of BTV from Africa and Asia into Mediterranean Europe (Guis et al., 2012; Purse et al., 2005, 2008; Wilson and Mellor, 2008).

Coincident with the invasion of multiple serotypes of BTV into Europe, at least 10 novel serotypes of BTV have been identified in the southeastern United States (US) i.e. in addition to the 5 serotypes (BTV-2, 10, 11, 13 and 17) previously recognized to be enzootic in extensive portions of North America (Gibbs et al., 2008; Johnson, 2007; Ostlund, 2009). These novel viruses have now been identified in multiple states in the region, as far north as Arkansas and as far west as Texas. The incursion of multiple novel BTV serotypes into the southeastern US, which to date has not led to any notable reported increase in the occurrence of clinical BT, is

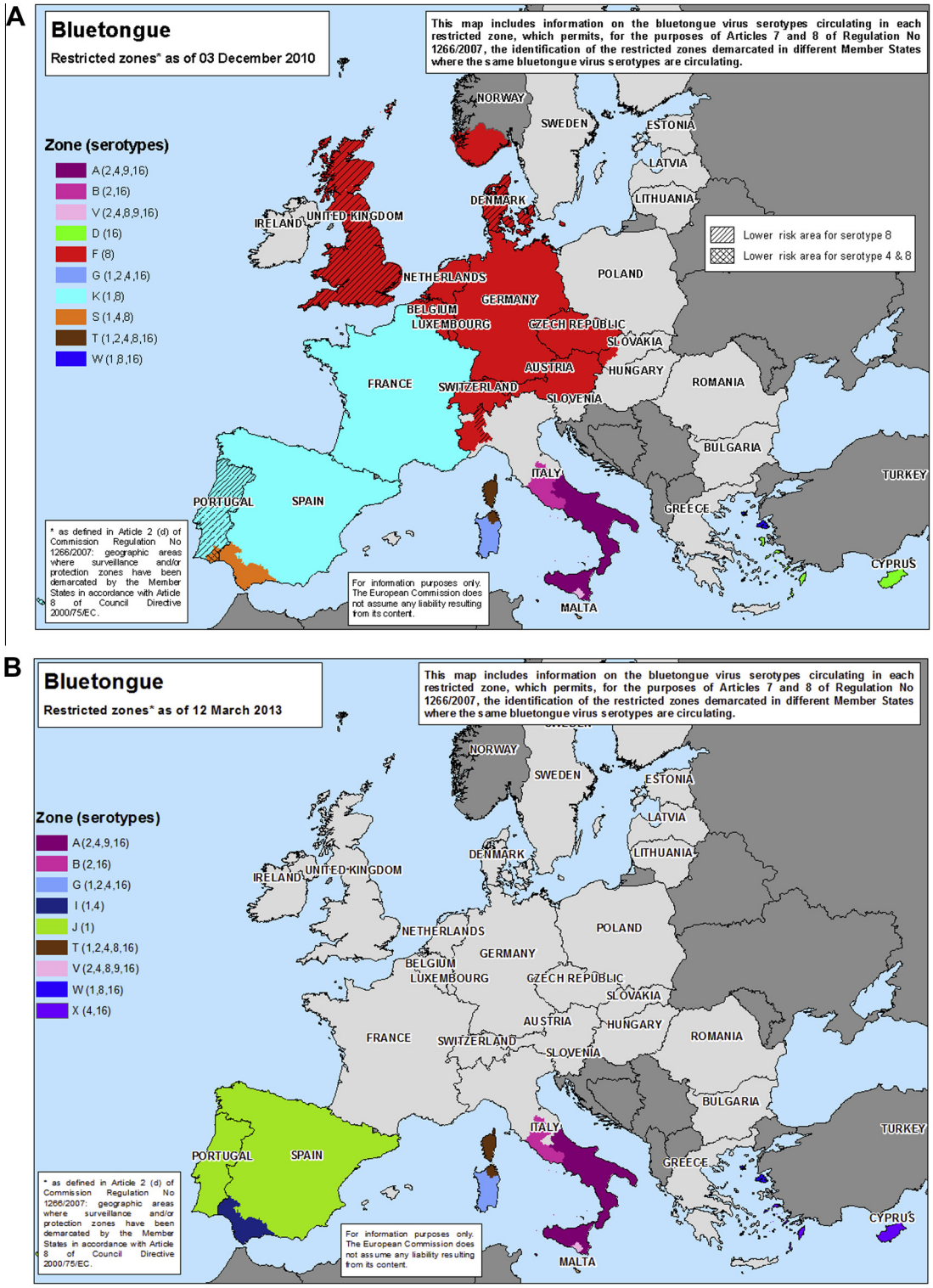

Fig. 1. (A) Restricted zones for different serotypes of bluetongue virus in Europe as of December, 2010. (B) Restricted zones for different serotypes of bluetongue virus in Europe as of March, 2013. From the European Commission. Restriction zones established by the Member States. Available at: http://ec.europa.eu/food/animal/diseases/controlmeasures/bt_restrictedzones-map.jpg and http://ec.europa.eu/food/animal/diseases/controlmeasures/bt_restrictedzones-map_2012.jpg. Copyright European Communities, Accessed March 22, 2013.

speculated to reflect northward expansion of the margins of the Caribbean/Central American BTV episystem where *Culicoides insignis* is the predominant vector, whereas *C. sonorensis* is the principal North American vector (Gibbs and Greiner, 1994; Maclachlan, 2011; Tabachnick, 2010). A considerably greater number of BTV serotypes have been historically identified within the Caribbean Basin than in North America, so the recent identification of multiple novel BTV serotypes in the US is indicative of a profound change in the distribution/dynamics of BTV infection within the southeastern US –– perhaps, as in Europe, driven by climate

change. Ongoing surveillance elsewhere in the world has also identified novel BTV serotypes in historically enzootic countries, Australia and Israel for example (Maclachlan, 2011). Perhaps because of enhanced surveillance following the recent highly publicized and economically devastating epizootics of BT in Europe, putative new BTV serotypes (BTV-25 and 26) have been identified recently in Switzerland and the Middle East (Hofmann et al., 2008; Maan et al., 2011).

Although climate change has been proposed by some investigators to be the cause of this recent expansion in the global range and dynamics of BTV infection, other environmental and anthropogenic factors might also be important or even responsible for these events (Gould and Higgs, 2009; Tabachnick, 2010). Independent of the incursion of multiple serotypes of BTV into Mediterranean Europe, an especially virulent strain of BTV serotype 8 (BTV-8) appeared and quickly spread throughout much of northern Europe (Elbers et al., 2008; Gibbens, 2010; Makoschey et al., 2009; Saegerman et al., 2008; Wilson and Mellor, 2008; Velthuis et al., 2010; Zientara and Sanchez-Vizcaino, in press). This virus spread as far north as the British Isles and Scandinavia, and although it quickly spread throughout virtually all of Europe, it appears to have since established significant enzootic infection in only very limited southern regions of the continent (Fig. 1A and B). The incursion of BTV-8 into northern Europe occurred following an unusually hot summer, and the virus was spread by Palearctic species of *Culicoides* midges that were not previously considered to be vectors of BTV (Enserink, 2006; Guis et al., 2012). However, climate change alone cannot explain the event, as this particular virus did not encroach from directly adjacent regions – indeed, it is unknown where the strain of BTV-8 originated, as there were no reported outbreaks of BTV-8-associated disease elsewhere in the world at the time. Furthermore, to gain access to northern Europe this strain of BTV-8 clearly had to "leapfrog" the entire Mediterranean Basin where at least 5 other virus serotypes were actively circulating at the time.

Other BTV serotypes subsequently have appeared transiently in Europe, first BTV-6 and BTV-11 in northern Europe and, most recently, BTV-14 in Russia and the adjacent Baltic states. These latter incursions (BTV-6, 11, 14) are all believed to have resulted from the introduction of South African live-attenuated BTV vaccine viruses but, like the BTV-8 epizootic (which was not caused by any known live attenuated vaccine virus strain), their route and method(s) of introduction remain uncertain (de Clercq et al., 2009; van Rijn et al., 2012b). Similarly in North America, BTV-2 was recently (2010) isolated in California, representing trans-continental spread of this virus serotype that was first identified in the US in Florida in 1982 and that had previously been considered to be confined to the southeastern US (Maclachlan et al., 2013). The strain of BTV-2 isolated in California is a reassortant of BTV-2 and BTV-6, the latter a serotype that was previously exotic to North America. These multiple and independent events in Europe and North America confirm that anthropogenic factors, and not climatic factors alone, can contribute to spread of BTV.

Agricultural practices also can influence the occurrence of BT; for example, BTV is enzootic in extensive areas of northern Australia where cattle are commonly infected subclinically (Eagles et al., 2012; Kirkland, 2004). Although sheep infected experimentally with some Australian strains of BTV develop typical BT, confirming virulence of these viruses, clinical BT is currently not significant in Australia because sheep production is largely limited to areas of the country that are south of the area of virus circulation. In a different vein, the extensive waste-water effluent lagoons that are used for crop irrigation at contemporary intensive dairy production units in portions of California provide a fertile environment for vector *C. sonorensis* midges, which potentially leads in turn to higher rates of BTV infection of local livestock (Gerry and Mullens, 2000; Mayo et al., 2010, 2012a,b). Lastly, studies in both North America and Europe confirm that live, attenuated BTV vaccine viruses (or individual gene segments thereof) used to vaccinate livestock can be acquired and transmitted by vector midges in the field, and so contribute to the gene pool of circulating viruses (Batten et al., 2008; Ferrari et al., 2005; Osburn et al., 1996). Some of these viruses have distinctive phenotypic properties, notably their high propensity to cross the ruminant placenta (Flanagan and Johnson, 1995; Maclachlan et al., 2000; Maclachlan and Osburn, 2008; Savini et al., in press; Shultz and DeLay, 1955).

3. Mechanisms of virus circulation and persistence

With the possible exception of goat-adapted BTV strains/serotypes (BTV-25 and 26) and rare instances of neonatal infection of livestock via colostrum and other possible instances of oral transmission, spread of BTV does not usually occur in the absence of its *Culicoides* midge vector (Batten et al., 2013; Mayo et al., 2010; Verwoerd and Erasmus, 2004). Few regions/countries of the world are free of *Culicoides* midges, and therefore BTV, only those that are geographically isolated and/or have inhospitable climates e.g. Antarctica, New Zealand, and Hawaii (Mellor et al., 2000). Interestingly, BTV is enzootic only in regions of the world where more than one virus serotype circulates, thus prior incursions of single BTV serotypes into the Iberian Peninsula, the Okanagan Valley of Canada, the Greek islands, and portions of northern Europe, the British Isles and Scandinavia, amongst other examples, were transient, and the invading virus disappeared within a few years with or without vaccination of livestock and other control measures (Clavijo et al., 2000; Maclachlan, 2011; Mellor et al., 2008; Fig. 1A and B). In contrast, BTV has persisted in other recently invaded areas, such as much of central/southern Italy, where multiple serotypes now circulate despite intensive vaccination of livestock and the implementation of other control strategies. The mechanism determining this phenomenon remains poorly studied however it is abundantly clear that whereas incursions of BTV typically involve a genetically distinct virus strain, the strains of BTV that circulate in enzootic regions are remarkably heterogeneous.

The extensive genetic heterogeneity of field strains of BTV in enzootic areas arises as a consequence of both genetic drift and

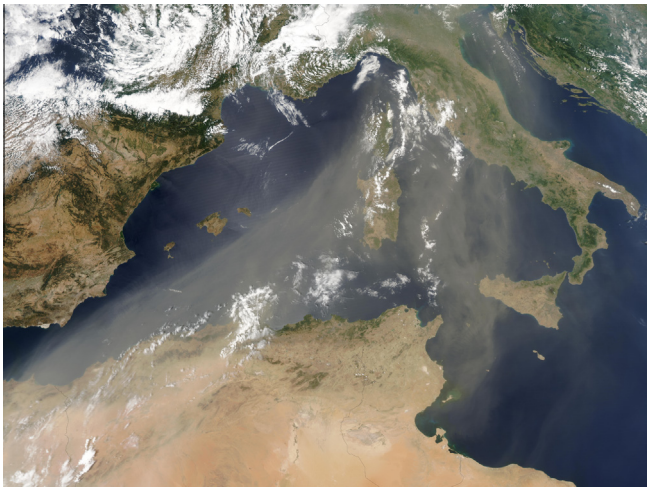

Fig. 2. Dust storm over Sardinia on 18 July, 2000, originating in Algeria and Tunisia, immediately prior to the outbreak of BT on Sardinia. The outbreak was caused by a virus that already was causing BT in Algeria and Tunisia, and is now attributed to long-distance, wind-borne dispersal of infected *Culicoides* midges. Source: 'SeaWiFS Project' (NASA/Goddard Space Flight Center) and ORBIMAGE, OrbView-2 (Calistri et al., 2004), courtesy of Veterinaria Italiana.

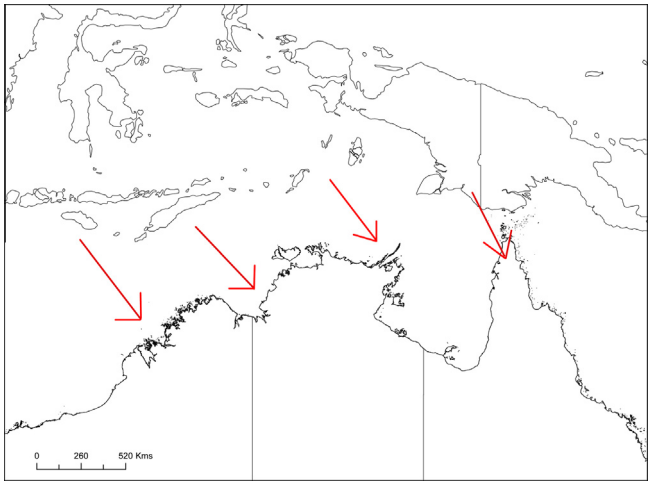

Fig. 3. Proposed long-distance spread on monsoonal winds of BTV-infected *Culicoides* midges from Timor to northern Australia (Courtesy of D. Eagles and P. Daniels).

shift (Bonneau and Maclachlan, 2004; Coetzee et al., 2012). The former occurs as a consequence of quasispecies evolution and founder effect during serial passage of the virus between its vertebrate and invertebrate hosts, whereas genetic shift occurs by reassortment of viral genes during mixed infections of these same hosts (Bonneau et al., 2001; Osburn et al., 1996; Pierce et al., 1998; Pritchard et al., 2004; Samal et al., 1987a,b). Thus, although the factors responsible for long-term maintenance of BTV in historically enzootic regions remain poorly characterized, the presence of multiple serotypes and genetically divergent strains of BTV in a region might contribute and there is no clear example of eradication of BTV from an historically long-term enzootic region.

Both virus-infected animals and insect vectors may contribute to the spread of BTV during epizootics, as shown recently in Europe. Specifically, *Culicoides* biting midges are capable of high-altitude flight, and long-range distribution of BTV via wind-borne midges was confirmed from North Africa to Sardinia (Fig. 2), continental Europe (Belgium) to the British Isles, and, perhaps, from Sardinia to the Balearic Islands (Agren et al., 2010; Burgin et al., 2013; Calistri et al., 2004; Garcia-Lastra et al., 2012; Hendrickyx et al., 2008; Sanders et al., 2011). Similarly, the regular incursion of BTV into the "top end" of Australia is attributed to the spread of virus-infected midges from adjacent islands of Southeast Asia by monsoonal winds (Eagles et al., 2012; Fig. 3). Long-distance wind-borne dispersal of infected vectors has also been incriminated in the spread of novel BTVs to the southeastern US from the Caribbean islands (Sellers et al., 1989). In contrast, sequential short-distance spread is likely to be more important in disseminating BTV during epizootics (Sedda et al., 2012) At the local level, BTV-8 progressively moved through portions of Europe at an estimated rate of >5 km/day (Pioz et al., 2011).

3.1. Interseasonal maintenance of BTV

An additional challenge to eliminating BTV from within a region is that the mechanism responsible for interseasonal maintenance (so-called "over-wintering") of the virus is unknown. This is especially important in temperate regions where BTV infection is distinctly seasonal, with virus transmission and disease occurring in the late summer/autumn months. The potential mechanisms of interseasonal maintenance are those proposed by Nevill (1971):

1. Vertical virus transmission in the vector.
2. A complicated overwintering cycle that involves some as yet unrecognized intermediate host, analogous, for example, to eastern equine encephalitis virus.
3. Survival of long-lived, virus-infected midges.
4. Prolonged survival of the virus in a livestock reservoir (cattle in particular).
5. Low-level circulation of the virus between its vector and animal hosts, the mechanism favored by Nevill, based on his findings in South Africa.

To this end, vector insects are persistently infected for their entire life span, but it is unlikely that individual insects could survive long enough to maintain the virus between transmission seasons –– a period of 6 months or more in temperate regions. Vertical transmission of BTV has not been proven in vector insects, although BTV nucleic acid has been detected in midge larvae (White et al., 2005). Similarly, claims of truly persistent BTV infection of ruminants have never been adequately substantiated, thus it is unlikely that ruminant livestock are a permanent source of virus, consistent with the fact that BTV has not persisted among livestock in transiently incursional areas such as the United Kingdom, much of northern Europe, or the Okanagan Valley of Canada (Clavijo et al., 2000; Gibbens, 2010; van Rijn et al., 2012b). Experimental studies claiming long-term persistent BTV infection of livestock following *in utero* infections were not replicated by other investigators (Walton, 2004).

The international debate on the potential role of ruminant livestock in maintenance of BTV infection has, in part, led to the convening of three international symposia, the last in 2003 (Maclachlan and Pearson, 2004). A more recent claim of a putative mechanism of persistent BTV infection of livestock (Takamatsu et al., 2003) also was not reproduced or supported by data from other investigators (Lunt et al., 2006; White and Mecham, 2004). Thus, current international trade policies of the World Organization for Animal Health (OIE) are based on the premise that duration of viremia in BTV-infected livestock is finite (less than 60 days [Singer et al., 2001]), but can be prolonged as a consequence of the virus's association with circulating blood cells (OIE, 2012). Although cattle and sheep may remain positive for the presence of viral nucleic acid by BTV-specific reverse transcription polymerase chain reaction (RT-PCR) assay for 4–8 months, infectious viremia is substantially less (Bonneau et al., 2002; Maclachlan et al., 1994; van Rijn et al., 2012a). Exhaustive studies have failed to show truly persistent BTV infection of livestock (reviewed: Maclachlan, 2004; Maclachlan et al., 2009; Melville et al., 2004; Walton, 2004).

Definition of the relative importance of insects and animals, potentially including non-livestock species, in maintaining BTV between seasons would better facilitate logical control strategies. Despite the importance of interseasonal maintenance of BTV, little progress has been made in the last >40 years in characterizing the specific mechanism thereof.

4. Control strategies and supporting diagnostic tests

Whereas many strains of BTV cause only subclinical or asymptomatic infections of susceptible animals, other virus strains cause serious disease in both livestock and wild non-African ungulates. The adverse economic impact of BT on sheep production in the Cape Colony (South Africa) region, where the disease was first recognized, was sufficiently profound that it stimulated a substantial effort to develop effective preventive and therapeutic strategies (Spreull, 1905; Verwoerd, 2012). In contrast, other countries have since adopted only reactive approaches that are based on outbreak crisis response and, as a result, vaccination strategies remain

essentially adaptations of those originally developed in South Africa by Sir Arnold Theiler over a century ago (Oya Alpar et al., 2009; Sumner et al., 2013; Verwoerd, 2012). Similarly, there is substantial folklore regarding strategies that might be used to protect livestock from attack by the insect vector of BTV, much of it reflecting observations made in South Africa in that same early era of discovery: for example, the utility of approaches such as stabling of livestock to protect them against vector attack clearly depends on the endophilic feeding behavior of the individual species of *Culicoides* midge vector (Viennet et al., 2012).

Furthermore, a problem inherent to the control of BTV and related orbiviral infections is the fact that *Culicoides* midges remain relatively poorly studied despite their broad global distribution and remarkable species divergence, their high population densities in many areas, and the fact that some species are especially voracious blood feeders (Carpenter et al., 2008; Mellor et al., 2000). The biology of *Culicoides* midges is clearly very different than that of mosquitoes, thus characterization of the unique aspects of the lifecycle and transmission dynamics of each midge species within its particular habitat is essential if logical efforts are to be instituted for vector control within individual BTV episystems. Importantly, optimal control of rapidly evolving arbovirus diseases such as BT will require integrated countermeasures that mitigate all aspects of the virus's transmission cycle, and optimal control will best be accomplished using preventative rather than purely reactive strategies.

The reactive strategies to control outbreaks of BT or unanticipated incursions of BTV on which most countries currently depend rely on rapid detection of either virus (virological surveillance) or disease (clinical surveillance). As mandated by the OIE (2012), this is best accomplished, especially in putatively BTV-free areas and in light of the likelihood of subclinical or mild infections of livestock and/or wildlife, by ongoing field surveillance for viral infection, not merely disease surveillance. It should include the evaluation of sentinel livestock and, ideally, surveillance of the potential vector midge species resident in the region. It should utilize appropriately sensitive and specific diagnostic assays for virus detection and, similarly, vector surveillance must include the most correct methods for trapping of midges, which may vary depending on the midge species of interest (Souza Monteiro et al., 2012).

Excellent diagnostic assays are now available for the detection of BTV, specifically group-reactive quantitative RT-PCR (RT-qPCR) assays that reliably detect all BTV serotypes and strains with high sensitivity and specificity (Hofmann et al., 2008; Mayo et al., 2010, 2012a,b; van Rijn et al., 2012a). Serotype-specific RT-qPCR assays increasingly are also used for rapid and specific determination of virus serotype, without the requirement for expensive and time-consuming virus isolation (Maan et al., 2012; Maclachlan et al., 2013; Mayo et al., 2012b). RT-qPCR assays are also considerably more sensitive than conventional virus isolation, and RT-qPCR can detect viral nucleic acid long after infectious virus cannot be isolated from the blood and tissues of exposed animals. Highly sensitive and specific competitive ELISA assays are available commercially for serological detection of BTV infection of livestock. Entomological surveillance of BTV infection, however, is complicated by the different behaviors of vector *Culicoides* midges in different regions and, with the notable exception of countries such as Australia and Italy, comprehensive vector surveillance is not the reality in most countries, even those that are at high risk for occurrence of the disease.

Logical BT control strategies must be based on a sound understanding of how the virus is introduced and spread, which can be difficult to ascertain. For example, recent experiences in Europe confirm repeated introduction of South African live-attenuated vaccine virus strains, but it is unknown how they got to Europe (de Clercq et al., 2009; van Rijn et al., 2012b). Similarly, the route whereby a virulent and novel strain of BTV-8 with unusual and distinctive properties was introduced into Northern Europe in 2006 remains uncertain, whether from movements of infected animals or vectors, biological products, or even bioterrorism. The precise role of climate change in mediating the expansion of BTV into Mediterranean Europe and the incursion of numerous novel BTV serotypes into the southeastern US also requires better clarification. Specifically, how climatic changes coupled with anthropogenic factors such as patterns of land use alter the vectorial capacity equation for the populations of the vector midges resident in affected regions must be quantified if accurate predictive mathematical models are to be developed. Importantly, countries in historically BTV-enzootic regions of the world should not ignore the infection, as many currently do, as BT occurs only sporadically in such regions, likely in part because of population immunity. The reality is that, as long as BTV is present in a region, there is always the potential for substantial disease outbreaks to occur, either from the emergence of a virulent virus from less pathogenic ancestors, or following the introduction of a novel virus strain or serotype into a region conducive to its spread.

The genetic basis of BTV strain virulence remains largely undefined, as does the genetic basis of distinctive phenotypic properties, such as the ability of some BTV strains to cross the ruminant placenta to cause fetal infections (reviewed: Coetzee et al., 2012). This latter property is especially characteristic of BTV strains propagated *in vitro,* such as live-attenuated vaccine virus strains (Flanagan and Johnson, 1995; Kirkland and Hawkes, 2004; Maclachlan et al., 2000; Shultz and DeLay, 1955; Savini et al., in press). Without accurate delineation of the virulence determinants of BTV, it is impossible to predict when and how virulent virus strains might emerge and what factors favor their emergence. The advent of laboratory animal infection models coupled with reverse genetic systems that facilitate manipulation of the BTV genome offers promise for effective strategies with which to better define the genetic basis of BTV strain virulence and phenotype (Caporale et al., 2011; Matsuo and Roy, 2013). However, unraveling the complex process of evolution of these determinants amongst field strains of the virus will be a substantial undertaking.

5. Potential strategies for control

It is logical in the future that mathematical modeling based on, and validated with, reliable data from each specific region (i.e., each BTV episystem) will be important to guide disease outbreak prediction and logical mitigation strategies. Control of BTV infection of wild and domestic ungulates hypothetically can be achieved, either through elimination of the midge vector or by protecting the virus's animal hosts from infection and/or disease.

5.1. Animal-based control strategies

5.1.1. Vaccination

Vaccination is currently central to the response of most at-risk countries to any BT outbreak. Vaccination, however, can be problematic given the plurality of BTV serotypes, coupled with apparent serotype-specific immunity in livestock (reviewed: Noad and Roy, 2009; Oya Alpar et al., 2009; Zientara et al., 2010). Thus, effective vaccines potentially must be developed to all 26 currently recognized BTV serotypes. Furthermore, there is a glaring lack of choices in terms of currently available commercial vaccines for BTV.

Live, attenuated (modified live virus [MLV]) vaccines are routinely used to prevent BT among sheep in the US, South Africa and Israel, and MLV vaccines were used for compulsory vaccination of cattle and sheep in Italy following the incursion of BTV into that

country in 1999 (Calistri et al., 2004). MLV vaccine viruses clearly can be acquired and transmitted by insect vectors, then circulate as field strains, and they can reassort gene segments with field viruses to generate novel progeny (Batten et al., 2008; Ferrari et al., 2005; Osburn et al., 1996). MLV vaccines also have the capacity to cross the placenta to infect the fetus (Flanagan and Johnson, 1995; Maclachlan et al., 2000; Maclachlan and Osburn, 2008; Savini et al., in press; Shultz and Delay, 1955).

Inactivated BTV vaccines, which were not commercially available at the beginning of the European epizootic, enjoy several potential advantages over MLV vaccines. Specifically, inactivated vaccines cannot revert to virulence, reassort genes with field or MLV viruses or cross the placenta to cause reproductive losses. Inactivated vaccines were exclusively used in response to the outbreak of BTV-8 in Europe. However, inactivated vaccines suffer from their relative slow onset of immunity, as compared to MLV vaccines, and the lack of commercial products for most serotypes. New-generation products such as baculovirus-expressed virus-like particles (VLPs) and vectored recombinant vaccines, including a canarypox virus recombinant expressing the VP2 and VP5 outer capsid proteins of BTV, have been shown to be effective experimentally, but their inherent cost and limited market potential have prevented their commercial use to date (Noad and Roy, 2009; Boone et al., 2007). Subunit vaccination strategies are clearly viable for BTV as the neutralization epitopes are clustered on VP2, although the expression of immunogenic VP2 can be challenging, given the conformational nature of individual epitopes (DeMaula et al., 2000).

5.1.2. Livestock housing, movement restrictions, and culling

BT typically occurs in the late summer and autumn in temperate areas, and South African farmers quickly recognized that minimizing exposure of sheep to biting insects was an effective method of reducing its impact (Nevill, 1971; Spreull, 1905; Verwoerd, 2012). Disease control strategies that were accepted as beneficial included the stabling of animals at night, the addition of a few cattle to sheep herds, based on a perception that the insect vector preferred to feed on cattle rather than sheep, and the relocation of susceptible sheep to areas less suitable for the biting insects that were assumed to be vectors of the disease. It is to be stressed that these strategies were developed long before it was determined and definitively shown that the major vector of BTV in South Africa was a *Culicoides* midge, specifically *C. imicola*. The same control strategies were subsequently adopted in other areas of the world in which BT later was recognized. However, because the behavior of *C. imicola* can be quite different from that of vector midges that populate other global episystems, the utility of such management strategies is entirely dependent on the feeding behavior of the species resident in each episystem.

Regardless of the vector species in an area, complete protection of livestock could conceivably be achieved, either by stabling livestock in insect-proof quarantine or the use of a universally effective insect repellant. Insecticides are typically not effective if they require the vector to first feed on livestock, as any feeding midge would have the opportunity to transmit BTV before succumbing to the insecticide. Similarly, the use of protective housing is often impractical for extensive livestock production systems, and the utility of stabling is somewhat dependent on the degree of endo/exophilic activity exhibited by the vector species resident in each area (Cheah and Rajamanickam, 1991; Doherty et al., 2004; Napp et al., 2011).

Animal movement restrictions are also central to the control of BT outbreaks, although it must be stressed that these can be logistically challenging and economically devastating (Papadopoulas et al., 2009). During the recent epizootic in Italy, for example, the imposition of restrictions on the movement (transhumance) of livestock from Sardinia to Tuscany led to social unrest and a potential animal welfare crisis that was only resolved by a mass vaccination campaign coordinated with the passage of emergency legislation to allow the movement of vaccinated livestock (Giovannini et al., 2004). There is a slow but progressive international acceptance that BTV-immune (seropositive) livestock are likely the safest for trade purposes. Historic reluctance of regulatory authorities to allow the movement or trade of immune animals was based on now-discredited proposals suggesting the occurrence of persistent BTV infection of ruminant livestock following vertical transmission of the virus (Walton, 2004). It now is clearly evident, and increasingly accepted internationally, that immune competence of fetal ruminants to BTV arises before mid-gestation (Enright and Osburn, 1980; Maclachlan et al., 1984); thus, infection in early gestation typically leads to the development of teratogenic defects of the central nervous system and a robust immune response, and not to the birth of persistently infected animals (reviewed: Maclachlan and Osburn, 2008; Maclachlan et al., 2009).

Finally, animal culling has previously been used in an effort to "stamp out" epizootics of emerging transboundary diseases, notably foot and mouth disease (Papadopoulas et al., 2009). Such strategies have been used previously in efforts to control BT, most recently in Europe, but there is increasing and justifiable societal resistance to the large-scale slaughter of healthy livestock and wildlife. Furthermore, arboviral diseases like BT are not readily managed by culling, because of the high rate of subclinical infections in wild and domestic animals, and because vector midges that harbor the virus for prolonged periods can be spread widely.

5.2. Vector-based control strategies

Strategies to reduce or eradicate populations of either adult or immature stages of the midge vector could theoretically be used to control infection and limit the spread of BTV. Such strategies are challenging, however, given the enormous midge populations in many regions coupled with their associated extensive breeding sites. The challenge to effective vector control is further exacerbated by the fact that different species of midge vector predominate in different areas, and the ecology of individual species may vary substantially in critical features such as their host feeding preferences, the frequency with which they enter livestock stabling facilities (endo-/exophilic activity), and the location and type of their breeding sites, which are poorly defined for many species.

5.2.1. Targeting immature life stages of Culicoides midges

Control of immature life-stages (e.g., larvae) can be used to reduce populations of adult *C. sonorensis* midges at the local, farm-based level, but few data are available regarding the effect of such an approach on subsequent BTV transmission to livestock in areas with diverse landscapes or with other species of midge vector. Such control strategies reduce larval habitat by removing breeding sites, e.g. by the drainage of marshlands, waste-water lagoons, or standing pools of water (Carpenter et al., 2008; Mullens and Rodriguez, 1989). A similar strategy was used previously to control populations of the salt marsh midge, *Culicoides furens*, in areas of Florida and the Caribbean (Linley et al., 1970).

Other strategies that target larval stages of the midge vector include the use of organochlorine, organophosphate (OP), or pyrethroid insecticides (Holbrook, 1986; Woodward et al., 1985). However, their limited efficacy in areas that require broad application limits the utility of this strategy, as do concerns regarding the potential environmental ramifications of such an approach. For these reasons, there is increased interest in alternative methods of vector control such as the use of "biorational" pesticides that utilize hormonal or microbiological agents to limit development of *Culicoides* midges, such as bacteria (e.g., *Bacillus thuringiensis*),

mermithids (*Heleidomermis magnapapula*), iridescent viruses, and fungi (*Lagenidium giganteum*) (Apperson and Yows, 1976; Kelson et al., 1980; Kline et al., 1985; Mullens et al., 1999; Paine and Mullens, 1994; Wirth, 1980; Wright and Easton, 1996). To date, although these agents and strategies have proven effective in the laboratory, their utility for broader application in the field remains largely unknown.

5.2.2. Targeting of mature Culicoides midges

Insecticides can be used to reduce populations of adult vector *Culicoides* midges and thereby reduce the risk of BTV transmission. However, in light of the vast areas involved and the likelihood of human exposure, saturation spraying strategies analogous to those used to reduce mosquito populations during outbreaks of human arboviral diseases are not realistic for non-zoonotic, *Culicoides*-transmitted animal diseases like BT. Insecticides can be used at least temporarily to protect individual or groups of animals against attack by vectors, and insecticide impregnated nets can help to protect stabled or housed livestock against attack by adult midges (Jamnback, 1963; Porter, 1959). A variety of compounds used to control both ecto- and endo-parasites of cattle have potential efficacy against *Culicoides* midges. These include topical administration of "pour-on" products, impregnated ear tags, or dipping of animals in synthetic pyrethroid or OP compounds. Locally administered products tend to have reduced insecticidal efficacy on the belly and legs of treated animals (Carpenter et al., 2007; Mullens et al., 2001). The effectiveness of systemically administered (injectable) products such as the macrocyclic lactones (avermectins) has not been adequately assessed, but these are unlikely to be useful in reducing regional populations of *Culicoides* midges (Holbrook and Mullens, 1994; Standfast et al., 1984).

Repellents and attractants have also been evaluated as "decoys" to artificially lure *Culicoides* midges and thereby reduce the biting rate among animals. Several compounds, such as *p*-menthane-3,8-diol, *N*,*N*-diethyl-*m*-methylbenzamide, DEET and KBR2023, have demonstrated efficacy as repellents and to reduce *Culicoides* biting rates on humans. However, their use in livestock is complicated and limited by the tedious daily application regimen, combined with the limited information available regarding the withdrawal period for the active ingredients that are rapidly absorbed through the skin (Carpenter et al., 2005; Trigg, 1996). The utility of insect attractants including semiochemical cues (i.e. 1-octen-3-ol, 3-*n*-propyl-phenol, 4-methylphenol) remains largely unexplored. Although studies in Florida and Scotland suggest that insect trapping using attractants can influence populations of *Culicoides* midges, there are no available data showing that this strategy has a significant impact on either animal biting rates or virus transmission (Blackwell, 2001; Cilek et al., 2003).

Other potential strategies to reduce biting rates on livestock include trapping of adult *Culicoides* midges or the use of decoy animal hosts (Du Toit, 1962; Nevill, 1978). In the same context as the use of synthetic attractants and repellents, it has been recommended that cattle, which are generally resistant to BT, be placed in pastures of sheep to act as "decoy hosts" to reduce the incidence of BT disease in the latter species. While this may be somewhat effective in reducing transmission of BTV to sheep by *C. imicola*, it is increasingly evident that many species of *Culicoides* midges are opportunistic feeders so that increasing animal density on a farm might also increase the vector population and so increase the risk to the entire animal population (Martinez-de la Puente et al., 2012; Santiago-Alarcon et al., 2012). Furthermore, cattle serve as amplifying hosts of BTV with resultant infection of vector midges (Barratt-Boyes and Maclachlan, 1995; Mayo et al., 2012b; Spreull, 1905; Verwoerd and Erasmus, 2004).

6. Potential benefits of control

Control of BTV infection of livestock has become increasingly important because of the economic and animal welfare impacts of the disease and because livestock from enzootic regions continue to be impacted by animal movement/trade restrictions (Giovannini et al., 2004; Maclachlan and Osburn, 2006; Papadopoulas et al., 2009). Although BT is most likely to occur at the margins of the virus's global range, it also occurs sporadically in enzootic regions. There are several imperatives to the rapid implementation of efficacious control strategies in response to outbreaks of BT and related orbiviral diseases:

1. The most pressing initial need during outbreaks of BT is typically to reduce the morbidity and mortality among susceptible livestock species, as outbreaks of BT can have serious economic consequences to producers.
2. BT outbreaks or unanticipated incursions of BTV can interrupt or even prevent the movement and trade of ruminant livestock, which can have disastrous economic and social consequences to affected regions and/or countries. Thus, any interventional program should include a strategy to allow safe movement of susceptible livestock species.
3. Limiting the spread of virus is a general goal of any properly instituted control program in a previously BTV-free region. Furthermore, veterinary authorities would strive to prevent the virus from becoming enzootic following incursion.
4. BTV is the prototype virus of a substantial group of genetically heterogeneic viruses, and it therefore serves as a tangible and contemporary example of just how difficult the control of any *Culicoides*-transmitted infectious disease might be. Other orbiviruses, notably African horse sickness virus, are even more pathogenic, and expansion of the range of some of these other agents – or the appearance of novel viruses transmitted by the same midge vector – could be disastrous. Lastly, although human populations have been largely spared to date from any *Culicoides*-transmitted diseases, certain instances of filariasis and the hemorrhagic fever caused by Oropouche virus being the notable exceptions, there is always the possibility that such a disease will emerge in the future. This potential is highlighted by the high human biting rates of some *Culicoides* species, coupled with their broad host-feeding behavior that favors the emergence of zoonotic infections. Experience gained in controlling BT and related diseases will clearly be critical to the future control of any similar, newly emergent disease of humans.

7. What might the future hold?

Dramatic recent changes in the global distribution and nature of BTV infection could serve as a warning of things to come, in terms of the combined effect of climate change and anthropogenic factors on the emergence of arboviral diseases. However, because BT and related animal orbiviruses are not zoonoses, these diseases and the *Culicoides* midges that transmit them have received little attention from the biomedical research community. An important theme for the future emergence of these diseases is the interaction of climate and human factors. Thus BTV-8 first appeared in 2006 in the Netherlands, following an unusually hot summer in northern Europe and, similarly, extensive outbreaks of EHD and BT occurred among cattle in the mid-western US in 2012, the hottest summer on record in the region. Still unresolved, however, is how BTV-8 was introduced to northern Europe, and from where? Similarly, it is uncertain why strains of the serotypes of BTV and EHDV that were already enzootic in the US emerged during an especially hot summer in 2012 to cause notable outbreaks of disease in cattle,

which are typically infected only subclinically with these viruses. The potential future ramifications for human health of these ubiquitous hematophagous midges and the animal diseases they transmit are self-evident.

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