

Review

Bluetongue in Europe: past, present and future

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The recent arrival in Northern and Western (NW) Europe of bluetongue virus (BTV), which causes the ruminant disease ‘bluetongue’, has raised the profile of this vector-borne ruminant disease and sparked discussions on the reasons for its sudden emergence so far north. This expansion has not happened in isolation and the disease has been expanding into Southern and Eastern Europe for the last decade. This shifting disease distribution is being facilitated by a number of different introduction mechanisms including the movement of infected livestock, the passive movement of infected *Culicoides* on the wind and, in NW Europe, an unknown route of introduction. The expansion of BTV in Europe has forced a re-evaluation of the importance of Palaearctic *Culicoides* species in transmission, as well as the importance of secondary transmission routes, such as transplacental transmission, in facilitating the persistence of the virus. The current European outbreak of BTV-8 is believed to have caused greater economic damage than any previous single-serotype outbreak. Although attempts are being made to improve the capacity of European countries to cope with future BTV incursions, the options available are limited by a lack of basic entomological data and limited virological surveillance.

Keywords: bluetongue virus; *Culicoides*; climate; environmental change; emerging infectious disease

1. INTRODUCTION

Bluetongue (BT) is a disease of ruminants caused by bluetongue virus (BTV), a non-contagious vector-borne *Orbivirus*. The first comprehensive clinical profile of the disease was published by Spreull (1905), and during the century since this first description its distribution across the world was shown to comprise a broad band between approximately 40°N and 35°S (see figure 3). More recently, there has been a dramatic northwards extension of the virus in Europe. A number of different mechanisms have been involved in the introduction process, including the movement of infected livestock, the passive movement of infected *Culicoides* on the wind, and an unknown route of introduction into Western Europe. In this review we discuss the epidemiology of BT, highlighting its mechanisms of spread and persistence, and recent advances in our understanding of these aspects of its epidemiology. We then summarize recent BT activity in Europe, examine how Northern and Western (NW) Europe has fared during its first encounter with this infectious disease, and suggest some directions for future research that could improve the capacity of European countries to cope with future incursions.

2. TRANSMISSION AND ECOLOGY OF BLUETONGUE VIRUS

BTV is the type species of the genus *Orbivirus* in the family Reoviridae, and consists of a double-stranded

RNA genome of 10 segments contained within three concentric shells of structural proteins that form the subcore, outer core and outer capsid. Variation in the proteins that make up the outer capsid—VP2 and, to a lesser extent, VP5—determine the serotype, of which 24 have been identified to date. BTV can infect any ruminant species, including cattle, sheep, deer, goats and camelids, although severe clinical signs are most commonly seen in improved breeds of sheep. Infection with BTV can result in a wide range of clinical signs or can be entirely sub-clinical. The majority of infected animals develop no detectable clinical signs (Anon 2005) but, in others, signs may include fever, depression, lameness, oedema of the lips, tongue and head, conjunctivitis, coronitis, excessive salivation, nasal discharge, hyperaemia and pain at muco-cutaneous junctions such as the gums and vulva, and death (Darpel *et al.* 2007). In pregnant animals abortion may also occur. The blue tongue after which the disease was named is seen only rarely and in more serious clinical cases. Following recovery, animals may also exhibit a number of long-lasting secondary effects, such as reductions in milk yield and weight gain, severe wool break and temporary infertility.

BTV is transmitted between its ruminant hosts by certain species of biting midges of the genus *Culicoides*. These are small (<3 mm) haematophagous insects that occur in every inhabited continent in the world and breed in a wide variety of semi-aquatic sites including tree holes, rotting vegetation, pond margins, damp soils and certain sorts of herbivore dung. Most *Culicoides* species of veterinary importance tend to breed in organically enriched, damp soil such as is

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found adjacent to their hosts in and around farm holdings. As with mosquitoes, blood-feeding is limited to females, which require protein for the production of eggs. The host range of *Culicoides* can be very wide with some species being insectivorous and others blood-feeding upon birds, mammals or amphibians. Only around 50 of the 1500 known *Culicoides* species have been shown to be capable of developing a fully disseminated transmissible BTV infection because of the existence of a series of barrier systems, as described below. The ability to act as an effective vector of BTV further requires that a species feeds mainly or wholly upon ruminants and occurs in large numbers, and as a result only a handful of *Culicoides* species are thought to be able to act as effective vectors in the field. These include *C. imicola* (the principal vector in Africa, the Middle East, much of southeast Asia and parts of Southern Europe), *C. sonorensis* (the principal vector in North America) and *C. brevitarsis* (the principal vector in Australia).

The first suggestion that Palaearctic species of *Culicoides* might be able to transmit BTV in the field was indicated by the isolation of BTV-4 from *C. obsoletus*-group females during an outbreak in Cyprus (Mellor & Pitzolis 1979), and later by the isolation of African horse sickness virus (AHSV), which is closely related to BTV, from a mixed pool of *C. obsoletus*- and *C. pulicaris*-group females in Spain (Mellor *et al.* 1990). The larvae of *obsoletus*-group *Culicoides* have been collected from dung, waterlogged soil, compost and leaf litter, while those of *pulicaris*-group *Culicoides* have generally been found in waterlogged soil (Kettle & Lawson 1952; Campbell & Pelham-Clinton 1960).¹ Both *obsoletus*- and *pulicaris*-group *Culicoides* bite livestock and occur in large numbers on farms, making them potentially effective vectors of BTV. However, the distribution of BTV in Europe prior to 1998 corresponded closely to the known distribution of the Afro-Asiatic species *C. imicola*, and it was therefore assumed that, despite the early findings of Mellor and Pitzolis, Palaearctic species could not sustain the transmission of BTV in the field. The expansion of BTV since 1998 into areas of Europe where *C. imicola* is absent demonstrated the fallacy of this assumption and, coupled with the research described above (Mellor & Pitzolis 1979; Mellor *et al.* 1990), suggested that at least one species in the *obsoletus* group and possibly one or more in the *pulicaris* group were able to act as effective vectors of BTV. Both groups are ubiquitous across the Palaearctic region. Because females of many of the species within these two groups cannot be distinguished on the basis of morphology (Carpenter *et al.* 2009), it had until recently been impossible to explicitly incriminate individual species within these groups. However, the development of a polymerase chain reaction assay to differentiate the species within both groups (Nolan *et al.* 2007) will enable future studies to quantify competence to the species level.

Culicoides are biological vectors of BTV and, as such, ingested virus must infect the insect midgut cells, replicate in them, escape into the body cavity of the insect (haemocoel) and infect and replicate in the salivary glands before it can be transmitted to a new host. The time required for ingested virus to

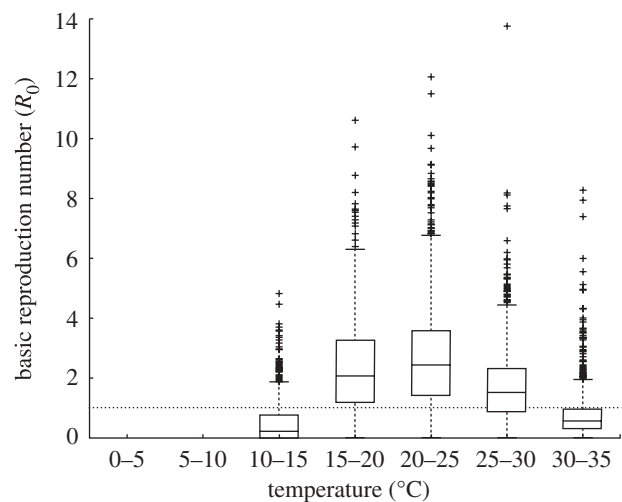


Figure 1. Estimated effect of temperature on the transmission of bluetongue virus on mixed holdings (cattle + sheep). Reproduced from Gubbins *et al.* (2008).

spread from the midgut cells to the salivary glands of a vector is termed the extrinsic incubation period (EIP), and its duration is largely determined by the activity rate of the RNA polymerase of the virus, which depends on the ambient temperature. *Culicoides* kept at 15°C require several weeks to complete the EIP, while those kept at 30°C may complete incubation in a couple of days (Wittmann *et al.* 2002). At temperatures below around 12°C replication apparently ceases altogether, although virus may persist in infected midges and resume replication if the temperature is later raised (Wellby *et al.* 1996). The time required by an insect vector to digest a blood meal is also reduced at higher temperatures, increasing the frequency of blood-feeding. The most immediate effect of a temperature rise is therefore to shorten the generation time of the virus and increase the opportunities for transmission, as shown in figure 1.

A brief rise in temperature may also have a further effect on the transmission of BTV by local *Culicoides* populations. Even within the handful of species that are known or suspected to have the capacity to transmit BTV, only a fraction of individuals within a population are capable of supporting a fully disseminated infection. In the remainder, one or more barrier systems appear to prevent the initiation of infection or dissemination of virus beyond the gut cells (Mellor 1990, 2000). In a recent study to characterize the competence of UK field populations of *obsoletus*-group *Culicoides*, as few as 0.4 per cent of individuals were competent in some populations (Carpenter *et al.* 2006). While the molecular basis of these barrier systems is not known, exposure to high temperatures during larval rearing appears to disrupt their development, substantially increasing the proportion of orally infected *Culicoides* capable of developing a disseminated infection. An increase in rearing temperature of 5–10°C is capable of increasing the oral infection rate of colony-reared *C. sonorensis* with AHSV more than 10-fold (Mellor *et al.* 1998). Although a similar study of BTV did not detect a significant effect of temperature on competence (Wittmann *et al.* 2002), this was based on a very small dataset and further

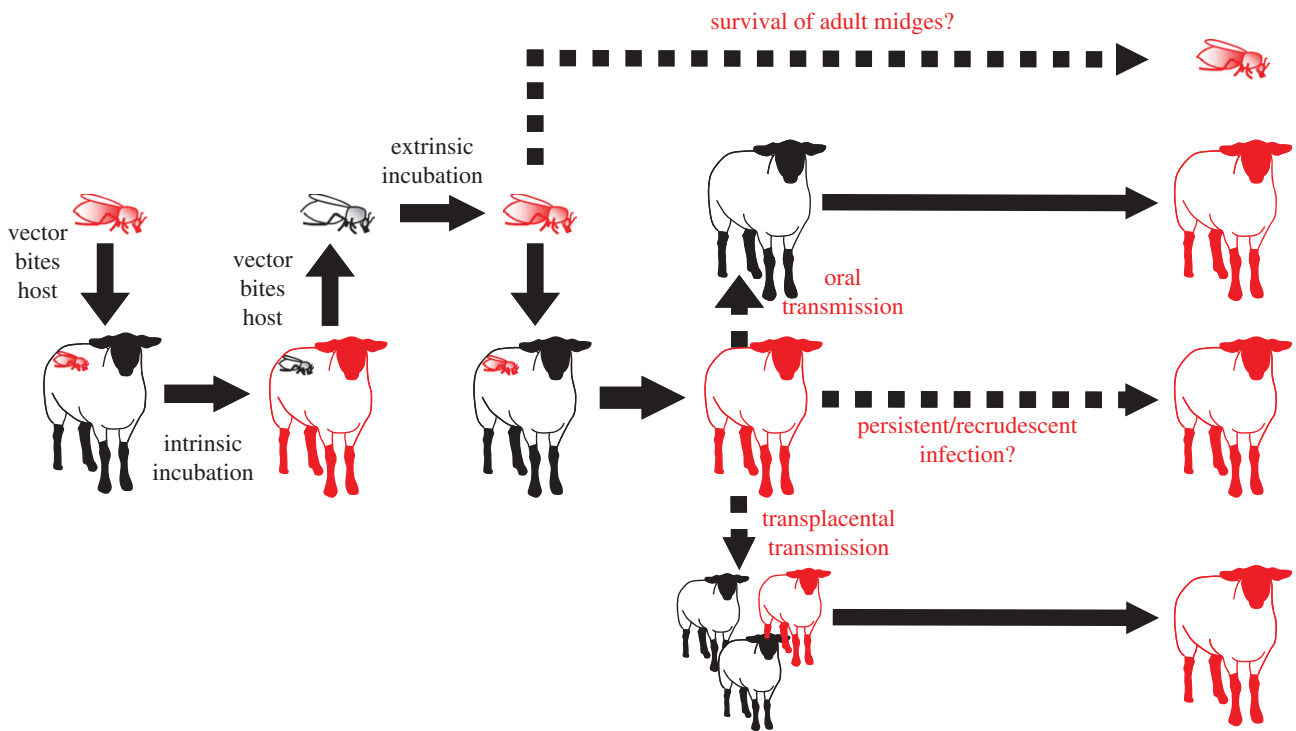


Figure 2. The transmission of bluetongue virus, showing putative secondary transmission routes. Figure modified from Wilson *et al.* (2008). *PLoS Biol.* 6(8); distributed under a Creative Commons Attribution License.

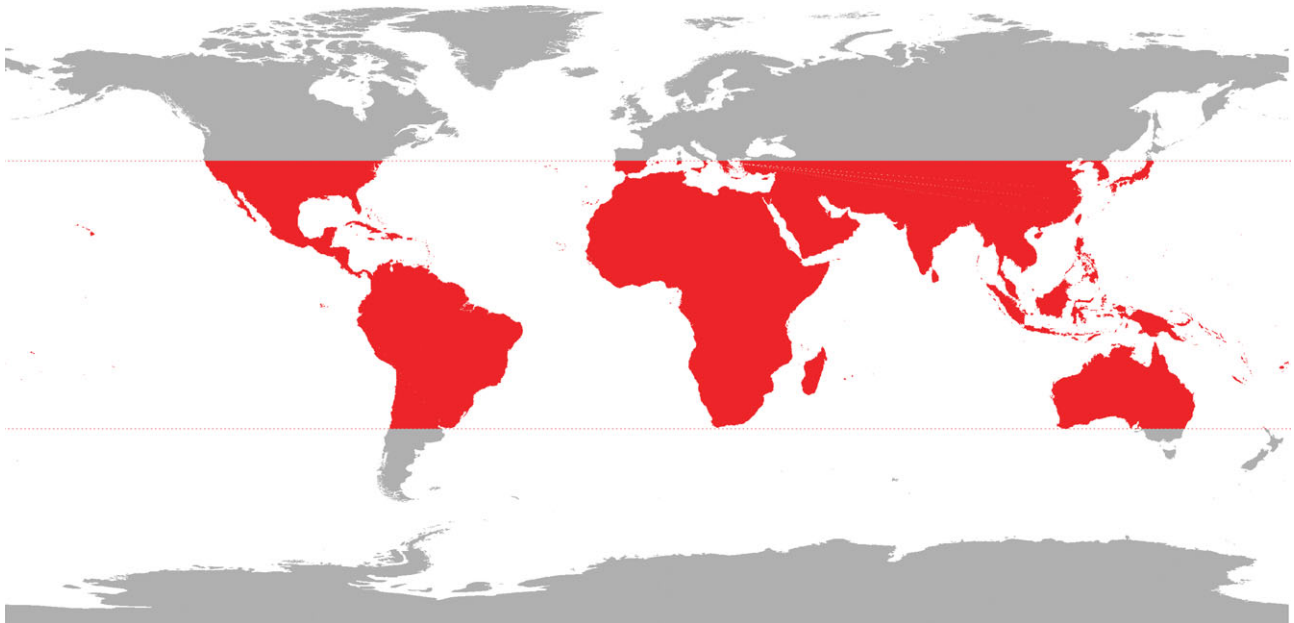


Figure 3. Map of the estimated global range of bluetongue virus prior to 1998.

work is required in this area. At the same time, high temperatures will increase vector mortality, placing an upper limit on the extent to which increasing temperatures increase the transmission potential of BTV and, consequently, the likelihood of BTV transmission by *Culicoides* is a function of the interaction between these antagonistic effects of temperature.

Different species of *Culicoides* have different environmental tolerances, and the optimal temperature and rainfall levels for populations of Afro-Asiatic species such as *C. imicola* are different to those for

Palaearctic species such as the *obsoletus* and *pulicaris* groups. Thus, long-term environmental change may alter the suitability of a geographical region for some *Culicoides* species, changing its vector fauna and consequently the potential for the transmission of BTV and other *Culicoides*-borne arboviruses. The distribution of *C. imicola* in Southern Europe is believed to have expanded in recent years as a result of higher temperature and lower rainfall (Purse *et al.* 2005). It was also recently suggested that the distribution of *C. dewulfi* in Belgium may have expanded over recent decades (De Deken *et al.* 2008), although this is more likely

to be attributable to changes in sampling methods and effort (Meiswinkel *et al.* 2008).

In addition to being spread between ruminants by *Culicoides*, evidence suggests that at least some strains of BTV can be transmitted directly from host to host by one or more secondary mechanisms, including transplacental, iatrogenic and oral transmission, as well as potentially being transmitted mechanically between hosts on the mouthparts of biting flies. Virus may also be able to persist in either vector or host for longer than a normal transmission cycle via long-lived infected *Culicoides*, persistently infected ruminants or other methods (figure 2). The evidence for each of these mechanisms has been reviewed by Wilson *et al.* (2008). As described above, BTV appears unable to replicate at temperatures below 12°C, and the activity of *Culicoides* vectors also reduces or ceases at low temperatures. As a result, in many temperate regions classical BTV transmission is almost completely interrupted for several months of the year by cold weather, but outbreaks often resume after interruptions far longer than the typical lifespan of an adult vector or the normal period of host infectiousness, a phenomenon termed 'overwintering'. Although the secondary routes described above are likely to be of minor significance during normal transmission, they may become disproportionately important for the survival of the virus when normal transmission is interrupted by cold weather (Wilson *et al.* 2008), and one or more of these mechanisms is likely to be responsible for the handful of BTV transmission events confirmed to have occurred during the winter in NW Europe (e.g. De Clercq *et al.* 2008; Hoffmann *et al.* 2008; Menzies *et al.* 2008).

3. GLOBAL BLUETONGUE VIRUS DISTRIBUTION AND ACTIVITY (PRE-1998)

For the first half of the twentieth century, BTV was reported only from parts of Africa and Cyprus, but in 1951 an outbreak of BT was also confirmed in Israel. The detection of BTV in the USA the following year represented an even more dramatic expansion in the known distribution of the virus, and one initially deemed so unlikely that this outbreak was assumed to represent an entirely new disease, termed 'soremuzzle' (Hardy & Price 1952). BTV was first confirmed in Asia during 1961 when an outbreak of the disease was detected in India (Sapre 1964), and then in Australia where the virus was isolated from *Culicoides* collected near Darwin in 1975 (St George *et al.* 1978). In Europe, meanwhile, a major outbreak owing to BTV-10 ravaged the sheep populations of Spain and Portugal between 1956 and 1960 causing the deaths of almost 180 000 animals (Manso-Ribeiro *et al.* 1957) and a smaller outbreak of BTV-4 occurred on several Greek islands near the Anatolian Turkish coast in 1979 (Vassalos 1980). The massive mortality experienced in Iberia demonstrated that while BTV incursions into Europe were infrequent at that time, parts of Southern Europe were capable of supporting the transmission of BTV and European livestock were highly vulnerable to the virus.

By the late-twentieth century, the known distribution of BTV had expanded dramatically and the virus was

reported from most regions within a broad band around the world stretching from approximately 35° S to 40° N (figure 3). In some regions, BTV was detected as far as 50° N (Mellor *et al.* 2000; Mellor & Wittmann 2002), and a study of seasonal temperature conditions in these locations during the early-1990s suggested that the virus had the potential to be transmitted considerably further north in other locations, including Europe and North America, than had hitherto been seen (Sellers & Mellor 1993). Combined with the evidence described above that one or more Palaearctic species of *Culicoides* might be able to transmit BTV in the field, and that many breeds of sheep in Europe developed severe disease on infection with the virus (as seen during the 1956–1960 outbreak in Iberia), this information might have encouraged European countries to take the risk of BTV emergence more seriously. However, the fact that only two outbreaks had occurred previously in Europe led most of them to believe that the risk was low (Carpenter *et al.* 2009).

4. 1998–2005: EMERGENCE OF BLUETONGUE VIRUS IN SOUTHERN AND EASTERN EUROPE

In 1998, BTV was detected on several Greek islands close to the Anatolian Turkish coast (Anon 1998). Over the following 3 years, this strain, identified as BTV-9, spread northwards and westwards through mainland Greece and beyond, eventually reaching as far as Kosovo in 2001 (Osmani *et al.* 2006). This was followed by incursions of BTV-4 and BTV-16 in 1999 and BTV-1 in 2001 into mainland Greece. The introductions of BTV-1, 9 and 16 were probably attributable at least in part to animal movements along the Eurasian ruminant street, a contiguous region with high densities of ruminants stretching from India and Pakistan through Afghanistan, Turkey, Iraq and Iran to the southeast of Europe, which is also believed to contribute to the spread of other livestock diseases such as foot-and-mouth disease (FMD) (Slingenbergh *et al.* 2004). BTV-16 was isolated in Turkey in 2000 and occurs regularly in Israel, suggesting a Middle Eastern origin, while the strain of BTV-1 detected in 2001 is similar to isolates from India and Malaysia. Although BTV-4 was also detected in Greece in 1999, the virus isolated was very closely related to strains isolated from Cyprus and Turkey decades earlier, suggesting that it has been circulating locally for many years.

Although *C. imicola* was not identified in Europe until 1982 (Boorman & Wilkinson 1983; Mellor *et al.* 1983), outbreaks of BTV (as described above) and of AHSV (Diaz Montilla & Panos Marti 1967) suggest that it had been present in parts of Southern Europe considerably earlier. BTV activity in Europe prior to 2001 was apparently restricted to the known range of *C. imicola* (Mellor & Pitzolis 1979; Boorman & Wilkinson 1983; Jennings 1983; Mellor *et al.* 1984; Boorman *et al.* 1985; Mellor *et al.* 1985; Boorman 1986; Rawlings *et al.* 1997; Ortega *et al.* 1998). The spread of BTV-9 as far north as Kosovo where *C. imicola* is absent was therefore a milestone in the epidemiology of BT in Europe, as it represented the first incontrovertible confirmation that field

transmission by local northern Palaearctic vector species of *Culicoides* was possible on a large scale outside the distribution of *C. imicola*. The pattern with which BTV spread in the Aegean area also emphasizes the importance of mechanisms by which BTV may spread other than by animal movement. While *Culicoides* are typically capable of active flight over short distances (1–2 km), they can also be blown passively on the wind for long distances because of their small size. The transport of BTV-infected *Culicoides* on the wind, particularly over bodies of water, has been implicated as the most likely source of a number of introductions of BTV (e.g. Sellers *et al.* 1978, 1979), as well as of other *Culicoides*-borne viruses. The spread of BTV to islands (e.g. from Anatolian Turkey to several Greek islands) in the absence of correlated movements of livestock strongly suggests that several of these introduction events were attributable to the aerial movement of infected *Culicoides*. This mechanism is particularly important from a control perspective, because it renders restrictions on the movement of susceptible animals considerably less effective at limiting the spread of disease outbreaks. As a result, following confirmation of BTV infection, European Union (EU) legislation requires movement restrictions to be imposed over a minimum radius of 100 km (European Commission 2000).

Meanwhile, BTV-2 was detected in Tunisia in 2000, and spread further in North Africa during the year (to Algeria and Morocco). It also spread across the Mediterranean Sea, and was isolated from animals in the Spanish Balearic islands, Corsica, Sardinia, Sicily and southern Italy by December. BTV-2 is common in areas of sub-Saharan West Africa (Herniman *et al.* 1980, 1983), and its initial introduction into North Africa may have occurred via the illegal movement of cattle from sub-Saharan West Africa into the Maghreb, a route implicated in the origins of an FMD virus outbreak in Algeria in the same year (Knowles & Davies 2000). A strain of BTV-4 unrelated to that active in the eastern Mediterranean Basin also apparently spread from North Africa to Spain, Portugal and Corsica in 2003–2005. At a finer geographical resolution, the pattern of these outbreaks suggests that their origins fall into one of two categories: the movement of virus across the strait of Gibraltar, through either livestock movement or the aerial introduction of infected *Culicoides*, and the movement of virus across the strait of Sicily from Tunisia to Sicily, probably primarily via aerial introduction.

Prior to 2001, only live attenuated vaccines had been developed for BTV. The use of any vaccine which includes live virus is unavoidably associated with some risk, as there is the potential for a vaccine strain to re-assort with a wild-type strain (potentially introducing new genetic diversity into the virulent population), to revert to a higher degree of virulence and resume field transmission, and to result in clinical disease if used with highly susceptible breeds of ruminant. The attenuated vaccines existing prior to 2001 had been developed for use in regions where multiple strains of BTV were already endemic and where relatively resistant breeds were used, where many of these risks are clearly of lower concern. In a European

context, the risks associated with the use of such vaccines were clearly of greater concern, and were sufficient to discourage the use of live attenuated vaccines in several of the countries in Southern and Eastern Europe that experienced BTV incursions since 1998, as well as prompting the EU to fund a project to develop inactivated BTV vaccines for future use in the area (Mellor *et al.* 2001). However, the arrival of BTV-2, BTV-4, BTV-9 and BTV-16 in Italy within a short span of time led the Italian authorities to consider that the risks were outweighed by the cost of not vaccinating, and wide-scale use of live attenuated BTV vaccines in Italy began in 2001. During this vaccination campaign several of the above concerns were confirmed, including the re-assortment of attenuated vaccine strains with other strains in co-infected hosts (Batten *et al.* 2008) and the transmission of the vaccine strains in the field (Ferrari *et al.* 2005; Barros *et al.* 2007). Subsequent experimental work also confirmed the potential for attenuated BTV vaccine strains to be transmitted by *Culicoides* under laboratory conditions (Venter & Paweska 2007), and to generate clinical signs in European breeds of sheep (Veronesi *et al.* 2005).

In summary, the pattern of BTV activity in Europe prior to 2006 appeared to indicate two things. First, most European outbreaks followed the introduction of a novel strain of BTV into either Anatolian Turkey or the Maghreb by livestock movement, followed by further spread into Eastern or Southern Europe through one of the three 'gateways' shown in figure 4. Spread into Europe could occur either via the transport of infected livestock or the introduction of infected midges on the wind. Secondly, despite previous studies suggesting that BTV had the potential for dramatic expansion further into Europe, and evidence from Kosovo that *Culicoides* species occurring throughout Europe were capable of sustaining widespread transmission in the field, the lack of such expansion led policymakers to assume that other as yet unknown factors were largely continuing to restrict the spread of the virus into NW Europe, and also that any eventual incursion into these regions would, like previous outbreaks, begin at one of the viral gateways shown in figure 4 and spread incrementally, allowing plenty of time for policymakers in NW Europe to formulate a response. The complacency resulting from these assumptions meant that funding for areas such as entomological surveillance and vector control was relatively neglected, although the need for safe and efficacious vaccine technologies for use in future outbreaks was recognized and addressed by EU funders as described in the previous paragraph.

5. 2006 TO PRESENT: CURRENT EUROPEAN SITUATION

In August 2006, the Dutch Central Institute for Animal Disease Control (CIDC) in Lelystad² confirmed the first ever case of BTV infection in Western Europe. The serotype of the virus responsible for this outbreak was rapidly identified at the UK Institute for Animal Health (IAH) as BTV-8, and subsequent sequence analysis suggested a close similarity to strains



Figure 4. The three principal routes by which bluetongue virus is introduced into Europe. A, Morocco-Spain; B, Tunisia-Sicily; C, Turkey-Greece/Bulgaria. Figure reproduced from Wilson & Mellor (2008).

of this serotype previously isolated from sub-Saharan Africa (Anon 2006). During the following months, this outbreak spread to infect animals on over 2000 holdings in the Netherlands, Belgium, Germany, France and Luxembourg, before cold weather interrupted transmission by preventing the completion of viral incubation in the vector and reducing the activity of adult *Culicoides* as described above. The last clinical case was reported on 15 January 2007 (Losson *et al.* 2007), although several further outbreaks were reported retrospectively as a result of serological testing.

How BTV-8 was originally introduced into Western Europe remains unknown, although the distance of the index case from any of the normal gateways of introduction into Europe described in the previous section and the apparent absence of the serotype from intervening regions suggests that none of these routes was responsible, and while various alternative suggestions have been made these remain entirely speculative (EFSA 2007b). As mentioned above, previous research had already suggested that BTV transmission was theoretically possible in NW Europe. In this context, and given the effects of temperature on BTV transmission, it is relevant to note that the summer and autumn of 2006 were exceptionally warm; the average European land-surface air temperature in July was the warmest on record, and autumn (September–November) was more than 3°C warmer than normal between the north side of the Alps and southern Norway (van Oldenborgh 2007). In many countries it was the warmest autumn since official measurements began. As a result, conditions in Western Europe in the summer and autumn of 2006 were probably particularly favourable for BTV to become established in the event of an introduction.

Because typical winter temperatures in Western Europe are considerably lower than the minimum temperature required for BTV transmission, many hoped that the outbreak would be extinguished in

the winter of 2006. However, as described above, the ability of BTV outbreaks to re-emerge after long periods of winter absence has been recognized for decades, and on 13 June 2007, the German National Reference Laboratory at the Friedrich Loeffler Institut confirmed that a sentinel animal on a holding in North-Rhine Westphalia had seroconverted between early April and early May (International Society for Infectious Diseases 2007a), indicating that the virus had successfully overwintered in the region. The virus subsequently resurfaced in all countries affected in 2006, with new cases occurring for the first time in Denmark (International Society for Infectious Diseases 2007b), Switzerland (International Society for Infectious Diseases 2007c), the Czech Republic (World Organisation for Animal Health 2007d) and the UK (Defra 2007).

The 2007 outbreak was far more extensive than that of 2006, and by the end of 2007 nearly 60 000 holdings had been infected (table 1). In Belgium, the increase in sheep and goat deaths between July and October 2007 when compared with the same period in 2006 represented a sixth of the national sheep flock (Wilson & Mellor 2008). However, major steps towards effective control were also taken during the year. Because of the safety concerns about the use of live attenuated BTV vaccines described in the previous section, affected Member States in NW Europe were unwilling to use such vaccines, while at the same time because of the previously low profile of BTV-8, no inactivated vaccine had yet been developed for this serotype, and for commercial reasons vaccine companies were unwilling to begin development without firm orders. The first such orders were placed during October, enabling companies to begin vaccine development and testing.

Large numbers of suspected cases of BTV infection were announced in the winter of 2007–2008, but these almost entirely represented delayed notifications rather than ongoing or resumed transmission. The first

Table 1. Bluetongue virus activity in Europe (2006 to present).

country	holdings affected, 2006	holdings affected, 2007	holdings affected, 2008
The Netherlands	BTV-8, 460 ^a (up to 1 February 2007)	BTV-8, 6442 ^b (up to 4 January 2008)	BTV-8, 58 ^c ; BTV-6, 14 ^c ; BTV-1, 1 ^c (up to 2 December 2008)
Belgium	BTV-8, 695 ^a (up to 1 February 2007)	BTV-8, 6870 ^b (up to April 2008)	BTV-8, 27 ^c ; BTV-6 ^{c,d} (up to 2 December 2008)
Germany	BTV-8, 952 ^a (up to 1 February 2007)	BTV-8, 23 443 ^b (up to 28 March 2008)	BTV-8, 2487 ^c ; BTV-6 ^{c,d}
France	BTV-8, 7 ^a (up to 1 February 2007)	BTV-8, 19 322 ^b (up to 4 April 2008)	BTV-8, 24 469 ^c ; BTV-1, 4469 ^c (up to 2 December 2008)
Luxembourg	BTV-8, 8 ^a (up to 1 February 2007)	BTV-8, 1315 ^b (up to 20 December 2007)	BTV-8, 19 ^c (up to 11 November 2008)
Denmark	—	BTV-8, 1 ^b (up to 4 April 2008)	BTV-8, 15 ^c (up to 3 December 2008)
Switzerland	—	BTV-8, 7 ^b (up to 4 April 2008)	BTV-8 ^d (TOV; see above)
Spain	—	BTV-8, 12 ^b (up to 4 April 2008)	BTV-8, 12 ^c ; BTV-1, 1918 ^c (up to 11 November 2008)
Czech Republic	—	BTV-8, 2 ^b (up to 4 April 2008)	BTV-8, 21 ^c (up to 2 December 2008)
UK	—	BTV-8, 128 ^f (up to 4 April 2008)	BTV-8 ^{c,d} ; BTV-1 ^{c,d} (up to 2 December 2008)
Italy	—	BTV-8, 3 ^b (up to 4 April 2008)	BTV-8 ^d
Portugal	—	BTV-1, 1 ^g (up to 25 September 2007)	BTV-1, 78 ^h (up to 2 December 2008)
Austria	—	—	BTV-8, 2 ^c (up to 13 December 2008)
Greece	—	—	BTV-16, 43 ^f (up to 30 January 2009)
Sweden	—	—	BTV-8, 28 ^f (up to 30 January 2009)
Hungary	—	—	BTV-8, 1 ^f (up to 30 January 2009)
Ireland	—	—	BTV-8 ^d

^aEFSA (2007a).^bProMed-Mail post 20080422.1430.^cProMed-Mail post 20081214.3934.^dNo confirmed instances of local transmission.^e<http://www.defra.gov.uk/animalh/diseases/monitoring/pdf/bt-update-081114.pdf>.^fOIE WAHID Interface: Summary of Immediate notifications and Follow-ups. Accessed 2009-01-30.^gProMed-Mail post 20070930.3229.^hhttp://ec.europa.eu/food/committees/regulatory/scfcah/animal_health/presentations/bt_08012009_pt.pdf.

new case of BTV-8 in Europe during 2008 was probably that detected on 8 January in Cantabria, northern Spain (World Organisation for Animal Health 2008b), which represented a considerable southerly expansion in the range of the serotype. However, its importance was rapidly eclipsed by a series of events in Northern Ireland, beginning with the importation of 21 dairy heifers from continental Europe on 11 January. Post-import testing suggested that eight had previously been infected with BTV but had cleared the virus. Four of the heifers then calved, and retesting a few days later showed that three calves and two antibody-negative heifers now tested positive by PCR, indicating recent infection. These results indicated that BTV transmission had been able to occur despite the apparent absence of an infection source and the lack of *Culicoides* vectors, as confirmed by a light trap operated throughout the period in the same building, and was the first indication that vertical and horizontal (oral) transmission of this strain might be possible (Menzies *et al.* 2008), a finding that was supported by several subsequent studies (e.g. De Clerq *et al.* 2008).

Several inactivated BTV-8 vaccines also became commercially available early in 2008, and vaccination programmes were rapidly initiated throughout most of the affected area. Although within the EU, government-led compulsory vaccination programmes are eligible for financial support covering 100 per cent of the cost of vaccine purchase and 50 per cent of the cost of delivery (European Commission 1990), such schemes must first be approved by the EU and may carry additional administrative requirements. Furthermore, some EU members such as the UK receive only a fraction of this amount in real terms as the remainder is offset against existing EU rebates. In combination with the additional cost and delay that may result from compulsory government-led vaccination programmes, this led several countries (including England, Wales and the Netherlands) to opt for voluntary schemes while others (including Belgium, Germany, Luxembourg and the Czech Republic) opted for compulsory schemes partially subsidised by the EU, and some (such as France) operated a mixture of voluntary and compulsory schemes.

The success of vaccination varied considerably from one country to the other. In countries such as Belgium and the Netherlands which were hit hard by BTV-8 in both 2006 and 2007, transmission during the 2008 season was restricted to a handful of cases, but it is difficult to estimate the importance of vaccination in achieving this result as the vast majority of susceptible animals in these areas would be expected to already possess BTV antibodies as a result of natural infection and recovery. In Germany, approximately 70 per cent of cattle and 90 per cent of sheep in the infected areas were vaccinated by the end of August, a fact which the Federal German Ministry of Food, Agriculture and Consumer Protection believed to be responsible for the far fewer cases of BTV-8 seen during 2008 (Anon 2008). Finally, despite the voluntary nature of the vaccination programme in the UK, sales data suggest that a coverage of 80 per cent or higher was achieved within areas where BTV

transmission had been confirmed in 2007, although coverage in areas where no BTV had been reported and that were brought within the protection zone (PZ) for trading reasons was as low as 40 per cent in some areas.³ To summarize, where uptake was high and vaccine was administered in time for protection to develop before the seasonal peak of transmission, vaccination appears to have been broadly effective in controlling BTV transmission, an outcome best exemplified by the UK where no cases of BTV-8 infection were recorded during 2008.

In the meantime, a second serotype of BTV had been spreading incrementally northwards in Europe. BTV-1 activity was first reported from several countries in the Maghreb during the autumn of 2006 (International Society for Infectious Diseases 2006a,b,c), and the serotype subsequently spread to southern Spain, where it was detected in the summer of 2007 (World Organisation for Animal Health 2007c). Although BTV-1 had previously entered southeast Europe, this represented the first isolation of BTV-1 in the southwest and no inactivated vaccine for the serotype had at that time been developed. However, in Spain, government and industry were already collaborating over the development and production of inactivated BTV-4 vaccine, and this facilitated the rapid development and production of an inactivated vaccine for BTV-1, which became commercially available by December only five months after the serotype was first detected in Spain. BTV-1 continued to spread during 2007 and was detected in Portugal by September (World Organisation for Animal Health 2007a) and southwest France in November (World Organisation for Animal Health 2007b). By November 2008, BTV-1 had spread as far as Brittany in northern France (International Society for Infectious Diseases 2008), and may pose an additional risk to other NW European countries in 2009. Both Portugal and France have since begun widespread vaccination against BTV-1 using inactivated vaccine.

In October 2008, the Dutch National Reference Laboratory at CVI Lelystad confirmed the presence of a third serotype of BTV in Western Europe. This isolate was subsequently identified at IAH as BTV-6 (World Organisation for Animal Health 2008c). This BTV-6 strain, which was also subsequently detected in Germany in the region bordering the Netherlands (World Organisation for Animal Health 2008a), appeared to be closely related to the live attenuated vaccine strain produced in South Africa (SA), and was associated with less severe clinical signs than the NW European BTV-8 strain. A strain of BTV-11 was also detected in Belgium during January 2009, and also appeared to be closely related to the corresponding SA vaccine strain (International Society for Infectious Diseases 2009). In both cases, temporary restriction zones were put in place following virus detection, but lifted following reports submitted to the EC by the relevant authorities concerning the potential of the strains to cause clinical signs (Anon 2009; Houdart 2009). Finally, during surveillance for BTV in Switzerland by the national reference laboratory, a number of unusual infections were detected in goats. Subsequent investigation revealed the

presence of a novel BT-like virus provisionally termed Toggenburg Orbivirus (TOV), genetically distinct from any other BTV, which results in subclinical low viraemia infections in goats (Hofmann *et al.* 2008). Although official classification of TOV as a 25th BTV serotype or as a new virus will depend upon whether it can be demonstrated to be capable of reassortment with other BTV serotypes, a BTV-like orbivirus that could have been circulating silently in Europe for a relatively long period of time may offer insights into the ways in which newly arrived BTVs might adapt to the local environment, and requires further study.

To summarize, during 2008 over 27 000 holdings in Europe were affected by BTV-8 and over 6000 by BTV-1. In addition, two new serotypes (BTV-6 and BTV-11) arrived in the region, and an entirely new BT-like virus had been detected. The availability of inactivated vaccines against BTV-1 and BTV-8 by the end of the year means that most affected countries are likely to be using vaccination as their preferred method of controlling the disease in future years.

The number of holdings affected in each country each year since 2006 is shown in table 1.

6. DISCUSSION AND FUTURE RESEARCH DIRECTIONS

(a) *What have we learned since bluetongue virus arrived in NW Europe?*

BT was first described at the start of the twentieth century in southern Africa. Subsequently, the virus causing this disease was identified widely across the world in a broad band stretching from approximately 40° N to 35° S. This distribution was thought to be limited to the range of susceptible vector species of *Culicoides* and the temperatures under which these midges could transmit the virus. However, since 1998 the virus has undergone a massive expansion in range, and it has established itself in these regions. Since 2006, BTV has expanded further into NW Europe reaching as far north as 58° N, representing a new wave of expansion. As stated above, the route by which BTV-8 was introduced into Western Europe in 2006 remains unknown, but in light of the detection of BTV-6 and BTV-11 in the same region in 2008, it seems plausible that introductions of BTV into Western Europe via this route are presently occurring with relatively high frequency. Whether a new route for virus introduction has recently been established or whether conditions have previously been less suitable for the establishment of introduced viruses remains to be seen, but in our view further such introductions into Western Europe are likely.

One predictable but poorly anticipated difference between the current outbreak in NW Europe and previous European outbreaks has been the much greater relative economic importance of the indirect costs associated with BTV infection in NW Europe, such as animal movement restrictions. Farming in NW Europe relies far more upon animal transport than in Southern Europe, and the area over which restrictions are imposed on confirmation of BTV infection is one hundred times greater than following the confirmation

of, for example, FMD virus (compare European Commission 2000, 2003). As can be seen from the findings of a report conducted into the economic impact of BTV infection in the Netherlands (Hoogendam 2007), while the direct costs of BTV infection rose considerably between 2006 and 2007, the indirect costs remained roughly constant and far outweighed either (figure 5). Detailed study of the frequency and cost of the hitherto underappreciated secondary effects of BTV infection in recovered animals, such as weight loss and reduction in milk yield, has also revealed that the economic costs of BTV infection for the cattle industry are likely to be considerably higher than those for the sheep industry despite the higher levels of disease and mortality in the latter (Gunn *et al.* 2008).

Finally, since the arrival of BTV in Western Europe in 2006, our understanding of the epidemiology of the disease has evolved considerably. Previous advice regarding the effectiveness of housing as protection from vector attack may need to be revised as recent information suggests that some vector *Culicoides* species may enter housing, particularly late in the season when outside temperatures are falling, while evidence of transplacental and contact routes of transmission for BTV-8 have partially explained the ability of this virus to survive periods of vector absence. Concerning the latter, it is important to note that the vast majority of previous studies have failed to detect transplacental transmission of wild strains of BTV, and that virtually all evidence to the contrary thus far relates to the particular strain of BTV-8 currently circulating in NW Europe. Although it is not yet clear whether this and other characteristics of the NW European strain of BTV-8 represent unique adaptations to the local environment, the co-circulation of two or more additional BTV serotypes within its range means that reassortment can now occur and any adaptations that have helped to facilitate the transmission of BTV-8 in the environment of NW Europe are likely to spread to other strains in the region.

(b) *Improving responses to future bluetongue virus outbreaks within the European Union*

The experience of 2008 has indicated that the widespread use of an appropriate inactivated vaccine can be a highly effective method for controlling an outbreak of BTV. However, delays in development and production and problems with the speed of delivery and the extent of uptake also indicate considerable room for improvement. The commercial nature of vaccine production and the high costs of developing and producing a new vaccine mean that the process is unlikely to begin while any uncertainty remains over the purchase and use of the product, while the existence of 24 BTV serotypes and the lack of cross-protection mean that the EU currently considers it uneconomical to establish a BTV vaccine bank (Vassiliou 2009). New BTV vaccines can however be developed relatively rapidly where close links between government and industry exist and, where the safety and effectiveness of a technology has already been proven, as was

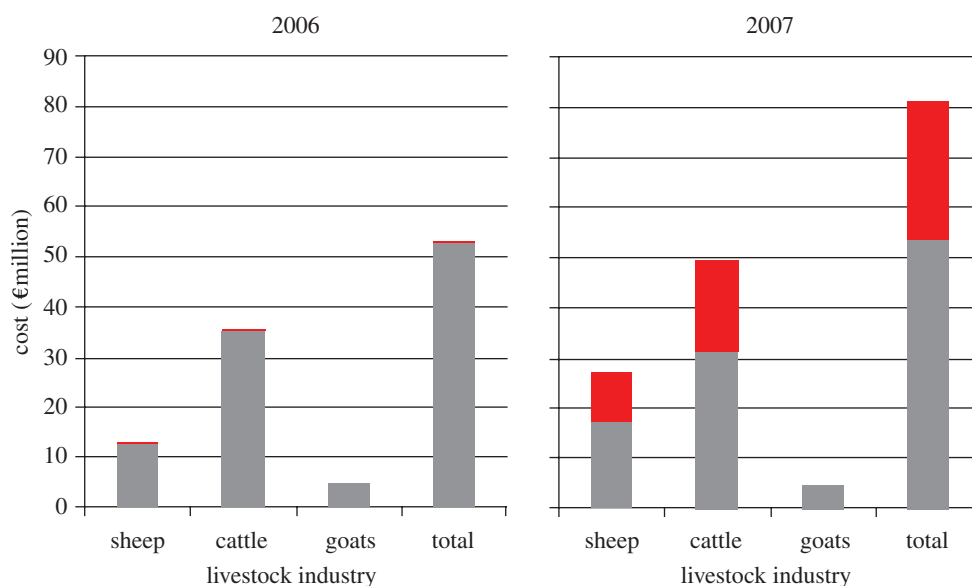


Figure 5. Direct and indirect costs of BTV-8 to the Dutch farming industry in 2006 and 2007. Data from Hoogendam (2007). Figure reproduced from Wilson & Mellor (2008). Red, direct; grey, indirect.

shown by the development and production of an inactivated vaccine against BTV-1 in Spain in 2007. One clear area for improvement may be in government action where placing pre-orders for vaccines is likely to result in more rapid delivery and earlier vaccine deployment. Meanwhile, problems with vaccine uptake can be reduced by improving the provision of information to livestock keepers about the effectiveness and safety of a given vaccine and the economic risk of not vaccinating.

Current EU legislation has discouraged the use of vaccination as a preventive measure in regions at risk of BTV but not yet affected, such as Scotland. Under current legislation, vaccination can only be undertaken within a PZ, into which the importation of animals from areas where the same serotype of BTV is actively circulating cannot be prevented. Countries currently wholly or partially free of BTV that wish to initiate preventive vaccination programmes must therefore first declare the region to be vaccinated as a PZ, allowing the unrestricted importation of potentially infected animals and substantially increasing the likelihood of the precise situation that they are attempting to prevent. Despite this situation, countries such as Scotland have decided that this risk was outweighed by that of doing nothing, and have initiated compulsory vaccination programmes. The possibility of modifying the existing legislation to permit the declaration of 'vaccination-only' zones has been raised at recent meetings of the Standing Committee on the Food Chain and Animal Health and may be implemented in the future. The status of such zones would be most easily monitored using DIVA (Differentiating Infected from Vaccinated Animals) tests, which do not currently exist for BTV and would therefore represent a logical priority for future research. The only way to monitor the status of vaccination zones using present technology would be via the regular testing of unvaccinated sentinel animals.

BT is a vector-borne disease and, as such, several aspects of its epidemiology could be better understood by improving our knowledge of the distribution, activity and behaviour of its vectors. The seasonal nature of BTV transmission in NW Europe is partly because of the seasonal activity of its *Culicoides* vectors, and under current EU legislation Member States have the option of declaring a 'vector-free period', based on entomological surveillance, which reduces the testing requirements for animal movement. Although many Member States have taken advantage of this legislation, others have been discouraged by the possibilities of intermittent vector activity during the winter and inside buildings, as well as uncertainty about the timing of the springtime resumption of vector activity. Entomological data gaps have also made it difficult to design appropriate and proportionate legislation on the optimal size of restriction zones, the safe movement of animals through those zones, the protection of susceptible livestock from vector attack, and the implementation of effective vector control, among other areas. The design of appropriate responses to future European outbreaks of BT and other *Culicoides*-borne viral diseases would therefore be supported by a greater commitment to basic entomological data collection by Member States, as described by Carpenter *et al.* (2009).

As described above, the potential for strains of BTV to spread via the windborne movement of infected *Culicoides* has been recognized for a long time, and is partially responsible for the relatively large restriction zones imposed on an area following the confirmation of BTV transmission. More recently, attempts have been made to quantify the risk of specific introduction events via this method, adapting existing models of atmospheric dispersion to predict locations and periods at relatively high risk of introduction in order to inform policy-making. Such models have been used operationally during the current BTV-8 outbreak to assess the risk of BTV-8 spreading from continental

Europe to the UK (Gloster *et al.* 2007) and to assess the impact of wind-borne vector dispersal on the overland spread of the outbreak (Hendrickx *et al.* 2008). Similar models could be used during future outbreaks to determine how best to allocate resources for surveillance or together with regional vaccination to establish 'fire-breaks' preventing introduced virus from becoming established. At the same time, within- and between-farm models of the transmission of BTV may also help to anticipate the risk of future virus establishment (Gubbins *et al.* 2008) and explore the cost-effectiveness of control options (Gunn *et al.* 2008).

Finally, most introductions of BTV into Europe in the last decade are thought to have occurred via one of the three 'gateways' illustrated in figure 4. It would be sensible to make the most efficient use of limited surveillance resources by targeting known routes of viral introduction into Europe. However, the arrival in Western Europe of BTV-8 in 2006, BTV-6 in 2008 and BTV-11 in 2009 suggests that at least one other route or mode of introduction may exist. Although attempts have been made to identify the route(s) of introduction of these viruses by tracing animal imports, analysing sequence data and modelling meteorological conditions, their mechanism of introduction into Western Europe, presumably from sources remote to the continent, remains uncertain and clearly requires elucidation before appropriate measures can be put in place to prevent others following their lead.

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ENDNOTES

¹Comprehensive information on the specific habitats from which each species in this group has been reared can be found on <http://www.Culicoides.net>.

²CIDC Lelystad became part of the Centraal Veterinair Instituut van Wageningen UR (<http://www.cvi.wur.nl/>) on 1 January 2008.

³House of Commons Hansard Written Answers for 17 November 2008 (HC Deb 17 November 2008, vol. 483 c92W).

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