

Infection of the vectors and bluetongue epidemiology in Europe

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Summary

The author describes some of the factors controlling the infection and transmission of bluetongue (BT) virus (BTV) by vector species of *Culicoides*. Also outlined are certain important features of the recent BT epizootic in the Mediterranean Basin, concentrating on those aspects involving vector transmission and overwintering of the virus. The regions affected by the outbreaks and the BTV serotypes involved are set out, the distribution of the major vector, *C. imicola* is described and the impact of novel vector species of *Culicoides* and a possible overwintering mechanism for the virus in Europe, are discussed.

Keywords

Bluetongue – *Culicoides* – Epidemiology – Europe – Infection – Novel vectors – Overwintering.

There are some 1 250 named species of *Culicoides* biting midges, in the world but only very few have been shown to act as competent vectors of bluetongue (BT) virus (BTV) (29). In North America, *C. sonorensis* is the major vector with *C. insignis* also being important in parts of some of the southern states (e.g. Florida). Further south in the Caribbean areas and in South America *C. insignis* and *C. pusillus* are thought to be the major transmitters of BTV. In Australia several vectors are thought to exist, with *C. brevitarsis* the most important but *C. wadai*, *C. actoni* and *C. fulvus* being of local significance. In most of the Old World, *C. imicola* is by far the most important BTV vector although recently one of its sibling species in Africa, *C. bolitinos*, has also been shown to be competent and, in Europe, *C. pulicaris* and *C. obsoletus* have both recently been implicated in BTV transmission.

Vector competence

The reasons why some species of *Culicoides* contain some individuals that are competent to support the replication and transmission of BTV subsequent to oral ingestion, and why others are not, are complex (reviewed in 22). Basically, when an arbovirus like BTV is ingested by a haematophagous insect during blood feeding, the virus passes into the lumen of the hind part of the mid-gut. It then has to gain access to the body of the insect proper before the potentially hostile environment in the gut lumen inactivates it or before it is excreted. If the virus is to be orally transmitted by the vector, as is BTV, it must reach the salivary glands with or without amplification in

other susceptible tissues, multiply in them and finally be released with the saliva into the salivary ducts where it is available to infect a second vertebrate host during a subsequent bite. The details of this cycle (its duration, the tissues infected, the titre of virus produced, the proportion of insects infected, the transmission rate) are controlled by a range of inter-dependent variables (the virus, the insect host and environmental factors – particularly temperature). In the case of BTV, a series of barriers or constraints are known to exist within the bodies of non-vector species of *Culicoides* and even within a variable proportion of individuals within vector species, that act to prevent infection or else restrict infection in such a way as to prevent transmission. Briefly, the major barriers that an arbovirus may have to surmount upon being deposited in the mid-gut of an haematophagous insect in order to develop a fully patent infection and so be available for oral transmission are as follows:

- infection of the mid-gut cells: mid-gut infection barrier (MIB)
- escape of progeny virus from the mid-gut cells into the haemocoel: mid-gut escape barrier (MEB)
- dissemination of virus through the haemocoel to the salivary glands (and ovaries if transovarial transmission is to occur): dissemination barrier (DB)
- infection of the salivary glands: salivary gland infection barrier (SGIB)

- release from the salivary glands into the salivary ducts: salivary gland escape barrier (SGEB).

In addition, there is a further barrier to surmount if the virus is to be transmitted transovarially, i.e. the transovarial transmission barrier (TOTB). Figure 1 depicts a summary of these barriers to infection and transmission, and highlights those that have so far been identified in the BTV-*Culicoides* system.

Within a vector species of *Culicoides* susceptibility to infection is a genetically heritable trait. This means that different populations of the same species may have widely varying oral susceptibilities to infection and transmission of a particular serotype or strain of BTV dependent upon the genotypes prevalent in the parental populations from which they were derived. Consequently, results obtained by testing one or several populations of a suspect vector species of *Culicoides* cannot necessarily be extrapolated across all or most populations of that species. This situation can make it difficult to estimate the importance of a suspect vector species unless exhaustive testing has been undertaken across many populations of that species using a range of BTV serotypes and strains. As may be seen when considering the current outbreaks of BT in Europe, in practice, this can result in incorrect assessments being made of the significance of such novel vectors.

Regions affected by the 1998-2003 epizootics in Europe

The current epizootic of BT in Europe is presumed to have started in October 1998 on four Greek islands adjacent to the Anatolian coast of Turkey. The serotype involved was identified as BTV-9 and this was the first occasion that this serotype had ever been identified in Europe (30).

As expected, transmission of BTV in the Greek islands seemed to end during December 1998, probably because of zoosanitary measures introduced by the Greek Veterinary Authorities and because adult vector populations of *C. imicola* in this region are at a minimum from this time of the year (M. Patakakis, personal communication). However, in June 1999, BTV 9 was again reported from eastern Europe, first in south-east Bulgaria and then in rapid succession from European Turkey and from the north-east of mainland Greece (30). The outbreaks in Greece were particularly active and extended across the north of the country from Evros on the Turkish/Bulgarian border to west of Thessaloniki and then south to Larissa, Magnisia and Evia. These outbreaks also continued sporadically in Greece during the summer of 2000. Of particular concern in Greece was the discovery that, in addition

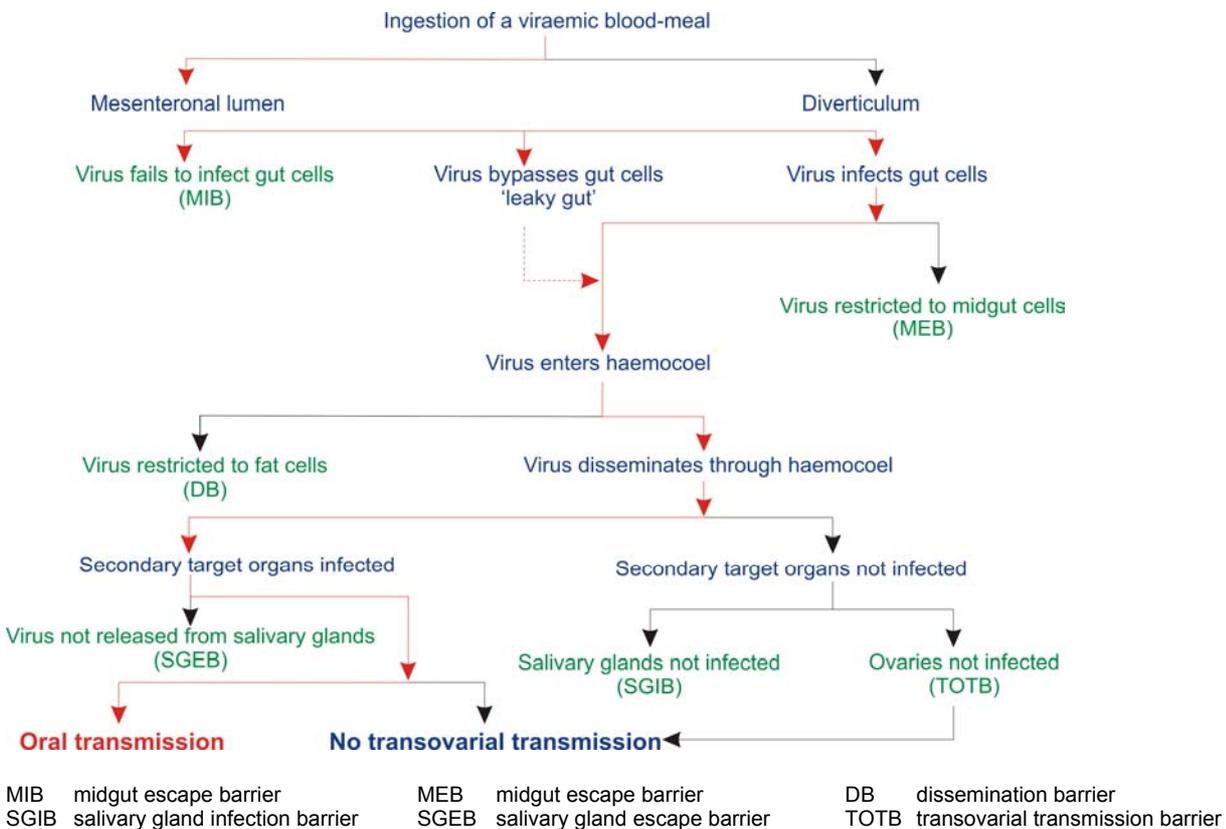


Figure 1
 Barriers to the infection and transmission of arboviruses by insect vectors
 Route of virus dissemination in the *Culicoides*-BTV system highlighted in red

to BTV-9, serotypes 4 and 16 were also active in the country. All three serotypes have been identified historically from locations further east (e.g. Anatolian Turkey, Syria, Jordan and Israel), and BTV-16 was also isolated from Izmir Province in western Anatolian Turkey in August 2000 (30) (C. Hamblin, personal communication).

Meanwhile, in January 2000, BT was reported for the first time ever from Tunisia and the virus involved was identified as BTV-2 (30). The origins of this incursion are obscure but are likely to be separate from the one involving Turkey, Greece and Bulgaria. As foot and mouth disease virus had also entered Tunisia (and Algeria) during 1999, probably via cattle imported from Côte d'Ivoire and Guinea into Algeria (18) it is possible that BTV could have followed a similar route. Cattle in Africa often experience sub-clinical infections with BTV, and BTV-2 is common in several areas of sub-Saharan West Africa (15, 16).

From June 2000, additional, more widespread outbreaks of BT due to serotype 2 of the virus were reported from Tunisia and in July 2000 the same serotype was also identified in Algeria. These outbreaks continued until September and October 2000. In addition, seropositive animals were also recorded from 18 provinces stretching across northern Morocco (30). Then, in August 2000, came a new and rather dramatic turn of events, when BTV-2 was confirmed for the first time ever in Italy. The island of Sardinia was affected first but by October, BTV-2 had also spread to Sicily and southern mainland Italy. Outbreaks continued in Italy into December 2000 but were not reported later in the winter of 2000-2001, presumably because the cooler temperatures prevailing at those times significantly reduced vector abundance. Unfortunately the outbreaks in Italy were not the end of the matter and, in October 2000, BTV-2 was also recorded for the first time on the French island of Corsica, and on the Spanish islands of Menorca and Mallorca. Just as in Italy, outbreaks in the Balearics continued into November and December 2002 but were not recorded later in that winter (30).

So at the end of 2000 there were two main foci of BTV infection established in Europe. One to the east, centred on Turkey, Bulgaria and Greece involving BTV serotypes 4, 9 and 16, and the second to the west, centred on Italy and certain French and Spanish islands (and parts of North Africa) involving BTV-2. Unfortunately both BT foci continued to be active in 2001 and 2002.

Eastern focus

In 2001, the eastern focus spread to new areas in north-west Greece and Lesbos during August and October, and also extended northwards to involve Kosovo and south-east Serbia in August, west Bulgaria and Macedonia in September and Croatia in December. Unofficially, seroconversions were also reported in sheep and cattle in European Turkey. Importantly in 2001 the Greek Veterinary Authorities identified an additional BTV serotype (BTV-1) in Greece. This brought the number of BTV serotypes active in Europe to 5 (BTV-1, -2, -4, -9 and -16). The origin of the new serotype is difficult to determine but recent studies by Mertens and his colleagues (iah.bbsrc.ac.uk/dsRNA_virus_proteins/orbivirus-phylogenetic-trees.htm) have shown that, phylogenetically, the BTV-1 reported in Greece is much closer to topotypes of this virus from the east (India) than from Africa. This suggests that this incursion, like those involving serotypes 4, 9 and 16, originated to the east of Europe, unlike the incursion of BTV-2.

In 2002 Greece was reported free from BTV but disease due to BTV-9 was still widely reported from the Balkans (southern Bulgaria, Kosovo, Montenegro and Bosnia). The presence of BTV-9 in the Balkans in 2002 makes this the fourth consecutive year that this serotype has been present in this region of Europe. Furthermore, the outbreaks in Serbia in 2001 and those in Bosnia in 2002 are, at 44°30'N, the most northerly ever reported in Europe.

Western focus

In May 2001, Italy recorded renewed BTV activity in Sardinia. Further activity was reported in June (Calabria), August (Basilicata) and September (Lazio and Tuscany). The virus was also active for the second year running on the French island of Corsica but the Spanish authorities reported that the Balearics were free from infection. The vast majority of these outbreaks were due to BTV-2 but significantly and for the first time in this western focus, BTV-9 was also identified in the regions of Calabria and Basilicata in Italy.

In 2002, BT continued to be widespread in Italy (Lazio, Tuscany, Campania, Abruzzo, Puglia, Basilicata, Calabria, Sardinia and Sicily) but was not reported from any other country previously involved in the western focus. Once more, most of the outbreaks in Italy seemed to be due to BTV-2 but BTV-9 was again detected and more widely so than in 2001 (Calabria, Puglia, Basilicata, Campania and Sicily) and a third serotype (BTV-16) was also reported from southern Italy (Calabria) (G.Savini 2002, personal communication). The occurrence in the western focus of BTV serotypes 9 and 16 that had previously only been reported from the eastern

focus is a matter of concern and represents a clear link between the two foci.

In the Mediterranean Basin, the general trend of BTV movement seems to be in a westerly direction. In the eastern focus, this is seen with all of the serotypes involved, which were first detected in the extreme east of the region but spread across Turkey, Greece and Bulgaria, and at least two of the serotypes have now moved as far west as Italy. In the western focus, BTV was first detected in Tunisia but then infected animals were reported in Algeria and Morocco in North Africa and, subsequent to Italy, on Corsica and on two of the Balearic islands of Spain. Despite the widespread outbreaks of BTV-2 in Italy, this virus has not yet been identified in Greece, to the east, despite the geographical proximity of the two countries.

The vector situation

Culicoides imicola

C. imicola is the major Old World vector of BTV and African horse sickness virus (AHSV). It has long been considered to be the only major vector of these viruses in the Mediterranean Basin because, prior to 1998, every incursion of BTV or AHSV into the Mediterranean Basin has been restricted to regions where *C. imicola* is known to be present.

The known distribution of *C. imicola* in the Mediterranean Basin prior to 1999 is shown in Figure 2. This distribution is a composite of the findings of many investigations conducted since 1974 (3, 4, 5, 6, 11, 17, 21, 24, 25, 26, 31). However, following the 1998 incursion of BTV into Europe, further investigation into the distribution of *C. imicola* in the Mediterranean Basin has been galvanised and

workers in Greece, Italy, France, Spain, Turkey and Bulgaria have all made significant contributions to our knowledge of where this species exists and, equally importantly, where it is apparently absent (1, 2, 4, 7, 8, 14, 20, 32) (J.-C. Delécolle 2002, personal communication; S. de La Rocque 2002, personal communication; M. Patakakis 2002, personal communication; I. Burgu 2002, personal communication; A. Martinez 2002, personal communication; J. Delgado and P. Collantes 2002, personal communication; N.K. Nedelchev and G. Georgiev 2002, personal communication). The second line on Figure 2 has been derived from these accumulated findings and depicts the northern limits of *C. imicola*, the so-called '*imicola*-line', as known in 2002. It is likely that in the future this '*imicola*-line' will move even further northwards.

When comparing the distribution of *C. imicola* in 2002 with earlier findings, it is at once apparent that the species is present in major areas of Europe where it was previously thought to be absent (e.g. south-eastern Spain, the Balearics, Corsica, Sardinia, Sicily, much of mainland Italy and much of mainland Greece). In Italy *C. imicola* has now been recorded as far north as 44°N (14). Whether this represents a real and general movement of *C. imicola* northwards and westwards perhaps in response to climate-change, or whether it is merely a reflection of more intensive sampling, is difficult to say although in some regions, at least, the former seems to be the most likely option. For example, in mainland Greece, a survey in 1983 included 16 collections of *Culicoides* comprising 19 species from locations where there was intense BTV transmission in 1999 but not a single isolate of *C. imicola* was recorded (25).



— Known northern limits of *Culicoides imicola* distribution prior to 1999
 — Known northern limits of *Culicoides imicola* distribution in 2002

Figure 2

The known northernmost limits of *Culicoides imicola* in the Mediterranean Basin prior to 1999 and in 2002

However, more recent surveys conducted between 1999 and 2001 from locations in the same regions have found *C. imicola* to be common (M. Patakakis 2001, personal communication). Be that as it may, the newly discovered presence of populations of *C. imicola* in Menorca, Mallorca, Corsica, Sardinia, mainland Italy, mainland Greece (and Tunisia) are clearly the main reason that BTV was able to be transmitted in these regions.

However, between 1999 and 2002, BTV was also transmitted in many other locations (northern Greece, European Turkey, Bulgaria, Serbia, Croatia, Macedonia, Montenegro and Bosnia) that are apparently beyond this new 'imicola-line'. In some of these areas, no vector *Culicoides* surveys have been performed, but in others, *C. imicola* has been sought for since the BTV outbreaks started but has not been recorded (e.g. northern Greece [Thrace], European Turkey and Bulgaria). Indeed, in Bulgaria, *Culicoides* surveys have been conducted at intervals for over 10 years, 29 species of *Culicoides* have been recorded but not a single specimen of *C. imicola* has been identified (10, 12, 13). These findings suggest in the strongest possible terms that in some parts of Europe as yet unidentified, or unconfirmed, BTV vector(s) are present.

Novel vector species of *Culicoides* in Europe

In all of the 'non-imicola' areas where BTV has been detected, *Culicoides* of the *C. obsoletus* and *C. pulicaris* groups are by far the most abundant and prevalent biting-midge species (30). *C. obsoletus* and *C. pulicaris* have long been suspected of being BTV vectors, mainly on the basis of BTV isolations from *C. obsoletus* made in Cyprus (23) and AHSV isolations from mixed pools of *C. obsoletus* and *C. pulicaris* made in Spain (28). In this context, it should be borne in mind that BTV and AHSV tend to utilise as vectors the same *Culicoides* species (21). However, vector competence studies carried out on a population of *C. obsoletus* and *C. pulicaris* in the United Kingdom (UK) during the 1980s recorded oral susceptibility rates of less than 2%, in comparison with 19.5% for a known major vector, *C. sonorensis* (= *variipennis* in part) (19, 27). At the time, these findings and the restriction of all previous BTV outbreaks in Europe entirely to 'imicola-areas' (see above) suggested that these other species were likely to be of only minor importance as BTV (or AHSV) vectors. Nevertheless, as is explained below, in hindsight perhaps, more significance should have been attributed to these early findings.

Firstly, the high abundance and high survival rates of *C. obsoletus* and *C. pulicaris* as exhibited in Bulgaria in 1999 (30) could compensate for low levels of vector competence. Precisely this situation exists in

Australia where *C. brevitarsis* is considered to be the most important BTV vector, mainly on the basis of its high abundance and prevalence, even though its vector competence levels (0.3%) are very low indeed (34). Secondly, as expression of competence by a vector species for a particular virus is an hereditary trait (see section on Vector competence), populations with high, intermediate and low levels of competence are to be expected. It may be, therefore, that populations of these species in Bulgaria, northern Greece and European Turkey express higher levels of competence for BTV than had hitherto been suspected. In this context, the only previous vector competence information about these species, which was derived from testing single populations of *C. obsoletus* and *C. pulicaris* in southern England some 20 years ago, is clearly insufficient to extrapolate meaningfully across the whole of Europe or even the UK for that matter.

Consequently, until very recently other populations of *C. obsoletus* and *C. pulicaris* throughout Europe have remained untested and their levels of competence for BTV were completely unknown. However, new and important information from workers on mainland Italy and in Sicily is now beginning to demonstrate the real significance of these two groups of *Culicoides* in the epidemiology of BT. Savini *et al.* (33) have drawn attention to the fact that outbreaks of BT have occurred in areas of southern Italy where *C. imicola* is scarce or absent. In these areas, Obsoletus Complex midges apparently comprised more than 90% of over 10 000 collected *Culicoides* and more than 95% were parous individuals (cf. parous midges have taken and digested a blood-meal and so are the only specimens likely to harbour virus). These authors also reported that three isolations of BTV were made from 5 800 tested Obsoletus Complex midges (estimated as 95% *C. obsoletus sensu stricto* and 5% *C. scoticus*) which gives a rate of one BTV isolation per approximately 2 000 insects. Elsewhere on mainland Italy, other workers have also isolated BTV from pools of Obsoletus Complex collected in BT outbreak areas, at locations where *C. imicola* was either scarce or absent (C. De Liberato 2003, personal communication). These findings of Italian workers are the first to positively incriminate *C. obsoletus* midges as BTV vectors since 1977 when BTV was first isolated from this species group in Cyprus (23).

Equally important are the recent findings of Sicilian scientists. Caracappa *et al.* (9) report that in Sicily, *C. imicola* is much less common than in most other regions of Italy where BT has occurred, and in 2002 he and his colleagues made five isolations of BTV from parous, non-engorged *C. pulicaris* at locations and times when *C. imicola* was absent. These are the

first such field isolations ever reported from this species. Importantly, these five isolations were made from just 987 specimens, which gives an isolation rate of about 1 per 200 insects and indeed 1 isolation was made from a pool of only 10 individuals. This is a far higher rate than has been recorded from *Obsoletus* Complex midges elsewhere and may imply the presence of a highly competent population of *C. pulicaris* in Sicily. Significantly, Caracappa and his colleagues made no BTV isolations from 724 *C. obsoletus* midges collected at the same locations and at the same times, suggesting that this species may be a less competent vector, at least in parts of Sicily.

These findings confirm the importance of *C. pulicaris* and *Obsoletus* Complex midges as probable BTV vectors in parts of Italy and suggest that these species are likely to be important in some locations, even within the latitudes where *C. imicola* occurs. Those responsible for implementing vector control campaigns might do well to be aware of this. The discoveries in Italy also strongly implicate *C. pulicaris* and *Obsoletus* Complex midges as the likely vectors in those more northerly parts of Europe where BTV has occurred beyond the 'imicola-line' (northern Greece, European Turkey and the Balkans).

The recognition of probable new vector species of *Culicoides* in Europe is a matter of considerable concern. Although expanding its range, *C. imicola* is still restricted, climatically, to the more southerly parts of the continent, *C. pulicaris* and *Obsoletus* Complex midges exhibit no such restriction and are probably the most common *Culicoides* species across the entire central and northern regions of Europe. Theoretically, this would seem to put at risk most of these areas. However, BTV has not yet occurred across Europe, so the question is, why not? The reasons are likely to be complex but will probably include some or all of the following:

- a) vector abundance may be lower further north
- b) most northerly populations of the novel vectors may be less efficient BTV transmitters than most populations of *C. imicola*
- c) ambient temperatures further north tend to be lower. As BTV is transmitted more quickly and more efficiently at higher temperatures than lower, transmission may not be possible or may only be possible for a brief part of the year or in restricted locales in more northerly climates
- d) adult vectors may be completely absent for part or much of the year in northerly areas due to the harsh winters so that BTV-infected animals either recover or die before the new vector season begins.

However, climate-change, through its effects upon the vectors, is likely to moderate all of these factors, allowing BTV to spread further northwards and be transmitted more efficiently over a greater proportion of the year than at present (30). Additionally, and specifically in connection with point (d) above, the apparent ability of BTV to overwinter in some of the more northerly, currently infected areas (e.g. Bulgaria, the Balkans, north-western Greece and European Turkey) where adult *Culicoides* are absent for much of the year is extraordinary (30). If confirmed, this suggests that a novel overwintering mechanism may be involved that could extend the area perceived to be at risk to BTV significantly further north, irrespective of climate change. Interestingly, such a mechanism has recently been described which postulates the presence of covertly infected, seropositive ruminants in which BTV-persistently infected $\gamma\delta$ T-cells are recruited into the skin during late summer and autumn in response to the biting activity of the new generation of vectors which thereby become infected and so initiate fresh transmission cycles (35).

All of these recent findings, taken in concert with climate change, suggest that the current outbreaks of BTV in Europe are unlikely to be a one-off aberration but may be a sign of things to come. The strong control measures presently being deployed against the virus in Europe may result in its temporary elimination from this continent but further, more frequent and widespread incursions are a real prospect.

Novel aspects of the 1998-2003 incursion into Europe

There are a number of factors that make the current BTV incursions into Europe unique. These are as follows:

- 1) For the first time, multiple serotypes of BTV have been involved (i.e. 1, 2, 4, 9 and 16), most of which are new to Europe.
- 2) In some areas, live BTV vaccines of several different types have been deployed for the first time (i.e. including serotypes 2, 3, 4, 8, 9, 10 and 11).
- 3) The major vector, *C. imicola*, has been found in many new locations in and around Europe (e.g. Sardinia, Sicily, mainland Italy, Corsica, mainland Greece, Majorca, Menorca, eastern mainland Spain, European Turkey and Tunisia).
- 4) BTV has entered almost all of these locations but in some areas has penetrated beyond the 'imicola line', reaching further north than ever before (e.g. 44°30' N in Serbia and Bosnia).

- 5) Novel vector species of *Culicoides* are therefore involved in certain outbreak areas (e.g. Bulgaria, northern Greece, European Turkey, Macedonia, Serbia, Kosovo, Albania, Croatia and Bosnia). Circumstantial evidence (e.g. abundance and prevalence) suggests that these novel vectors are likely to be members of the *Obsoletus* and *Pulicaris* species complexes. These are the most common *Culicoides* species across northern Europe.
- 6) Multiple isolations of BTV have now been made from *C. pulicaris* and species of the *Obsoletus* Complex in Italy, confirming their vector status and suggesting that they may also be involved in BTV transmission within the latitudes where *C. imicola* occurs.
- 7) In some of the more northerly areas, BTV has overwintered in the absence of adult vectors. A novel mechanism must be involved (e.g. the $\gamma\delta$ T-cell mechanism).

Acknowledgements

The author is grateful to many colleagues throughout Europe and North Africa for sharing their unpublished information and their thoughts. This paper was produced while the author was in receipt of grants from the European Union (Contract Nos. QLK2-CT-2000-00611 and QLK2-CT-2001-01722) and the Department for Environment, Food and Rural Affairs (DEFRA) (Reference SE 2610 – Effect of temperature and regional origin on the vector capacity of British and European *Culicoides* spp.).

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