Potential new *Culicoides* vector of bluetongue virus in northern Europe

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BLUETONGUE is an orbiviral disease that affects domesticated ruminant livestock, especially sheep and cattle (Erasmus 1990, MacLachlan 1994). The disease occurs almost worldwide (Tabachnick 2004) and is transmitted by approximately 30 species of biting midges of the genus *Culicoides* (Meiswinkel and others 2004). There are 24 known serotypes of bluetongue virus (BTV); in Europe, eight serotypes occur in the Mediterranean Basin, where they are confined to below the 45th parallel. Within this region, four species of *Culicoides* have been incriminated as vectors: the Afro-Asiatic *Culicoides imicola* (accounting for at least 90 per cent of disease transmission) and the palaearctic endemic species *Culicoides obsoletus*, *Culicoides scoticus* and *Culicoides pulicaris* (Mellor and Pitzolis 1979, Caracappa and others 2003, Savini and others 2005). Purse and others (2005) have suggested that under the influence of a warming global climate, *C imicola* may move further northwards into Europe. If this is the case, it poses an increased future risk to livestock production in the region.

Since 1998, five serotypes of BTV have swept across the Mediterranean Basin and affected 15 countries, many of which had never experienced the disease previously (Purse and others 2005). In August 2006, following an unprecedented warm summer, bluetongue took a significant 'leap' northwards, appearing unexpectedly in northern Europe and affecting parts of the Netherlands, Belgium, Germany and northern France. To date, the disease has spread across approximately 100,000 km^2 of territory and advanced almost to the 53rd parallel, the furthest north BTV has been identified anywhere in the world. The virus was identified by the European Community Reference Laboratory (Pirbright, UK) as BTV serotype 8 (BTV-8) and found to be closely related phyletically to a 1982 west African strain of the same serotype; importantly, it appears not to be descended from vaccine forms of BTV-8 used in parts of southern Europe some years ago (International Society for Infectious Diseases 2006). The link to sub-Saharan Africa, and the fact that BTV-8 is novel to Europe, indicates the virus was introduced over a long distance; however, the exact origin of the virus, and the route of introduction, is not known.

In September 2006, the veterinary authorities of the Netherlands, in conjunction with the Istituto Zooprofilattico Sperimentale (IZS), Teramo, Italy, commenced light-trapping (using the Onderstepoort-type blacklight trap) on a bluetongue-affected dairy in Gulpen, south-east Limburg, in an attempt to detect the virus in pools of identified *Culicoides* species midges. In 35 collections, made between September 14 and 24, 12,001 midges, representing 15 species, were captured. The average percentage prevalences and parity rates (as derived from a single, randomly chosen light-trap collection) of the seven most common *Culicoides* species captured in Gulpen are shown in Table 1. Those taxa that could not be identified reliably on external morphology were aggregated into mixed species pools; these are *C obsoletus/C scoticus* and *C pulicaris/Culicoides lupicaris,* and include species previously incriminated in the transmission of BTV in the Mediterranean Basin (Mellor and Pitzolis 1979, De Liberato and others 2005, Savini and others 2005). All the captured midges were age-graded as either nulliparous, parous, gravid or freshly blood-fed following the method of Dyce (1969); the parity rate is the percentage of older parous and gravid females in the population, that is, the individuals most likely to harbour BTV. After 11 days of continuous light trapping, a cumulative total of 3242 older parous and gravid females was obtained and subdivided among 40 species pools for virus detection (Table 1). A clear majority of pools comprised *C obsoletus/C scoticus* (77·1 per cent) and *Culicoides dewulfi* (18·2 per cent). The pools were code numbered and then submitted 'blind' to the Central Institute for Animal Disease Control, Lelystad, on September 29, to be tested individually using an in-house bluetongue serogroup-specific reverse transcriptase-PCR (based on segment 10 of the non-structural protein NS3).

On October 4, one pool of *C* (subgenus *Avaritia*) *dewulfi* was found to be PCR positive for BTV. This species had never before been linked to the transmission of bluetongue disease in Europe. While *C dewulfi* remains to be incriminated more conclusively as a novel vector, the result is of some interest for the following reasons.

C dewulfi is known to breed in '…cow dung lying naturally in the field' (Kettle and Lawson 1952). This dependence on bovid dung means that the distribution of *C dewulfi* will be determined by the distribution of cattle. This implies that *C dewulfi* will be found throughout most of the Netherlands, and the rest of Europe, where cattle are husbanded. This cattle-*Culicoides* association is paralleled in other parts of the world affected by bluetongue, such as south-east Asia (including Australia) and Africa, where the respective vectors, *Culicoides brevitarsis* and *Culicoides bolitinos,* also breed in cattle and buffalo dung (Meiswinkel 1989). It is also noteworthy that *C bolitinos* is an important vector of the devastating disease African horse sickness (AHS) (Meiswinkel and Paweska 2003) because, as demonstrated in Ireland, *C dewulfi* also feeds avidly on horses (Townley and others 1984) and breeds in its dung (Kettle 1962).

C dewulfi was described in 1936 from material collected in Belgium but, remarkably, has never before been reported from the Netherlands. It occurs principally in countries at temperate latitudes, and is less commonly reported further south, along the northern margin of the Mediterranean Basin. Throughout the region, very little is known about the local abundance levels and seasonal dynamics of *C dewulfi*.

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TABLE 1: *Culicoides* species (including subgenus and species complex designation) collected by light-trapping during September and October 2006 in Gulpen, Limburg province, south-east Netherlands and the number and size of pools tested for bluetongue virus nucleic acids using a serogroup-specific PCR

Species	Total number of parous/ gravid specimens	Total number of pools	Pool size	Light trap prevalence (%)	Parous/ gravid (parity rate) (%)	*Culicoides* subgenus	Species complex	PCR result
Culicoides obsoletus/scoticus	2500	25	100	68·5	38·6	*Avaritia*	Obsoletus	–
Culicoides dewulfi	590	12	40/50	17·4	42·0	*Avaritia*	Dewulfi	+
Culicoides chiopterus	36	1	36	0·4	50·0	*Avaritia*	Chiopterus	–
Culicoides pulicaris/lupicaris	96	1	96	12·5	11·1	*Culicoides*	Pulicaris	–
Culicoides punctatus	20	1	20	0·4	25·0	*Culicoides*	Pulicaris	–
Culicoides imicola	0	0	0	0	0	*Avaritia*	Imicola	NA

– Negative, + Positive, NA Not applicable

As a taxonomic entity, *C dewulfi* belongs within the subgenus *Avaritia*, which, across the world, is subdivided into at least 10 species complexes (Meiswinkel and others 2004). However, most authors erroneously place *C dewulfi* within the Obsoletus complex (or the *C obsoletus* group). Recent morphological and molecular studies (Gomulski and others 2005) conducted in Italy clarified further the position of *C dewulfi* within the Palaearctic sector of *Avaritia,* and showed it to be a monophyletic taxon within *Avaritia*, and only distantly related to the six species that comprise the Obsoletus complex sensu stricto. In the present study, the identification of *C dewulfi* was based primarily on the descriptions given by Campbell and Pelham-Clinton (1960) and Delécolle (1985).

Similarly, as an entity in the field, most researchers in virus isolation or vector competency studies are not able to confidently distinguish *C dewulfi* from other sympatrically captured species of the subgenus *Avaritia,* including *C obsoletus* and *Culicoides chiopterus*. This failure might explain the significant differences in BTV oral susceptibility measured recently among UK populations of *C obsoletus* by Carpenter and others (2006). Accurate identification, that is, a sound taxonomy, is fundamental to such studies, and exposes the lack of taxonomic expertise currently available in Europe (as was so clearly articulated by the Vector Working Group [Mellor and others 2004]).

The incrimination of *C dewulfi* as a potential vector of BTV does not exclude other species, or species complexes, from having played a role in the outbreak in central Europe. Judging from its high parity rate (38·6 per cent), it would be prudent to assume that the Obsoletus complex was also involved.

Finally, no specimens of the Afro-Asiatic species *C imicola* were found at Gulpen, or among over 30,000 *Culicoides* species captured at 104 additional sites across the Netherlands. Although *C imicola* appears not to have established itself in northern Europe, this does not exclude the possibility of BTV-8 having been brought into the region by a single infected *C imicola* for example, carried on an aeroplane or by some other route.

Although additional studies are required to more conclusively implicate *C dewulfi* as a vector of BTV, these findings support studies by earlier workers in confirming that *Culicoides* species endemic to northern Europe are capable of replicating and transmitting the virus and that they can do so without the 'help' of *C imicola*. Furthermore, because the vectorial capacity of *Culicoides* is likely to increase under the influence of a warming climate, it is important to note that the other most abundant species of *Culicoides* captured at Gulpen have already been implicated in the transmission of BTV elsewhere in Europe. Northern Europe must therefore be considered vulnerable to a variety of *Culicoides*-borne orbiviral diseases of livestock, including bluetongue and African horse sickness, which may be introduced adventitiously along any one of its numerous trade routes.

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