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The *Culicoides* 'snapshot': A novel approach used to assess vector densities widely and rapidly during the 2006 outbreak of bluetongue (BT) in The Netherlands

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Abstract

A novel method was developed and implemented during the recent outbreak of bluetongue (BT) in sheep and cattle in The Netherlands to obtain rapidly a 'snapshot' of *Culicoides* vector densities at the national level. The country was divided into 110 raster cells, each measuring 20 km \times 20 km; within 106 of these cells, a farm was selected with a minimum of 10 cattle and sampled for *Culicoides* for one night only using the Onderstepoort-type blacklight trap. Prior to deployment of the light traps in the field, local veterinarians were trained in their use and in the preservation of captured *Culicoides*. The collections were despatched daily by courier to a field laboratory where the *Culicoides* were counted and identified. The 'snapshot' commenced on 12 September 2006 and was completed on 28 September coinciding with the 5–7 weeks of BT virus (BTV) activity in The Netherlands and when the number of grid cells in which a taxon occurred is represented by the index 20² gFR (=20 km \times 20 km grid Frequency Rate); this index essentially reflects the percentage of examined raster cells found to contain the potential vector in question. The 'snapshot' results can be summarised as follows:

- A total of >35,000 Culicoides were captured ($\mu = 333$ midges/light trap) representing 16 species;
- Not a single specimen of *C. imicola*, the principal Afro-Asiatic vector of BTV in southern Europe, was captured;
- The three European species of *Culicoides* implicated previously as potential vectors in the Mediterranean region and the Balkans also occur in The Netherlands;
- The Obsoletus Complex (represented by both potential vectors *Culicoides obsoletus* and *Culicoides scoticus*) was the most prevalent taxon (20² gFR: 93.4%) followed by the Pulicaris Complex (76.4%);
- The Pulicaris Complex comprised at least six species. One of these, *Culicoides pulicaris sensu stricto* (*ss*), a potential vector in southern Europe, had a low 20² gFR of 17.9% indicating it played no role in the transmission of BTV;

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- The next most prevalent taxa were *Culicoides dewulfi* (70.8%) and *Culicoides chiopterus* (67.0%) both of which breed in cattle dung. This close association with livestock raises their potential as vectors of BTV;
- Culicoides achrayi, Culicoides circumscriptus, C. dewulfi, Culicoides halophilus, Culicoides lupicaris and Culicoides stigma are new species records for The Netherlands and demonstrates that the extant knowledge on the Culicoides fauna of northern Europe is incomplete;
- Cooling trends in the weather induced a >50-fold decrease in overall *Culicoides* numbers but with subsequent warming these would rebound rapidly indicating that more intense cold is required before heightened mortalities are induced amongst adult biting midges.

The northward advance of BT in Europe compels the competent authorities in affected and in neighbouring territories to acquire rapidly baseline information around which to plan sound vector surveillance and livestock movement strategies. The *Culicoides* 'snapshot' is a tool well suited to this purpose. It is stressed that a vector surveillance program must be built upon a firm taxonomic base because misidentifications will flaw the mapped seasonal and geographic distribution patterns upon which veterinary authorities depend.

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1. Introduction

Bluetongue virus serotype 8 (BTV-8) appeared in northern Europe in August 2006 and was first detected in sheep in the village of Kerkrade, south-east Netherlands (Van Wuijckhuise et al., 2006). Over the ensuing four months it spread across the southern half of The Netherlands, Belgium, western Germany, northern France, and Luxembourg affecting both sheep and cattle (Elbers et al., 2007). The outbreak was eventually to cover an estimated 170,000 km² of territory reaching almost to latitude 53°N, which is the furthest north BTV has penetrated anywhere in the world. It is assumed that the virus arrived in one or other infected animal but all avenues of enquiry have failed to identify either the port of entry or the exact location of the index case. Speculation around its mode of entry includes the postulate that infected specimens of the principal Afro-Asiatic vector of BTV i.e. *Culicoides imicola* entered the region on an aeroplane but has not been substantiated.

BTV is comprised of 24 serotypes. These are distributed throughout the tropics and a subtropics of the world with the majority of serotypes found in Africa and in India (\approx 20 each). Each affected region of the world has a specific number of serotypes and because they are more or less stable in their occurrence has led to them being divided amongst five world episystems (Tabachnick, 2004). The Mediterranean/Near East episystem comprises at least eight serotypes; normally one or other of these serotypes will emerge (mostly from the east) and cyclically, at 20-to 30-year intervals, spread westwards. But since 1998, this pattern appears broken because multiple serotypes of BTV have swept across the Basin in successive years and waves. In the process, the disease has affected at least 15 countries, a number for the first time; in addition, it has progressed further north than ever before causing tremendous losses both in terms of the number of sheep lost (close to one million) and through widespread blockages in live animal trade.

The 24 serotypes of BTV are transmitted by biting midges of the genus *Culicoides*; the 32 species that have been implicated in their transmission in various parts of the world were listed recently according to subgenus and species complex (Meiswinkel et al., 2004). In southern

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