Indoor activity of *Culicoides* associated with livestock in the bluetongue virus (BTV) affected region of northern France during autumn 2006

T. Baldet a,*, J.C. Delécolle b, C. Cêtre-Sossah c, B. Mathieu d, R. Meiswinkel e, G. Gerbier a

a CIRAD, Epidemiology and Ecology of Animal Diseases, Montpellier, France
b Université Louis Pasteur de Strasbourg, Musée zoologique, Strasbourg, France
c CIRAD, Control of Emerging and Exotic Animal Diseases, Montpellier, France
d EID-Méditerranée, Montpellier, France
e CICD-Lelystad, The Netherlands

Abstract

In August 2006, bluetongue virus (BTV) was detected in the Netherlands, Belgium, western Germany, Luxembourg and northern France for the first time. Consequently, a longitudinal entomological study was conducted in the affected region of northern France (Ardennes) throughout the autumn of 2006. Data on the spatio-temporal distribution of *Culicoides* (Diptera: Ceratopogonidae) associated with livestock were collected and an attempt was made to identify the vector(s) involved in BTV transmission by means of virus detection in wild-caught biting midges. Weekly sampling using standardized Onderstepoort-type blacklight traps were performed simultaneously both outdoors and indoors in one BTV-free and three BTV-affected farms between September and December 2006.

*Culicoides* were sorted according to farm, location (outdoors vs. indoors), time point (in weeks), species and physiological stage. BTV detection was conducted by RT-PCR on monospecific pools of non-bloodfed parous female *Culicoides*.

The principal results showed: (i) the absence of the Mediterranean vector, *C. imicola*, (ii) the relatively low abundance of *C. dewulfi* and *C. pulicaris*, (iii) the widespread occurrence and abundance of *C. obsoletus/C. scoticus* with longevity and behaviour compatible with BTV transmission, and (iv) all *Culicoides* pools tested for BTV were negative.

In France, the very low levels of BTV-8 circulation were probably due to the limited introduction of the virus from affected neighbouring countries, and not due to the absence of local vector populations. A key finding has been the substantiation, for the first time, that *Culicoides*, and particularly the potential vectors...
C. obsoletus/C. scoticus and C. dewulfi, can be active at night inside livestock buildings and not only outside, as originally believed.

The endophagic tendencies of members of the Obsoletus group are discussed in light of the prolonged period of BTV transmission during the autumn of 2006 and the risk of BTV overwintering and resurgence in the spring of 2007. Overall, there is an urgent need to improve our knowledge on the ecology of local Culicoides species before any clear, effective and reliable recommendations can be provided to the veterinary authorities in terms of prevention and control.

# 2008 Elsevier B.V. All rights reserved.

**Keywords:** Culicoides; Obsoletus group; Pulicaris group; Bluetongue; France

1. Introduction

Bluetongue (BT) is an infectious arthropod-borne disease affecting ruminants, mainly sheep. Bluetongue virus (BTV), the causative agent of BT, is transmitted amongst vertebrate hosts by certain species of *Culicoides* biting midges (Diptera: Ceratopogonidae), which are true biological vectors (Mellor et al., 2000). Commencing in 1998 the outbreaks of BT in the Mediterranean Basin have been fuelled largely by the classical Afro-Asian vector *C. imicola* (Mellor and Wittmann, 2002). In August 2006, northern Europe faced a very unusual situation due to the emergence of BTV8 in sheep and cattle in the Netherlands, Belgium and Germany. Because of the risk of the rapid spread of this vector-borne disease, the French Ministry of Agriculture implemented a BT monitoring programme in northern France towards the end of August. In parallel with clinical and serological surveillance, a preliminary entomological survey revealed the main Mediterranean vector of BTV, i.e. *C. imicola* to be absent in the region (Baldet and Dele¨colle, 2006). On 31 August France reported an outbreak of BTV8 in the region of Ardennes close to the border with Belgium (and within the 150 km restriction zone that had been demarcated previously in response to the outbreaks in The Netherlands, Belgium and Germany). This case occurred in Brognon and involved a cow with weak clinical signs. On 2 September two additional cases were reported: a clinically unaffected cow at Beaurieux and another at Auberive. Both animals were detected within the framework of the BT serological surveillance programme based on exhaustive blood sampling of susceptible species and antibody detection by a commercial competitive ELISA kit (Biteau-Coroller et al., 2006). It is important to mention that for each of these three French outbreaks, all the remaining animals in the herds tested negative for BTV, both in terms of virus detection and seroconversion. On 5 September France reported a 4th case of BT at Andevanne: a male calf showing limited clinical signs. Three more cows from the same herd tested PCR-positive but were serologically negative and showed no clinical signs.

The unexpected appearance of BTV at a such high northern latitude, i.e. over 500 km further north than reported previously, raises an important question about the insect vector(s): if *C. imicola*, the main vector of BTV in the Mediterranean Basin, is absent in this area then what is the role played by local species of *Culicoides* in the emergence of BT in northern Europe?

Therefore, at the end of September, a longitudinal entomological survey was conducted both on infected and on BTV-free farms with the following objectives:

(i) to collect data on the spatial and temporal distribution of livestock-associated *Culicoides* with emphasis on suspected local vectors, i.e. Palaearctic species of the Obsoletus and Pulicaris groups as they have been implicated recently in outbreaks of BTV in southern
Europe such as in the Balkans (Mellor and Wittmann, 2002; Purse et al., 2006) and in Italy (Torina et al., 2004; De Liberato et al., 2005) and have been also successfully infected with BTV-9 in the laboratory in the UK using field populations (Carpenter et al., 2006) and (ii) to attempt to detect BTV in wild-caught specimens of both vector groups as achieved during the outbreaks of BTV in Italy (Savini et al., 2003; Caracappa et al., 2003; De Liberato et al., 2005; Savini et al., 2005).

The role of both the Obsoletus and Pulicaris species groups in the transmission of BTV is of real concern because they are common and widespread across the whole of central and northern Europe. Other local species, such as C. nubeculosus have also to be considered as they occur abundantly near livestock and because they feed preferentially on mammals and could transmit BTV during climatically warmer periods (Wittmann, 1999).

In the Mediterranean region of southern France, light traps to sample Culicoides in farms are operated only outdoors within 10 m of livestock (Baldet et al., 2004) because ruminants are usually stalled outside all year round with most livestock buildings constituting only a roof and lacking walls. Moreover, C. imicola, the main Mediterranean vector of BTV is mainly exophagic and exophilic (preferring to bite and to rest outdoors).

In northern France, comparative indoor/outdoor trapping commenced once night-time temperatures outdoors dropped sharply in the autumn resulting in some animals being maintained permanently inside well built cattle sheds till the following spring.

The principal entomological findings obtained during this longitudinal follow-up conducted in the BTV8-affected region of Northern France during the autumn of 2006 are presented here.

2. Materials and methods

2.1. Study site

From week 39 onwards (end of September) to week 50 (mid-December) weekly trappings were performed on 4 cattle farms in the Ardennes region. Three of the farms were BTV-infected: Brognon (49°56'20"N, 4°18'04"E), Aubervive (50°06'00"N, 4°46'08"E) and Andevanne (49°26'06"N, 5°04'53"E) while the last one Remonville (49°24'00"N, 5°19'48"E) was BTV-free. The first two farms are located in the forested hills of the northern Ardennes region; the remaining two occur in the zone of transition between the forested hills and the plains of the Paris Basin. As midge abundance and activity are related to the prevailing weather conditions, the maximum and minimum temperatures, total rainfall and wind speed were recorded using PC-link portable weather station at each site during trap operation to ensure that the weather conditions were appropriate for midge activity. Regional meteorological data recorded by nearby weather stations of the French National Weather Service from September to December 2006 were also obtained to provide information pertinent to longer term midge survival. Four national weather stations were located in the trapping areas.

2.2. Trapping protocol

Culicoides were captured using Onderstepoort-type blacklight traps equipped with 8W UV-light bulbs and downdraught suction motors (and powered by a 12 V car battery). Insects attracted by the light were sucked into a plastic collection beaker containing 300 ml of 70% ethanol. Mosquito netting (mesh size: 1–2 mm) was used to prevent all large insects from
entering the light trap. Specimens were preserved in 70% ethanol at 4 °C and were dispatched to the laboratory every two weeks maintaining the cold chain.

Traps were set one night per week under favourable climatic conditions (absence of heavy rain and/or wind) from approximately 1 h before sunset to 1 h after sunrise. Two traps per farm were hung at 1.5–2 m above ground level and were located both inside and outside buildings and within 10 m of livestock.

Farms were chosen according to a range of criteria: (i) located at least 5 km apart, (ii) with a minimum of ten cattle maintained both indoors and outdoors at night, (iii) livestock buildings well constructed with only one entrance for the animals, and (iv) no use of insecticides.

2.3. Culicoides identification

In the laboratory, all Ceratopogonidae (including Culicoides) were first separated from all other insects using a binocular microscope. Culicoides were identified based on wing pattern (Delecolle, 1985) with identifications confirmed subsequently, when necessary, by the mounting of specimens on microscope slides (Wirth and Marston, 1968).

During morphological identification all female Culicoides were pooled according to farm, location (outdoor or indoor), date, species and physiological stage (Dyce, 1969): (i) nulliparous, (ii) parous bloodfed, and (iii) parous non-bloodfed. It must be mentioned that the reliable identification of many species, in particular the more closely related ones such as sibling species inside group, is possible only by a small number of specialist taxonomists.

For some species, like C. obsoletus and C. scoticus both belonging to the Obsoletus group, females cannot be separated morphologically. Thus, these two undifferentiated species are recorded together and named C. obsoletus/C. scoticus.

2.4. BTV detection in Culicoides

RNA extraction was performed on monospecific pools of non-bloodfed parous female Culicoides using the RNeasy Tissue Kit (Qiagen, USA). The detection of BTV was attempted by RT-PCR based on the NTS1 gene using primers 11–31 and 284–265 (Katz et al., 1993). Parous females are the older members within a population of Culicoides (meaning they have sucked blood and laid eggs at least once previously) and thus are the ones most likely to harbour BTV in their salivary glands. Only BTV-positive non-bloodfed parous specimens indicate the replication of virus in the insect that may be transmitted subsequently to a ruminant host. Indeed, only the presence of multifeeding parous females will represent a high risk for BTV transmission.

3. Results

3.1. Relative abundance and mean catch of Culicoides species

A total of 92 light trap collections were made during 12 consecutive weeks at all 4 farms. Culicoides captured added up to 5335 Culicoides belonging to nine species (Table 1). Two species of the Obsoletus group, namely C. obsoletus/C. scoticus, comprised more than 90% of all individuals captured both outdoors (90.8%) and indoors (94.9%). The predominance of C. obsoletus/C. scoticus is constant in each studied farms (Table 2). Outdoors, four additional species of importance were C. dewulfi (3.2%), C. pulicaris (2.3%), C. chiopterus (1.8%) and C. punctatus (1.3%). The remaining four species (C. newsteadi, C. lupicaris, C. duddingstoni and
**Table 1**
Comparative outdoor and indoor *Culicoides* species abundances, parous rates, bloodfed rates and sex ratios on four farms (three infected and one BTV-free) in the Ardennes region (Northern France) during autumn 2006 based on 92 light trap collections (46 outdoors and 46 indoors) made over 12 consecutive weeks (W39 to W50).

<table>
<thead>
<tr>
<th>Rank</th>
<th>Species</th>
<th>Total</th>
<th>Mean catch(^a)</th>
<th>% total</th>
<th>Maximum catch</th>
<th>% P(^b)</th>
<th>% B(^c)</th>
<th>Sex ratio(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>C. obsoletus/C. scoticus</em>(^*)</td>
<td>963</td>
<td>20.9</td>
<td>90.8</td>
<td>286</td>
<td>58.7</td>
<td>0.42</td>
<td>0.02</td>
</tr>
<tr>
<td>2</td>
<td><em>C. dewulfi</em>(^*)</td>
<td>34</td>
<td>0.7</td>
<td>3.2</td>
<td>28</td>
<td>44.1</td>
<td>2.94</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td><em>C. pulicaris</em>(^**)</td>
<td>24</td>
<td>0.5</td>
<td>2.3</td>
<td>18</td>
<td>83.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td><em>C. chiopterus</em>(^*)</td>
<td>19</td>
<td>0.4</td>
<td>1.8</td>
<td>5</td>
<td>94.4</td>
<td>0</td>
<td>0.06</td>
</tr>
<tr>
<td>5</td>
<td><em>C. punctatus</em>(^**)</td>
<td>14</td>
<td>0.3</td>
<td>1.3</td>
<td>4</td>
<td>71.4</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td><em>C. newsteadi</em>(^*)</td>
<td>2</td>
<td>0.0</td>
<td>0.2</td>
<td>1</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>7</td>
<td><em>C. lupicaris</em>(^**)</td>
<td>2</td>
<td>0.0</td>
<td>0.2</td>
<td>2</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>8</td>
<td><em>C. duddingstoni</em></td>
<td>2</td>
<td>0.0</td>
<td>0.2</td>
<td>1</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>9</td>
<td><em>C. festivipennis</em></td>
<td>1</td>
<td>0.02</td>
<td>0.1</td>
<td>1</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td></td>
<td>Total outdoor</td>
<td>1061</td>
<td>23.1</td>
<td>100</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rank</th>
<th>Species</th>
<th>Total</th>
<th>Mean catch(^a)</th>
<th>% total</th>
<th>Maximum catch</th>
<th>% P(^b)</th>
<th>% B(^c)</th>
<th>Sex ratio(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>C. obsoletus/C. scoticus</em>(^*)</td>
<td>4057</td>
<td>88.2</td>
<td>94.9</td>
<td>881</td>
<td>55.1</td>
<td>4.4</td>
<td>0.08</td>
</tr>
<tr>
<td>2</td>
<td><em>C. chiopterus</em>(^*)</td>
<td>101</td>
<td>2.2</td>
<td>2.4</td>
<td>47</td>
<td>100</td>
<td>14.2</td>
<td>0.02</td>
</tr>
<tr>
<td>3</td>
<td><em>C. dewulfi</em>(^*)</td>
<td>93</td>
<td>2.0</td>
<td>2.2</td>
<td>68</td>
<td>57.6</td>
<td>13.1</td>
<td>0.01</td>
</tr>
<tr>
<td>4</td>
<td><em>C. pulicaris</em>(^**)</td>
<td>14</td>
<td>0.3</td>
<td>0.3</td>
<td>5</td>
<td>46.2</td>
<td>0</td>
<td>0.08</td>
</tr>
<tr>
<td>5</td>
<td><em>C. punctatus</em>(^**)</td>
<td>6</td>
<td>0.1</td>
<td>0.1</td>
<td>2</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>6</td>
<td><em>C. achrayi</em></td>
<td>3</td>
<td>0.1</td>
<td>0.1</td>
<td>2</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td></td>
<td>Total indoor</td>
<td>4274</td>
<td>92.9</td>
<td>100</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Nd: not determined (totalling < 10 specimens).

\(^*\) Obsoletus group.

\(^**\) Pulicaris group.

\(^a\) Mean *Culicoides*/trap/night.

\(^b\) Parous rate in % (total parous females/(total nulliparous females + total parous females)).

\(^c\) Bloodfed rate in % (total females bloodfed/total females caught).

\(^d\) Sex ratio (total males/total females).

*C. festivipennis* were captured at frequencies below 1% and constituted only 0.7% of all trappings made. *Culicoides obsoletus/C. scoticus, C. dewulfi* and *C. chiopterus* constitute those members of the Obsoletus group in the region, though the taxonomic status of *C. dewulfi* is currently under question. These species also belong to the subgenus *Avaritia* while *C. pulicaris, C. newsteadi, C. punctatus* and *C. lupicaris* belong to the subgenus *Culicoides* but are more commonly referred to as the *C. pulicaris* group (Meiswinkel et al., 2004).

Indoor collections also recorded *C. chiopterus* (2.2%) and *C. dewulfi* (2.0%) as still being present but *C. pulicaris* and *C. punctatus* were rare (both below 1%) while *C. newsteadi, C. lupicaris, C. duddingstoni* and *C. festivipennis* were absent. Three specimens of *C. achrayi*, absent in the outdoor collections, were caught indoors. The absence both indoors and outdoors was confirmed for *C. imicola, C. impunctatus* and *C. nubeculosus*.

During the autumn, the mean catches of *C. obsoletus/C. scoticus* recorded indoors was approximately four times greater than of the number captured outdoors and across a number of nights and under identical trapping conditions (paired *t*-test *t* = −2.69; *p* = 0.01). This ratio between indoor and outdoor trapping was reflected also in the maximum catch size: 881 specimens of *C. obsoletus/C. scoticus* indoors vs. 286 outdoors (Table 1). *Culicoides* can thus enter, and be active, inside livestock buildings in densities higher than those found outside. The indoor trapping rate (ITR) – defined as the total number of *Culicoides* caught indoors/total caught both indoors and outdoors × 100 – was 80.8 for the preponderant sibling species *C. obsoletus/
The ITR for *C. obsoletus*/*C. scoticus* were significantly different between the 4 studied farms: 87.0 in Brognon, 87.1 in Auberive, 69.0 in Andevanne and 59.7 in Remonville ($\chi^2 = 436.1; p < 0.0001$). Nevertheless, in each farms, the number of specimens found indoors was higher than outdoors independently of the total number of specimens caught per farm (Table 2). The global ITR was higher for *C. chiopterus* (ITR = 84.2) but lower for *C. dewulfi* (73.2). To the contrary, fewer specimens were trapped indoors than outdoors in the case of *C. pulicaris* (ITR = 36.8) and *C. punctatus* (ITR = 30.0).

The parous rates of the principal *Culicoides* species caught outdoors in autumn were significantly different: 58.7% for *C. obsoletus*/*C. scoticus*, 44.1% for *C. dewulfi*, 83.3% for *C. pulicaris* and 94.4% for *C. chiopterus* ($\chi^2 = 18.4; p < 0.001$).

More nulliparous females were caught indoors in the case of *C. obsoletus*/*C. scoticus* ($\chi^2 = 3.90; p = 0.048$) and *C. pulicaris* ($\chi^2 = 3.94; p = 0.041$). No differences were connected with the trap location for *C. chiopterus* and *C. dewulfi*.

For *C. obsoletus*/*C. scoticus*, bloodfed proportions ($\chi^2 = 33.93; p < 0.0001$) and sex ratio ($\chi^2 = 38.08; p < 0.0001$) were statistically higher inside the farm buildings. None of the *C. pulicaris* specimens caught both outdoors and indoors were freshly bloodfed.

### 3.2. Temporal variation of mean catch of Culicoides species

The weekly variations in the respective mean densities of *Culicoides* caught outdoors and indoors seemed to be related to external temperature (Fig. 1). *C. obsoletus*/*C. scoticus* was found

<table>
<thead>
<tr>
<th>Farms</th>
<th>Location</th>
<th><em>C. obsoletus</em>/<em>C. scoticus</em></th>
<th><em>C. chiopterus</em></th>
<th><em>C. dewulfi</em></th>
<th><em>C. pulicaris</em></th>
<th>Others</th>
<th>Mean total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brognon¹ BTV⁺</td>
<td>Outdoors</td>
<td>10.9</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>10.9</td>
</tr>
<tr>
<td></td>
<td>% total</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Indoors</td>
<td>65.1</td>
<td>0.6</td>
<td>6.5</td>
<td>0.6</td>
<td>0.3</td>
<td>73.2</td>
</tr>
<tr>
<td></td>
<td>% total</td>
<td>88.9</td>
<td>0.9</td>
<td>8.9</td>
<td>0.9</td>
<td>0.4</td>
<td>100</td>
</tr>
<tr>
<td>Auberive¹ BTV⁺</td>
<td>Outdoors</td>
<td>32.9</td>
<td>0.5</td>
<td>0.3</td>
<td>0.3</td>
<td>0.4</td>
<td>34.3</td>
</tr>
<tr>
<td></td>
<td>% total</td>
<td>96.0</td>
<td>1.3</td>
<td>0.8</td>
<td>0.8</td>
<td>1.1</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Indoors</td>
<td>221.7</td>
<td>7.3</td>
<td>1.7</td>
<td>0.4</td>
<td>0.3</td>
<td>231.4</td>
</tr>
<tr>
<td></td>
<td>% total</td>
<td>95.8</td>
<td>3.1</td>
<td>0.7</td>
<td>0.2</td>
<td>0.1</td>
<td>100</td>
</tr>
<tr>
<td>Andevanne² BTV⁺</td>
<td>Outdoors</td>
<td>8.3</td>
<td>0.6</td>
<td>0.3</td>
<td>0.3</td>
<td>0.8</td>
<td>10.1</td>
</tr>
<tr>
<td></td>
<td>% total</td>
<td>81.8</td>
<td>5.8</td>
<td>2.5</td>
<td>2.5</td>
<td>7.4</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Indoors</td>
<td>21.1</td>
<td>0.9</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
<td>22.4</td>
</tr>
<tr>
<td></td>
<td>% total</td>
<td>94.1</td>
<td>4.1</td>
<td>0.4</td>
<td>0.7</td>
<td>0.7</td>
<td>100</td>
</tr>
<tr>
<td>Remonville² BTV⁻</td>
<td>Outdoors</td>
<td>31.8</td>
<td>0.6</td>
<td>2.3</td>
<td>1.5</td>
<td>0.7</td>
<td>36.9</td>
</tr>
<tr>
<td></td>
<td>% total</td>
<td>86.2</td>
<td>1.6</td>
<td>6.3</td>
<td>4.1</td>
<td>1.8</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Indoors</td>
<td>54.1</td>
<td>0.3</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>54.6</td>
</tr>
<tr>
<td></td>
<td>% total</td>
<td>99.1</td>
<td>0.5</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>100</td>
</tr>
</tbody>
</table>

Results are given only for potential vector species—"Others" gather together 6 scarce and non-vector species (*C. punctatus***, *C. newsteadi***, *C. lupicaris***, *C. duddingstoni*, *C. festivipennis* and *C. achrayi*).

Environment: ¹Northern Forested Hills; ²Southern Transition zone.

BTV Status: + Infected; -Free.

* Obsoletus group.
** Pulicaris group.

*C. scoticus*. The ITR for *C. obsoletus*/*C. scoticus* were significantly different between the 4 studied farms: 87.0 in Brognon, 87.1 in Auberive, 69.0 in Andevanne and 59.7 in Remonville ($\chi^2 = 436.1; p < 0.0001$). Nevertheless, in each farms, the number of specimens found indoors was higher than outdoors independently of the total number of specimens caught per farm (Table 2). The global ITR was higher for *C. chiopterus* (ITR = 84.2) but lower for *C. dewulfi* (73.2). To the contrary, fewer specimens were trapped indoors than outdoors in the case of *C. pulicaris* (ITR = 36.8) and *C. punctatus* (ITR = 30.0).

The parous rates of the principal *Culicoides* species caught outdoors in autumn were significantly different: 58.7% for *C. obsoletus*/*C. scoticus*, 44.1% for *C. dewulfi*, 83.3% for *C. pulicaris* and 94.4% for *C. chiopterus* ($\chi^2 = 18.4; p < 0.001$).

More nulliparous females were caught indoors in the case of *C. obsoletus*/*C. scoticus* ($\chi^2 = 3.90; p = 0.048$) and *C. pulicaris* ($\chi^2 = 3.94; p = 0.041$). No differences were connected with the trap location for *C. chiopterus* and *C. dewulfi*.

For *C. obsoletus*/*C. scoticus*, bloodfed proportions ($\chi^2 = 33.93; p < 0.0001$) and sex ratio ($\chi^2 = 38.08; p < 0.0001$) were statistically higher inside the farm buildings. None of the *C. pulicaris* specimens caught both outdoors and indoors were freshly bloodfed.
to be predominant throughout autumn in September and in October and was virtually the only species recorded in November and December. The *Culicoides* mean densities outdoors was statistically correlated ($r = 0.82; p < 10^{-2}$) to daily external temperatures with no adult activity (based on <1 *Culicoides*/trap/night) when the maximum temperature fell below 10 °C and the minimum temperature below 5 °C such as occurred during weeks 44 and 50. Furthermore, *Culicoides* mean densities outdoors decreased markedly from the end of September (42.5 *Cul*/t/n in W39) to the beginning of October (5 *Cul*/t/n in W40) probably due to the decrease in night-time temperatures. Thereafter, the mean densities recorded outdoors in October remained relatively high (approximately 75 *Cul*/t/n from W41 to W43) only to decrease again in November (approximately 3 *Cul*/t/n from W44 to W47). This was due likely to the favourable temperature that prevailed in the region in October.

Importantly, it was found that even if outdoor *Culicoides* densities declined in response to the decrease in external temperatures, densities would increase once temperatures rose again (as shown in week 41 and to a lesser extent in W46 (Fig. 1)).

The indoor trapping rate seemed dependant on the outside temperature and not on the intrinsic behaviour of the insect (Fig. 1). The ITR increased when external temperatures decreased and vice versa. For example in W39, with a minimum temperature of >10 °C and a maximum temperature of >15 °C, the ITR was 35%. The following week (W40), with Tmin <10 °C and Tmax <15 °C, the ITR jumped to 98% meaning that only a few *Culicoides* were caught outdoors. In W41, when external temperatures increased (Tmin >10 °C and Tmax >15 °C) the ITR dropped back down to 41%. The same weekly variations in ITR determined by outdoor temperature were observed between weeks 39 and 43 at every studied farm. Nevertheless, the amplitude of these weekly variations changed from one farm to another.

![Weekly variation in outdoor and indoor *Culicoides* mean densities related to temperature in the Ardennes region (Northern France)—autumn 2006. Indoor trapping rate (ITR) as defined in the text is given for each week in italics.](image-url)
At the beginning of November (W44) the weather was cold with Tmin <5 °C and Tmax <10 °C; no *Culicoides* activity was observed outdoors but a few specimens were still caught indoors (mean density of 16.8 *Cult*/tn). One exception to this pattern was observed in mid-November (W46), when ITR increased along with the temperature. Even if this increment (from 77 to 92%) is not exceedingly high, future efforts have to be made to understand the role that temperature, setting and animal density may have on *Culicoides* endo/exophagic biting behaviour. At the end of November (W48) with seasonal temperatures abnormally high a mean of 54 *Cult*/tn was captured indoors whereas no *Culicoides* were captured outdoors. However, mean *Culicoides* densities at the end of November were still only half of those encountered in mid-November despite external temperatures having remained relatively constant.

**Fig. 2** presents the weekly variations in mean densities and parous rates for the predominant sibling species *C. obsoletus/C. scoticus*. In W42 and W43 (October) temperatures remained favourable for larval development and thus neonate specimens continued to emerge. As mean adult densities increased the parous rate declined indicating that fresh adult midges were being recruited into the existent population.

On the contrary, in W44 and W45 (beginning of November), cold temperatures induced a decline in adult activity and a reduction (or halt) in larval development resulting in lower mean adult densities and an increase in the parous rate (a sign of an ageing population). From then onwards, as the temperature increased between W45 and W46, *C. obsoletus/C. scoticus* mean densities peaked and the parous rate dropped, reaching a minimum of 5% at W47. It should be noted that the relatively low parous rate observed in W50 (25%), revealed that neonate adult females were able to emerge even in winter, once temperatures became mild again.

### 3.3. BTV detection in *Culicoides*

A total of 1778 non-engorged parous *Culicoides* females were captured and distributed amongst 97 pools (Table 3); 90% of the specimens belonged to *C. obsoletus/C. scoticus*. All of these pools were tested and found to be BTV negative by RT-PCR.

![Mean density (Cult/tn) vs Parous rate (%)](image)

**Fig. 2.** Weekly variation in *C. obsoletus/C. scoticus* mean density and parous rate in the Ardennes region (Northern France)—autumn 2006.
4. Discussion

4.1. Relative abundance of Culicoides found on livestock farms in autumn in northern France

Of the 5335 Culicoides caught in the study area, none were *C. imicola* (the Mediterranean vector of BTV); during the same period *C. imicola* was also not found in light trap collections made across The Netherlands, Belgium and Germany (Meiswinkel et al., this issue).

*C. impunctatus*, the “Scottish midge”, earlier suspected to be one of the potential vector in northern Europe because of its abundance, its mammophlic biting preferences and its oral susceptibility to BTV9 (Carpenter et al., 2006), was not captured either. This species is associated with wetland and peat bog habitats where it becomes abundant in spring and summer and helps explain its absence in the Ardennes in autumn.

*C. nubeculosus*, a potential BTV vector under climatically warm conditions (Wittmann, 1999), was also not captured.

However, the Palaearctic species considered to be the principal potential vectors of BTV in Europe, i.e. *C. obsoletus/C. scoticus* and, to a lesser extent *C. pulicaris*, were present on all the farms surveyed in the Ardennes. Indeed *C. obsoletus/C. scoticus*, both sibling species known to be abundant in deciduous forests of continental Europe, was found to comprise more than 90% of the Culicoides captured in autumn not only in northern France but also in The Netherlands (Takken et al., this issue; Meiswinkel, this issue) and in Belgium (De Deken et al., this issue). The relatively low abundances of *C. pulicaris* in northern France applied also to The Netherlands and to Belgium.

*C. dewulfi*, found RT-PCR-positive to BTV-8 in Gulpen, The Netherlands (Meiswinkel et al., 2007) was rare in northern France and comprised <3% of the total Culicoides captured both

---

**Table 3**

BTV detection by RT-PCR in taxon-specific pools of non-engorged parous Culicoides female midges, Ardennes region (Northern France)—autumn 2006

<table>
<thead>
<tr>
<th>Pools</th>
<th>Pools Total midges</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Outdoor</strong></td>
<td></td>
</tr>
<tr>
<td><em>C. obsoletus/C. scoticus</em></td>
<td>19</td>
</tr>
<tr>
<td><em>C. pulicaris</em></td>
<td>4</td>
</tr>
<tr>
<td><em>C. chiopterus</em></td>
<td>8</td>
</tr>
<tr>
<td><em>C. dewulfi</em></td>
<td>4</td>
</tr>
<tr>
<td><em>C. punctatus</em></td>
<td>5</td>
</tr>
<tr>
<td><em>C. newsteadi</em></td>
<td>2</td>
</tr>
<tr>
<td><em>C. lupicaris</em></td>
<td>1</td>
</tr>
<tr>
<td><em>C. festivipennis</em></td>
<td>1</td>
</tr>
<tr>
<td><em>C. diuddingstoni</em></td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
</tr>
<tr>
<td><strong>Indoor</strong></td>
<td></td>
</tr>
<tr>
<td><em>C. obsoletus/C. scoticus</em></td>
<td>25</td>
</tr>
<tr>
<td><em>C. chiopterus</em></td>
<td>12</td>
</tr>
<tr>
<td><em>C. dewulfi</em></td>
<td>6</td>
</tr>
<tr>
<td><em>C. pulicaris</em></td>
<td>5</td>
</tr>
<tr>
<td><em>C. punctatus</em></td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>52</td>
</tr>
</tbody>
</table>

* *C. obsoletus* group.
  **C. pulicaris* group.
indoors and outdoors, which is far lower than the overall 17% found in The Netherlands. In mid-September (W37), the mean density of *C. dewulfi* in northern France was 0.5/t/n out of a total mean of 168 *Cul/t/n* (Baldet and Delécolle, 2006); its mean density at Gulpen during the same period was 58/t/n (out of a total of 343 *Cul/t/n*). Trapping protocols (OVI trap), study period, weather conditions (temperature, rainfall) and diagnostic skills were comparable in both situations.

### 4.2. Seasonal distribution of Culicoides found near livestock in autumn in northern France

In northern France, it was observed during September 2006, and under favourable climatic conditions, that *Culicoides* activities increased in comparison to those encountered at the end of August (Baldet and Delécolle, 2006). The low temperatures and relatively heavy rains which characterised the climate in northern Europe in August 2006 likely had a negative impact on the populations of livestock-associated *Culicoides* inducing a decline in larval productivity (as flowing water will have disrupted breeding sites, decreased the egg hatching rate and slowed development in the larval stages) thereby suppressing also adult activity.

Subsequently, the exceptionally favourable climatic conditions that prevailed in northern Europe – particularly in October – will have facilitated a rebound of *Culicoides* activities, mainly those of *C. obsoletus/C. scoticus*. In Southern Europe, *C. obsoletus/C. scoticus* populations are known to peak in spring; decline abruptly thereafter, during summer; and re-attain a second, less important, peak towards the autumn season (Savini et al., 2005).

At the beginning of November, the dramatic decrease of temperature clearly induced a pause in the development and activity of *Culicoides* populations. From field data it has been estimated that the optimal temperatures for *C. obsoletus* range between 11 and 27.5 °C (Dzhafarov, 1964). Nielsen (1963) in Denmark captured *C. obsoletus* biting man just before sunset in June at temperatures ranging between 11 and 15 °C but included also a few individuals biting at 7.5 °C.

However, despite the rise in temperature, *Culicoides* activities did not rebound significantly after week 48 in late November due perhaps to the cumulative effect and duration of cold periods and the ever-shortening number of daylight hours. The reduction in photoperiod has a direct impact on the number of hot hours and so could reduce (or even halt) larval development thereby leading to complete cessation in adult activity.

Moreover, photoperiod – like temperature – influences the endocrine system, which controls insect growth and diapause (Lees, 1955). To halt larval development – or to induce diapause – both low day- and night-time temperatures, and a decrease in the length of the photoperiod, are required.

In northern France, the vector-free period was declared to commence in mid-December after the two previous weeks of light trapping had yielded <10 *Culicoides/trap/night* both outdoors and indoors (but with some residual *Culicoides* activity — notably indoors — on some farms) reflecting the cold advance of winter.

### 4.3. Indoor activity and overwintering Culicoides in northern France

In comparing *Culicoides* activity indoors and outdoors, the results must be interpreted with caution the buildings varied according to site and to the degree they could be considered as either ‘open’ or ‘closed’. Similarly, animal densities in the near vicinity of the trap varied, both in and outdoors depending on farm. Furthermore, factors other than cattle density such as moon phase, light intensity, temperature and wind speed would also have influenced the activity and numbers
of *Culicoides* caught, in particular outdoors, as has been observed for other vectors including *C. brevitarsis* (Bishop et al., 2000; Murray, 1987), *C. imicola* (Walker, 1977) and *C. impunctatus* (Blackwell, 1997). In this study these variables were controlled as far as practicably possible by avoiding sampling during the full phase of the moon and by not operating traps in the field when rain or wind was expected because adult midge activity is almost entirely suppressed at wind speeds greater than 2.2 m/s (Murray, 1987; Walker, 1977). Nevertheless, overall *Culicoides* species diversity was found to be lower indoors (six species) than outdoors (nine species). It is falsely assumed that *Culicoides* are purely exophagic and exophilic, i.e. that they feed only on animals maintained outdoors and that they will not enter and/or rest inside buildings.

This false assumption is mainly due to the fact that most detailed studies on *Culicoides* of veterinary interest, have been performed in tropical areas or in the Mediterranean, on exophagic species like *C. imicola*, or in a different context (i.e. animals resting outdoors all year round or in buildings partially open). Moreover, previous studies done in temperate regions such as Denmark (Nielsen and Christensen, 1975) and Canada (Anderson, 1993) had already underlined that certain *Culicoides* species, like *C. obsoletus*, could be active and feed indoors.

In northern France, it was observed in autumn that adult *Culicoides* activity, mainly in *C. obsoletus/C. scoticus*, was higher indoors than outdoors and included a high percentage of parous females with a relatively high proportion of them freshly bloodfed. *C. dewulfi* and *C. chiopterus* seemed to be also strongly endophagic whereas *C. pulicaris* and *C. punctatus* (of the Pulicaris group) appeared more exophagic. The endophagic tendencies of the Obsoletus group seemed to be influenced primarily by external temperatures and secondarily by animal husbandry practices (such as the stalling of animals indoors). In South Africa thousands of *C. bolitinos* are known to enter stables but it remains to be demonstrated whether the housing of all farm animals ‘forces’ even exophagic/exophilic species to enter cattle sheds to suck blood (Meiswinkel et al., 2000). In any case, the endo/exophagic biting behaviour of the main European *Culicoides* species of veterinary interest have been observed only at the four farms studied in Northern France and during a particular period, the autumn. It is thus imperative to explore more deeply the indoor biting activities of *Culicoides* all year round and to link the findings more conclusively to climatic factors (temperature and rainfall) and/or to husbandry practices (presence and density of animals and kind of setting—size of the building and size of the opening).

Moreover, the sporadic emergence of neonate females during the winter of 2006 (owing to the mild climatic conditions) and the persistence of adult *Culicoides* activity indoors have raised the question as to whether this may contribute to the maintenance of BTV through the winter (Losson et al., 2007). If so fresh outbreaks should be expected in summer 2007, once adult vector populations begin to increase in spring.

4.4. Potential vector of BTV8 in northern France

The implication of *C. dewulfi* as a potential vector of BTV-8 in The Netherlands (Meiswinkel et al., 2007) does not exclude other *Culicoides* from having been involved in the northern European outbreaks of BT. It would be prudent to consider *C. obsoletus/C. scoticus* to have also played a vectorial role not only because they have been implicated in southern Europe, firstly in Cyprus (Mellor and Pitzolis, 1979) and lately in Italy (De Liberato et al., 2005) and in the Balkans (Purse et al., 2006), but also because they were consistently found to be widespread and abundant on farms throughout the BT-affected area of northern Europe, particularly in the autumn. A high survival rate, multiple feeding events and a capacity to bite livestock indoors, will have further increased the vector potential of *C. obsoletus/C. scoticus*. Indeed, it is highly likely that evidence
for its vectorial involvement would have been obtained if more intensive investigations had been conducted in areas more heavily infected with BTV.

5. Conclusion

The emergence of BTV-8 in northern Europe is a significant development in the epidemiology of the disease as this serotype has heretofore never been encountered within the confines of the European Union (EU). In the affected Ardennes region of northern France, the entomological study conducted in autumn 2006 revealed:

- The absence of the principal Mediterranean vector, *C. imicola*, and has been confirmed by similar studies conducted simultaneously in affected neighbouring countries.
- The low abundance levels of both *C. dewulfi* (implicated as a potential vector in The Netherlands) and of *C. pulicaris* (implicated as a BTV vector in Italy).
- The widespread occurrence and heightened abundances of *C. obsoletus*/*C. scoticus* whose survival rate and endophagic behaviour are compatible with BTV replication and transmission.

Most importantly, the investigations in the Ardennes have made it possible to confirm that *Culicoides*, and in particular the potential vectors *C. obsoletus*/*C. scoticus* and *C. dewulfi*, are active at night inside livestock buildings but appears to be dependent on outside temperatures and on husbandry practices. To some extent this undermines, firstly, the recommendation of the competent authorities that livestock be housed at night to reduce *Culicoides* attack rates and, secondly, the elevated numbers of *Culicoides* found indoors compels us to review the definition of “vector-free period” according to the OIE, which at present is deemed to commence once “<10 *Culicoides* are found in a light trap suspended outdoors for one night”.

Research on the ecology of *Culicoides* breeding sites, adult dispersal (both local and regional), seasonal dynamics, biting activity and adult overwintering are required to facilitate the development of more effective control measures.

Conflict of interest statement

None of the authors (Thierry Baldet, Jean-Claude Delécolle, Catherine Cêtre-Sossah, Bruno Mathieu, Rudolf Meiswinkel and Guillaume Gerbier) has a financial or personal relationship with other people or organisations that could inappropriately influence or bias this paper.

Acknowledgements

This study was funded by the DGAL/French Ministry of Agriculture and by the European Food Safety Authority (EFSA), contracts CT/EFSA/SCAD/2006/01 and conducted by CIRAD-EMVT in collaboration with the University Louis Pasteur de Strasbourg. We would like to thank the owners of the farms and the local Veterinary Services for their support in the field, in particular A. Bronner, B. Thomas and L. Lamoureux (DDSV 08).

References


