



Schmallenberg virus RNA detected in *Culicoides* biting midges in the Netherlands in 2011[#]

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Background

Commencing in early summer of 2011, a novel Orthobunyavirus of the Simbu serogroup, namely Schmallenberg virus (SBV), spread silently across much of northern Europe, infecting ruminant livestock. Orthobunyaviruses are known for their teratologic effects (malformed new-borns) in ruminants and are arthropod-borne, the majority having been isolated from mosquitoes and *Culicoides* biting midges.

Objective

Preliminary reports have indicated one or more species of *Culicoides* to possibly act as field vectors for SBV in Europe. Our objective was to identify potential vectors for SBV. *Culicoides* collected in the autumn of 2011 in the Netherlands were tested retrospectively by RT-PCR.

Material and Methods

- Culicoides* were caught between Aug. – Oct. 2011 at a dairy herd and at two sheep flocks.
- A total of 610 pools of heads (10 *Culicoides* /pool) from 6,100 female *Culicoides* were analysed by RT-PCR.
- The number of pools assayed for each species was as follows: Obsolete Complex (230), *C. chiopterus* (144), *C. dewulfi* (130), *C. punctatus* (105), and *C. pulicaris* (1).



Figure 1. *Culicoides* midges were caught using a drop tent (sheep), OVI light trap and sweep-netting (cattle).

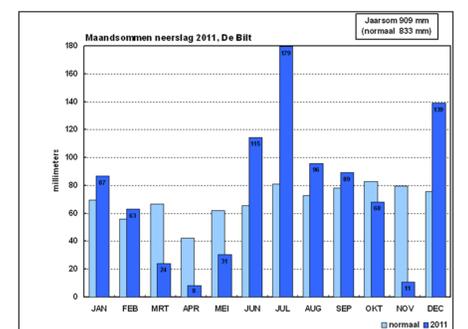
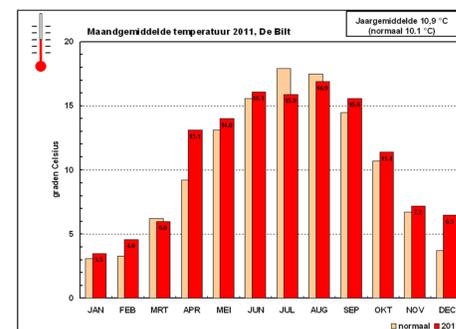
Results

- Fourteen (2.3%) of the 610 *Culicoides* pools (heads) tested SBV-positive by RT-PCR: eleven *C. scoticus*, one *C. obsoletus sensu stricto* and two *C. chiopterus*.
- Most PCR C_t -values < 30; C_t -values found in the heads of midges matched closely those obtained from their associated abdomens which renders it certain that **SBV had replicated to transmissible levels**.
- Almost all individual *Culicoides* body could be identified molecularly to species: ***C. scoticus* played a more prominent role than *C. obsoletus ss*** in the transmission of SBV in the Netherlands.
- SBV-prevalence in the two species that comprised the Obsolete Complex was **0.56%**. This is similar to the prevalence obtained for Akabane virus in *C. brevitarsis* from Australia in the 1970s, but about **ten times** higher than that reported for Bluetongue virus (BTV) in NW Europe. For *C. chiopterus* our results were **0.14%**, which is **five times** higher than reported for BTV in NW Europe.

Results

Table 1. PCR C_t -values of Schmallenberg virus positive *Culicoides* pools (heads) and individual *Culicoides* abdomen, and species identification by DNA sequencing.

Municipality (livestock species)	Pool (heads) identification	Morphological identification of <i>Culicoides</i> spp.	PCR C_t -value pools (heads)		<i>Culicoides</i> abdomen identification	C_t -values individual <i>Culicoides</i> abdomens				Species identification by DNA sequencing
			single	duplo		Body 1		Body 2		
						single	duplo	single	duplo	
Midden-Drenthe (sheep)	95-D	<i>C. chiopterus</i>	27.88		95-D	24.59				<i>C. chiopterus</i>
Ermelo (cattle)	501	<i>C. chiopterus</i>	35.36	34.98	501	36.45	35.07			<i>C. chiopterus</i>
Ermelo (cattle)	9-A	<i>C. obsoletus</i> complex	30.44		9-A	24.75				<i>C. scoticus</i>
Ermelo (cattle)	18-C	<i>C. obsoletus</i> complex	28.24		18-C	24.95				<i>C. scoticus</i>
Ermelo (cattle)	32-B	<i>C. obsoletus</i> complex	21.84		32-B	18.32				<i>C. scoticus</i>
Ermelo (cattle)	259	<i>C. obsoletus</i> complex	19.60		259	18.16				<i>C. scoticus</i>
Ermelo (cattle)	275	<i>C. obsoletus</i> complex	20.72		275	20.39				<i>C. scoticus</i>
Ermelo (cattle)	276	<i>C. obsoletus</i> complex	36.02	36.78	276	36.68				<i>C. scoticus</i>
Ermelo (cattle)	293	<i>C. obsoletus</i> complex	20.43		293	19.95				No reliable sequence
Ermelo (cattle)	294	<i>C. obsoletus</i> complex	24.60		294	20.06				<i>C. obsoletus sensu stricto</i>
Ermelo (cattle)	368	<i>C. obsoletus</i> complex	25.21		368	21.80				<i>C. scoticus</i>
Ermelo (cattle)	385	<i>C. obsoletus</i> complex	20.67		385	20.25				<i>C. scoticus</i>
Ermelo (cattle)	405	<i>C. obsoletus</i> complex	23.38		405	21.64				<i>C. scoticus</i>
Ermelo (cattle)	434	<i>C. obsoletus</i> complex	23.68		434-1 and 434-2	23.10		35.75	35.37	<i>C. scoticus</i>



Left figure: average monthly temperature (bright red bar) in 2011 in the Netherlands (light red bar is normal monthly average temp. based on last 30 years); Right figure: average monthly precipitation (bright blue bar) in 2011 in the Netherlands (light blue bar is normal monthly precipitation based on last 30 years)

Discussion

The high proportion of SBV-infected *Culicoides* midges is a result of the interplay between host, vector and environment. Lack of transport restrictions in 2011 might have spread infection by infected hosts over long distances and may have started several foci of local transmission. SBV might replicate more efficiently in *Culicoides* midges or specific *Culicoides* species compared to other viruses. This has to be determined by experimental vector competence studies, which is currently under investigation at Pirbright (UK) and Wageningen University (NL).

Vector biology might have been positively influenced by climatological circumstances in 2011 with a **prolonged vector season** (several weeks, due to high temperatures) and a **higher survival rate** and **increased vector abundance** (dry Spring and wet Summer and higher than normal temperatures in Autumn).

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