Schmallenberg virus monitoring in *Culicoides* trapped during 2011 and 2012 in Belgium.

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Introduction

In November 2011, a new virus was identified by researchers from the Friedrich Loeffler Institute (FLI, Germany) that had caused milk drop, diarrhea and fever in adult cattle during the summer of 2011 in Germany and the Netherlands (Hoffmann et al, 2012) and later was shown to be involved in congenital malformations in lambs, calves and goat kids. The virus was named Schmallenberg virus (SBV) and belongs to the Simbu serogroup of Orthobunyaviruses. Since its emergence the virus has rapidly spread all over Europe. The rapid and wide expansion of SBV, together with the knowledge that related viruses like Akabane and Aino virus are spread by midges and mosquitoes, led to the hypothesis that also SBV might be spread by these vectors. To examine the potential role of midges in the rapid expansion of SBV in Belgium in 2011 and evaluate a possible recirculation of SBV in midges in 2012 in the context of a high seroprevalence in the animal host population, midges caught in Belgium in 2011 and 2012 were screened for the presence of SBV RNA by realtime RT-PCR (rRT-PCR).

Material & Methods

Trapping, identification and pooling of *Culicoides:*

Culicoides were trapped with OVI traps at 4 different regions in Belgium (Figure 1) from July till November in 2011 and from May till November in 2012. All traps were situated in the neighborhood of sheep and cattle farms. The biting midges were morphologically identified at species level under the microscope using the key of Delécolle. For 2011, pools of maximum 25 heads of parous females without signs of a recent blood meal and identified at species level were prepared. For 2012, pools of maximum 20 whole parous female midges identified at group level (*Avaritia*, *Culicoides*, *Monoculicoides*, others) were prepared, except for Liège where species specific pools were made.

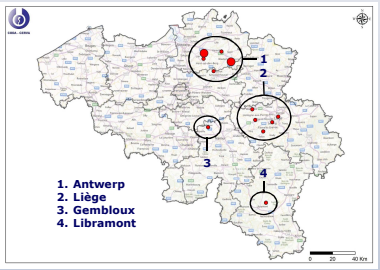

Figure 1: *Culicoides* trapping locations in Belgium during the monitoring of 2011 and 2012.

rRT-PCR analysis of pools of *Culicoides:*

Each pool was homogenized in 500 µl Trizol with a 5mm steel bead by high speed shaking (3 min, 25 Hz) in a TissueLyser. After phase separation following manufacturer instructions, total RNA in the aqueous phase was extracted using the MagMAX Total Nucleic Acid Isolation kit and the MagMAX Express-24 purification system. RNA was eluted in 90 µl elution buffer. The presence of SBV RNA was analyzed by using the AgPath-ID One Step RT-PCR kit following manufacturer's instructions in a duplex rRT-PCR for detection of the SBV-S segment and the 18S rRNA from Culicoides as an internal control for RNA extraction and amplification. Pools positive for the SBV-S segment were subjected to another rRT-PCR detecting the L segment of the virus using the same one step RT-PCR kit for confirmation. Only pools that were positive for the S and L segment were considered as SBV positive.

Results

SBV screening in *Culicoides* from 2011

In total 480 pools representing 7305 midges were tested (Figure 2). The first pool positive for both the S and L segment of SBV was caught in the region of Liège at August 23th and consisted of *C. dewulfi*. At Liège, two more S and L segment positive pools were found in September, both consisting of *C. obsoletus* complex midges. This number increased strongly in October with 17 out of 48 pools found S and L positive (15 pools of *C. obsoletus* complex, 1 *C. dewulfi* and 1 *C. chiopterus*). If it is assumed that each positive pool contained one SBV positive midge, this results in an infection rate of 2,4% in *C. obsoletus* complex midges at Liège in October. In the region of Antwerp, only one pool was found positive for the SBV S and L segment which consisted of *C. obsoletus* midges and was caught in September. In Gembloux the first and only S and L segment positive pool was caught on October 11th, but in contrast to all other positive pools, this pool consisted of heads from *C. obsoletus* complex midges that had blood and eggs in their abdomen. In the south of Belgium, at Libramont, no positive pools were found, indicating a lower circulation of SBV at that location. This is in line with the finding that a relative lower between herd seroprevalence was observed at the end of 2011 in the south of Belgium. The Ct values for the rRT-PCR detecting the S segment varied between 26.6 and 38.5 for the positive pools. Most of the pools with a Ct value for the S segment > 35 were not confirmed by the rRT-PCR detecting the L segment. One pool consisting of *C. pulicaris* was found positive for the S segment (Ct=37.9), but was not confirmed in the L segment.

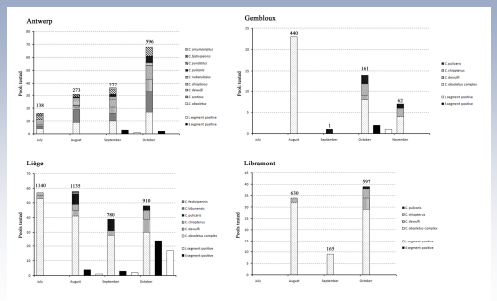

Figure 2: Overview of pools of *Culicoides* examined for the presence of Schmallenberg virus originating from four trapping regions in Belgium in 2011.

SBV screening in *Culicoides* from 2012

In total 904 pools representing 17461 midges were tested (Figure 3). The results show that in all 4 regions where *Culicoides* were sampled, SBV positive pools were found at the beginning of August 2012, confirming the presence of SBV at that time. At Libramont in the south of Belgium, the observed number of positive pools in 2012 was unexpectedly high with 57% (20/35) of *Avaritia* pools and 60 % (3/5) of *Culicoides* pools being rRT-PCR positive. If it is considered that only 1 midge per pool was SBV positive, this corresponds to an infection rate in August 2012 at Libramont of 2.86 and 3.26% in the subgenera *Avaritia* and *Culicoides* respectively. This is as high as the infection rate found in October 2011 at Liège. The most probable explanation for the high infection rate in midges in the south of Belgium is the presence of hosts that had not been infected during the SBV epidemic in 2011. This hypothesis is supported by the reported relatively lower seroprevalence in sheep and cattle in the south of Belgium at the end of 2011 and the presence of a partially seronegative population of wild cervids in southern Belgium at that time. The infection rates in August in the other regions were however clearly lower (0.4, 0.3, and 0.2% in *Avaritia* in Antwerp, Liège and Gembloux respectively and 0.4% in *Culicoides* in Gembloux). The Ct values for the rRT-PCR detecting the S segment in positive *Avaritia* pools varied between 19.9 and 35,8. In the 4 positive pools of subgenus *Culicoides* midges, the obtained Ct values were clearly higher, between 30.9 and 34.2, making it uncertain if these species could be competent vectors for SBV.

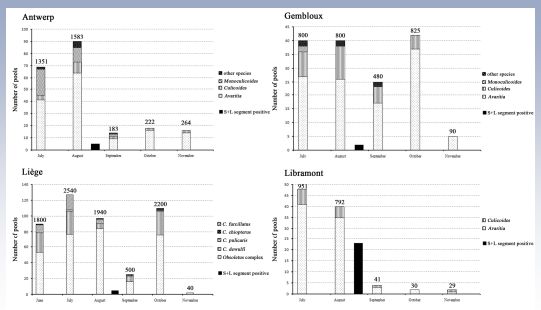

Figure 3: Overview of pools of *Culicoides* examined for the presence of Schmallenberg virus originating from four trapping regions in Belgium in 2012.

Conclusion

This study showed that Schmallenberg virus was first detected in *Culicoides* biting midges in Belgium in August 2011, coinciding with the period that regional Animal Health Care centers started receiving notifications of problems such as milk drop and diarrhea on cattle farms. Based on the detection of SBV RNA in heads of *C. obsoletus* complex, *C. dewulfi* and *C. chiopterus* midges, these species are proposed as putative vector species for this newly emerged virus. In 2012, a renewed but short-lived circulation of Schmallenberg virus in midges was observed despite the high seroprevalence rates that were present in the animal host population.

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