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Occurrence of Schmallenberg virus in Danish biting midges (Culicoides spp.)

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In autumn 2011, an unidentified disease of livestock was reported on both sides of the Dutch-German border. Using Metagenomics the etiological agent of this disease was identified as a novel orthobunyavirus and named Schmallenberg virus (SBV) (1). Other members of this virus genus (e.g. Akabane virus) are widespread in Africa and Asia; both biting midges (Culicoides spp.) and mosquitoes are responsible for transmission of these viruses. Hence it was a reasonable assumption that European Culicoides species may be responsible for the transmission of SBV within Europe.

The first find of SBV in Danish Culicoides were caught in October 2011 close (6 km) to the German border, and less than 10 km to an SBV-infected sheep farm in Germany (2) (on the map). After this initial find a thorough investigation of midge pools were initiated. Pools of midges caught in 2011 at sites scattered across the entire country (represented by on the map) were tested for the presence of SBV and Bluetongue virus (BTV). In 2012 investigations were focused on just 4 different areas (I, II, III, IV) where midges were collected on a weekly basis from the end of July until the middle of September. After a preliminary “rough and dirty” investigation, the midges were divided into Obsoletus group, Pulicaris group and others. Furthermore, were the heads and abdomen of the midges in the Obsoletus group tested separately.

Results 2011

After the initial finding of two SBV positive pools close to the German border, all other midge pools (approx. 250) tested negative for both SBV and BTB.

Results 2012

“Up close and personal”

In 2012 collection of Culicoides was focused on four areas. Midge were collected on a weekly basis from July 30th to September 1st and divided into the Obsoletus group, the Pulicaris group and "others". Furthermore, were the heads and abdomen of the midges in the Obsoletus group tested separately in pools of 10. The samples were homogenized using A Tissue Lyser II and RNA was purified by Magna Pure robot. RT-qPCR was performed using both S1 and L1 primers and probes. Only pools positive in both were considered positive for SBV. The mean Ct values in the tables are from the S3 PCR.

Discussion and Conclusions

In 2011, Culicoides containing Schmallenberg virus (SBV) was only found at the most southern location, of 44 catch sites located across the entire country, only 10 km from an infected German farm. In 2012 infected biting midges were found on all of the four catch sites which were all located in large distances from the infected site from 2011, and representing most of Denmark. In other words, the virus has managed to spread to midges in the entire country in just under a year and that without extensive reports of outbreaks. Only a single lamb was found infected with SBV.

The Obsoletus group were the most frequent caught and is also responsible for 36 of the 41 positive pools. In total 17% of the midges in the Obsoletus group tested positive for SBV. The mean Ct values in the tables are from the S3 PCR.

For Culicoides belonging to the Obsoletus group, heads and abdomen were tested separately for SBV (10/pool). The positive pools were subject to species specific multiplex PCR, detecting C. obsoletus, C. dewulfi, C. chiopterus and C. scoticus (13).

In 2011, Culicoides containing Schmallenberg virus (SBV) was only found at the most southern location, of 44 catch sites located across the entire country, only 10 km from an infected German farm. In 2012 infected biting midges were found on all of the four catch sites which were all located in large distances from the infected site from 2011, and representing most of Denmark. In other words, the virus has managed to spread to midges in the entire country in just under a year and that without extensive reports of outbreaks. Only a single lamb was found infected with SBV.

The Obsoletus group were the most frequent caught and is also responsible for 36 of the 41 positive pools. In total 17% of the Obsoletus group pools were positive for SBV. Only C. obsoletus and C. dewulfi were found in the positive pools. Both were found as the only species in the pool and in a mixture. This was the case for both head and abdomen and without the presents of ruminant blood which indicates that both species can act as vector for SBV.

It is still a puzzle how SBV manages to infect so many animals in so little time but these results more than indicate that Culicoides play a crucial role.