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

Lasse Dam Rasmussen¹, Carsten Kirkeby², Birgit Kristensen², Thomas Bruun Rasmussen¹, Graham J. Belsham¹, René Bødker², Anette Bøtner¹

¹DTU National Veterinary Institute, Lindholm, DK-4771 Kalvehave, Denmark ²DTU National Veterinary Institute, DK-1870 Copenhagen, Denmark

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 presents 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 Obsoleteus group, Pulicaris group and others. Furthermore, were the heads and abdomen of the midges in the Obsoleteus 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 BTV

Results 2012

“Rough and dirty”

“Up close and personal”

One pool pr. Sampling area pr. date containing 50 unsorted *Culicoides* spp.

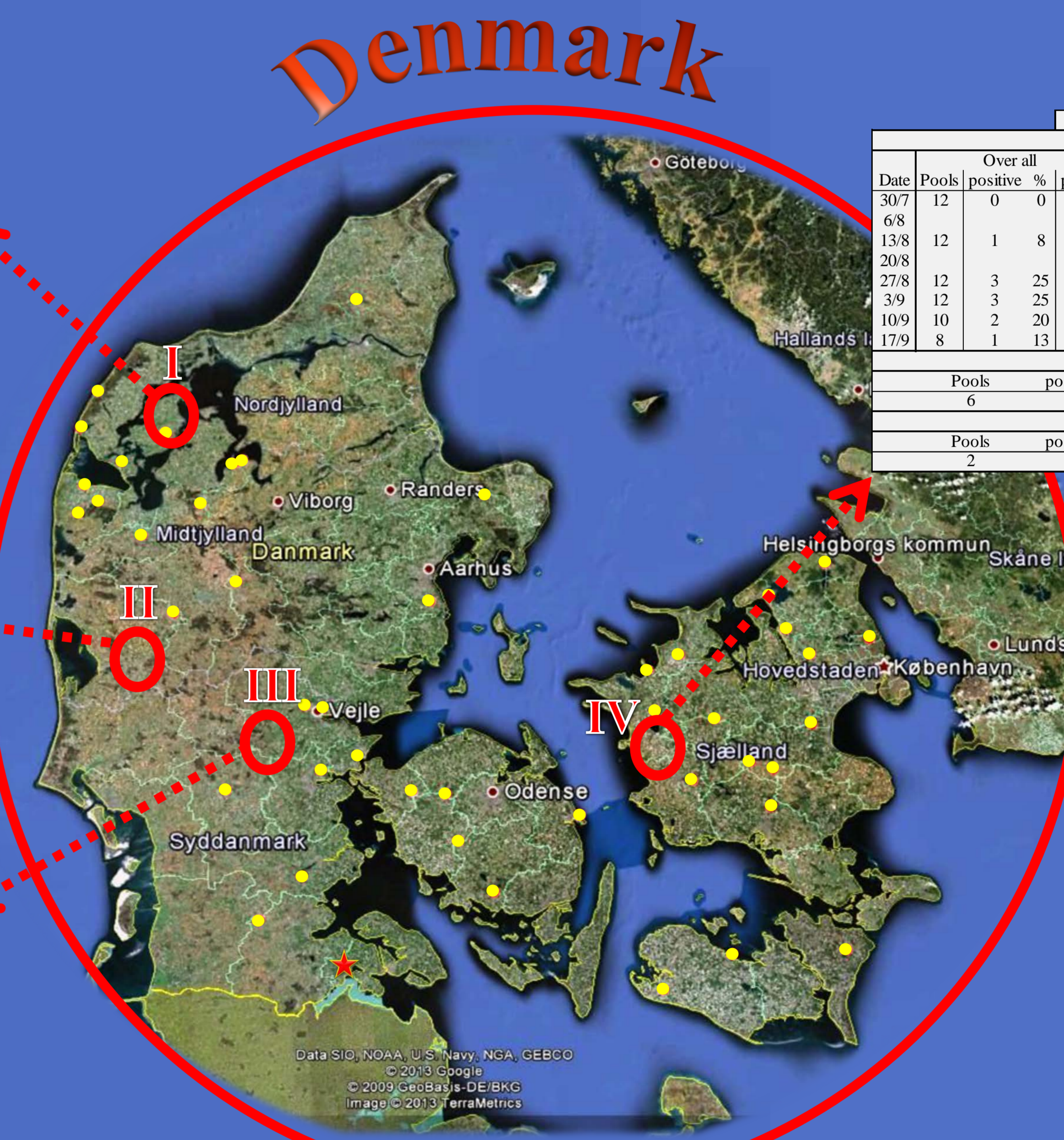
Sampling area	Collection date							
	30/7	6/8	13/8	20/8	27/8	3/9	10/9	17/9
I	na	negative	negative	positive	negative	negative	negative	positive
II	na	na	negative	negative	positive	na	na	na
III	na	negative	negative	negative	positive	positive	negative	positive
IV	negative	na	positive	na	positive	negative	positive	negative

In 2012 collection of *Culicoides* was focused on four areas. Midges were collected on a weekly basis from July 30th to September 17th and divided into the Obsoleteus group, the Pulicaris group and “others”. Furthermore, were the heads and abdomen of the midges in the Obsoleteus group tested separately in pools of 10. The samples were homogenized using A Tissue Lysar II and RNA was purified by MagNa Pure robot. RT-qPCR was performed using both S3 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.

II Obsoleteus group							
Date	Pools	Over all		Head		Abdomen	
		positive	%	positive	% mean Ct	positive	% mean Ct
30/7							
6/8							
13/8	3	0	0	0	0	0	0
20/8	11	0	0	0	0	0	0
27/8							
3/9							
10/9							
17/9							
Pulicaris group							
Pools		positive	frequency %	Mean Ct			
7		0	0				
unidentified							
Pools		positive	frequency %	Mean Ct			
6		2	33	29,2			

I Obsoleteus group							
Date	Pools	Over all		Head		Abdomen	
		positive	%	positive	% mean Ct	positive	% mean Ct
30/7							
6/8	12	0	0	0	0	0	0
13/8	12						
20/8	12	1	8	0	0	1	8
27/8	7	0	0	0	0	0	0
3/9	10	0	0	0	0	0	0
10/9							
17/9	8	2	25	0	0	2	25
Pulicaris group							
Pools		positive	frequency %	Mean Ct			
7		0	0				
unidentified							
Pools		positive	frequency %	Mean Ct			
6		0	0				

IV Obsoleteus group							
Date	Pools	Over all		Head		Abdomen	
		positive	%	positive	% mean Ct	positive	% mean Ct
30/7	12	0	0	0	0	0	0
6/8	12	1	8	0	0	1	8
13/8							
20/8	12	3	25	0	0	3	25
27/8	12	3	25	1	8	3	25
3/9	10	2	20	2	20	2	20
10/9	8	1	13	0	0	1	13
17/9							
Pulicaris group							
Pools		positive	frequency %	Mean Ct			
6		0	0				
unidentified							
Pools		positive	frequency %	Mean Ct			
2		0	0				



“Heads” or “Tails”

For *Culicoides* belonging to the Obsoleteus 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* (3).

Heads				Abdomen									
Date	Pools	blood		Species specific PCR		Date	Pools	blood		Species specific PCR			
		positive	%	<i>C. obsoletus</i>	<i>C. dewulfi</i>			both	positive	%	<i>C. obsoletus</i>	<i>C. dewulfi</i>	both
30/7	12	0				30/7	12	0					
6/8	24	2	100	Two unidentified pools		6/8	24	3	100	two unidentified			
13/8	27	0				13/8	27	1	0				
20/8	35	0				20/8	35	1	100				
27/8	31	3	33			27/8	31	9	56		3		
3/9	34	2	0			3/9	34	8	38		1		
10/9	22	4	0			10/9	22	6	17		1		
17/9	28	0				17/9	28	7	29		4		
Overall	213	11	27	4	3	2	Overall	213	35	43	17	6	10

Denmark 2012 Obsoleteus group							
Date	Pools	Over all		Head		Abdomen	
		positive	%	positive	% mean Ct	positive	% mean Ct
30/7	12	0	0	0	0	0	0
6/8	24	4	17	2	8	34,6	0
13/8	27	1	4	0	0	1	4
20/8	35	1	3	0	0	1	3
27/8	31	9	29	3	10	30,3	9
3/9	34	8	24	2	6	26,9	8
10/9	22	6	27	4	18	28,5	6
17/9	28	7	25	0	0	7	25
Pulicaris group							
Pools		positive	frequency %	Mean Ct			
27		2	7	31,5			
unidentified							
Pools		positive	frequency %	Mean Ct			
20		3	15	29,5			

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 Obsoleteus group were the most frequent caught and is also responsible for 36 of the 41 positive pools. In total 17% of the Obsoleteus 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.

1. Hoffmann B, et al. Novel orthobunyavirus in cattle, Europe, 2011. *Emerg Infect Dis.* 2012;18:469-72.
2. Rasmussen LD, et al. *Culicoides* as Vectors of Schmallenberg Virus. *Emerg Infect Dis.* 2012 18:1204-1206
3. Nolan DV, et al. Rapid diagnostic PCR assays for members of the *Culicoides obsoletus* and *Culicoides pulicaris* species complexes, implicated vectors of bluetongue virus in Europe. *Vet Microbiol.* 2007 124:82-94