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Background
The first case ever of bluetongue (BT) in Denmark was recorded in October 2007, so the biting midge season in spring 2008, was awaited with some anxiety, due to the rapid spread of the BTV serotype 8 epidemic in the countries south of Denmark.

First outbreak in 2008
The first outbreak in Denmark 2008 was detected on August 27 in a cattle herd in Brøndby, which is located in the southern part of Denmark, approximately 20 km north of the German border. Initially two animals were suspected of having BT based on clinical symptoms so EDTA blood and serum samples were submitted to our laboratory. One of the animals tested positive by ELISA (OD 15.54; 1.4) and real time PCR Ct 27.1 (0.6).

To evaluate the extent of this outbreak it was decided to test the remaining animals in pools from 5 samples.

Three pools were found positive for BT virus (BTV) by real time PCR. In each of these pools one sample of the five was positive for BTV RNA.

The animals were also tested individually for the presence of antibodies against BTV by ELISA.

Of the 75 animals tested, 19 were found positive for BTV specific antibodies. Of these 24 were cattle and the rest were sheep. The three PCR positive cows had high levels of anti-BTV antibodies (OD 4.12 2.67) whereas the 21 antibody positive but PCR negative cows had lower levels of antibody (ODs 3.31 0.49).

The latter results are probably due to the fact that vaccination against BTV took place 9 days prior to the collection of blood and several animals had succumbed in the intervening days.

In total in Denmark during 2008 some 15 outbreaks of BT were registered. Out of approx. 65 clinical suspicions 11 were found positive and the remaining four outbreaks were discovered by the routine surveillance of bulk milk. All outbreaks were located in the south western part of the country but several outbreaks were north of the original vaccination zone resulting in two extensions of the vaccination zone during fall 2008. All outbreaks in Denmark during 2008 were caused by BTV-8.

Vaccination experiment
The clinical symptoms in the first herd were observed in connection with vaccination against BT and the blood, from the two first animals, submitted for examination was sampled approx. 90 min. after vaccination, which raised the question of whether the positive PCR results could be due to the vaccine.

In order to address this issue, a study was performed in which blood samples were collected at short time intervals immediately before vaccination until 90 h after vaccination (sampling times: 0-25, 1, 4, 26-46 and 90 h).

Serotyping & sequencing
Bluetongue virus from all outbreaks were screened by real time PCR (Tropel BTV9, LIS, Lusin, France) and found to be BTV 8.

Sequencing of a part of segment 2 (using primer set IFW(1)) was performed on BTV RNA from three outbreaks which were believed to be representatives of all outbreaks. The most northern (Skjern, 08.06.2008), the most southern (Tonder, 09.06.2008) and the first outbreak (Brøndby, 27-08-2008). See map under the data for geographic location.

Sequences were identical from all three outbreaks and had 100% homology with the BTV9 isolate circulating in Ireland in 2006.

References