



landbouw, natuur en
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Epidemiological report BTV 6 in the Netherlands

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1. Introduction

On the 20th of October 2008 the Central authorities of the Netherlands reported a possible outbreak with a new serotype of bluetongue (BT) to the European Commission. On 4 farms (figure 1) in the eastern part of the Netherlands (one case in Gelderland and three cases in Overijssel) a PCR-signal was found that differed from the normal BTV 8 PCR-signal. Two animals showed non specific clinical symptoms.

Preventive measures were established effective immediately, to prevent further spread of this possible new virus type. Around the infected holdings a 50 km control zone was established and the whole country has been included in a restriction zone.



Figure 1: Location of BTV-6 cases in the Netherlands

On 24 October 2008 the community reference laboratory (CRL) for bluetongue in Pirbright reported that the BT-virus found was a BTV 6 strain and that the VP2 genome strongly resembled the VP2 genome of the vaccine virus used in South Africa for vaccine production, Onderstepoort Biological Products (OBP).

On 5 November Germany reported 4 BTV 6 infected animals in three different herds near the Dutch border. The locations and the distances from the cases in the Netherlands are presented in figure 2. Together with a European expert team the situation was evaluated. The main conclusion was that more information was necessary to determine whether this incident concerned an outbreak of a field virus or the spread of a vaccine virus. To answer this question a lot of tracing, monitoring and research was performed.

In this report the results of this work are presented. In this report the abbreviation BTV6/net2008 will be used. The main conclusion is that the BTV6/net2008 is not virulent and VP2 is similar to the South African vaccine virus. The rest of the genome shows strong resemblance with the original vaccine reference strain (available in the CRL) from which the vaccine virus has been derived. Monitoring in 2009 will give actual information on the disease situation constantly. Restrictions in relation to BTV6/net2008 are no longer required.

2. Tracing of a possible introduction route

2.1 First infection in the Netherlands

On the 27th of August 2008 a cow was tested for BT for export purposes. The cow originated from a farm in Oldenzaal. Later it was confirmed that this was the first case of BTV6/net2008 that was found.

2.2 The first four infected farms

To investigate the possible ways of introduction of the virus the four farms were visited. This led to the following observations:

- no exchange of animals between the farms, no supply contacts in common,
- no human contacts in common and they had all different veterinary practitioners,
- three farms vaccinated against BTV 8 and the batch number of first vaccination was identical,
- one farm had been visited in June by a South-African delegation of farmers.

The last two aspects were investigated further but did not lead to more insight in the introduction of BTV6. The observations were not alarming and did not reveal any commonalities between the farms

2.3 Investigation of the possibility of the use of BTV 8 vaccine contaminated with BTV6

In the Netherlands a BTV 8 vaccine of Intervet was used. To exclude a possible contamination of this vaccine Intervet was visited and audited by a team of experts from the Food and Consumer Products Safety Authority (VWA) and the national reference lab. No irregularities were found. The introduction of the virus in this way, was excluded.

2.4 Import of BTV sensitive animals from third countries

An other possible way of introduction is the import of an animal which was infected (or vaccinated) with the vaccine virus. For example lama's and alpaca's from Chile (although Chile is free from BTV 6 according to the OIE, it is present in the surrounding countries) were bled and tested for BTV. BTV6/net2008 was not found, only BTV-8 due to vaccination after arrival in the Netherlands.

2.5 Rumours

Rumours of illegal use of vaccine were investigated. No traces of vaccine use were found.

2.6 Possibilities of illegal delivery of OBP vaccine

According to the information of OBP no vaccine was delivered in Europe. In South Africa the vaccine is available in agricultural veterinary shops. A tourist can easily obtain small amounts of vaccine in South Africa. This way of introduction is possible and impossible to control.

2.7 Animal movements due to circuses, zoo exchanges and international horse events

Animal movements were investigated but did not give a lead to the possible introduction of BTV6

2.8 Monitoring 2009

In 2009 intensive monitoring activities will be in place:

- Around 500 BTV clinically suspected animals will be sampled and tested with PCR. Serotype is specified of each positive sample.
- Between 10.000 and 50.000 animals spread over the Netherlands will be PCR tested for export to third countries.
- Based on Regulation 2007/1266 a serological survey is executed on a yearly basis

This assures an alert monitoring and early warning in 2009 for BTV6/net2008 and other serotypes. For example the Netherlands were the first to detect and report BTV6 in 2008.

2.9 Conclusions of the tracing

- The BTV6/net2008 was introduced in the Netherlands before the 27th of august 2008 (in the middle of the vector season).
- No specific clues for a way of introduction of BTV6/net2008 were found. The most plausible introduction route is the illegal use of the OBP vaccine, obtained legally in South Africa. The Dutch authorities can only guess at the motivation/sense for the use. Bioterrorism is no a likely option.
- In 2009 an alert early warning and monitoring system for BTV is active in the Netherlands

3. Monitoring 2008; activities BTV 6

Different monitoring activities were done to specify the characteristics of the BTV6/net2008 incident. All animals of seven farms were fully investigated with a PCR test. On three of those farms serology and serum neutralization tests for BTV6 were performed. In the one kilometre zone of the seven farms all susceptible animals were PCRPCR tested. All suspected BT cases of 2008 that were PCR-negative and Elisa-positive were tested with a serum neutralization test. Thousands of animals were PCR tested for export to third countries.

3.1 Monitoring activities on seven infected farms

PCR-testing of all animals still present in the first 6 infected holdings and on a holding in Ophemert, where an imported animal from France (through Belgium) with BTV 1 was detected

Table 1: Monitoring of 7 holdings :

No holding	No of animals on holding	No of animals PCR-positive	% PCR positive	No of animals positive antibodies	% positive antibodies (SN-test)
1 Barchem	143	1	0,69 %	1	0,69 %
2 Heeten	125	1	0,80 %	1	0,80 %
3 Oldenzaal	52	1	1,92 %	0	0 %
4 Luttenberg	3	1	33,33 %	-	-
5 Holl. Rading	79	1	1,26 %	-	-
6 Basse	212	2	0,94 %	-	-
7 Ophemert	159	1	0,63 %	-	-
Total	773	7	5,65 %	2	0,75%

On the first six infected holdings all susceptible animals were tested with a PCR test (in total 773 animals). On the farm in Ophemert with the BTV 1 suspicion due to import from France, all animals were tested with PCR to detect possible spread of BTV1 on this farm..

In the screening two additional BTV6 cases were found, one on the farm in Basse and one on the farm in Ophemert. The BTV6 animal in Ophemert originated from the 50 km control zone.

All the animals of the first three farms were besides PCR also tested by a serum neutralization test. The serum neutralization test is always serotype specific and all samples were tested against serotypes 1, 2, 4, 6, 8, 9 15 and 16. This testing did not lead to any additional positive BTV serology for any type except for BTV8. On farm in Oldenzaal the infected animal was no longer available. Therefore there were no serological findings of BTV6/net2008 on this farm. The animals of the farm in Luttenberg were all slaughtered.

The farm prevalence of BTV6/net2008 is very low. The average was 0,75 %.

3.2 Monitoring in the 1 kilometre zone of five infected farms

All the susceptible animals in a circle of 1 km around primary four infected premises were PCR tested. In each kilometre zone one additional infected animal was found.

The 1 kilometre zone around the farm in Ophemert was PCR tested with regard to the screening activities for BTV 1. No additional BTV6/net2008 infected animals were found there.

The BTV6/net2008 prevalence in the one kilometre zones is low. The average prevalence is 0,4 %. In total 3426 animals were PCR tested.

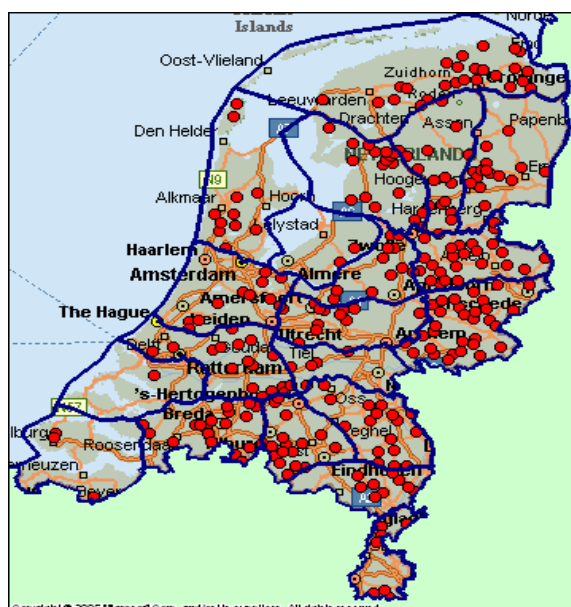
Table 2: Monitoring of the one kilometre zone of five farms

No	No. of holdings in 1 km	No of PCR-positive holdings	No of animals in 1 km	No of PCR-positive animals	% PCR positive holdings in 1 km	% PCR positive animals
1 Barchem	13	2	919	2	15,4 %	0,22 %
2 Heeten	20	2	1566	2	10 %	0,13 %
3 Oldenzaal	7	2	212	2	28,6 %	0,94 %
4 Luttenberg	24	2	632	2	8,3 %	0,32 %
5 Ophemert	9	0	197	0	0 %	0 %
Total	73	8	3426	8	12,4 %	0.40 %

3.3 SN-test of all suspected BT cases that were PCR-negative and Elisa-positive

In the passive monitoring of 2008 all PCR positive signals found in suspected animals were characterized for the serotype. No typing had been performed for PCR negative but serologically positive animals. They were considered to be BTV 8 infected or vaccinated animals. After the knowledge of BTV6/net2008 being introduced into the Netherlands, these cases were investigated with a serum neutralization test.

Table 3: SN-test of all suspected BT cases that were PCR-negative and Elisa-positive



No. of samples	No of BTV 6 positive	No of BTV 6 negative	% of BTV 6 positive
264	0	100%	0%

In the monitoring of the 264 BT suspected animals that were PCR-negative and Elisa-positive no BTV6/net2008 positive serology was found in the serum neutralization test.

3.4 Total PCR tests (all tests between 1 August 2008 and 31 January 2009)

In addition to the tests performed in relation to the BTV6/net2008 monitoring a huge number of PCR tests was performed for export purposes. In these tests eleven animals resulted positive for BTV6/net2008. Table 4 shows the results of all PCR tests performed at the NRL, including the monitoring tests and pre-export tests.

Table 4: Total PCR tests (all tests between 1 August 2008 and 31 January 2009)

No. of samples	No of BTV 6 positive	No of BTV 6 negative	% of BTV 6 positive
14393	22	14371	0.15 %

The average prevalence is 0,15 %.

3.4 Conclusions of the monitoring

- The average prevalence in the tested animals is very low (0,15 %).
- The BTV6/net2008 has spread over a limited area.
- No other BT serotypes than BTV 8 and BTV6/net2008 are circulating in the Netherlands

4. Research activities

To gain a better knowledge of the characteristics of BTV6/net2008 the following research projects were performed: longitudinal field study to determine duration of PCR-signal, animal trial to determine capacity to cause disease, molecular sequencing of BTV6/net2008 and a comparison with VP2 of the MLV vaccine in South Africa and with the complete the BT6 reference strain in the CRL (from which the MLV virus is derived).

4.1 Longitudinal field study to determine duration of PCR-signal

A naturally infected cow, that was BTV 8 vaccinated, was followed in the high containment unit of the national reference lab and 10 naturally infected animals in the field were monthly resampled. The majority of BTV6/net2008 infected animals (6 /10) remained PCR positive for more than 30 days after detection. Therefore PCR can be regarded as a good diagnostic tool to detect spread of BTV6 and it is not necessary to rely on the more difficult serum neutralization test.

4.2 Animal trial to determine capacity to cause disease

An animal trial was performed to determine the capacity of BTV6/net2008 to cause disease. The trial was divided into two parts. In the first part two calves, two sheep, two cattle were inoculated 2 days after arrival at the high containment unit on day 0 (D0), with two ml or three ml of (cattle) blood from the Heeten cow. The experiment ended at D38 post inoculation (D38pi). Blood samples were taken daily in the first week and thereafter every 2 days for PCR and weekly for serology. Daily clinical scores were kept.

On D7 pi one cow was found PCR positive,. The other animals remained PCR negative. On D10 pi blood from PCR positive animal was harvested for trial two. On D14 the PCR positive animal was found seropositive. The others remain negative in PCR and Elisa. At D38pi the experiment ended while the cow was still PCR and Elisa positive. The other animals remained negative and were therefore used in trial 2.

In the second experiment on D0 two new animals and the remaining animals from the first trial were used. Four sheep, four calves, three cows of the first trial were (re)infected with 10 ml or 15 ml blood of the experimentally infected cow from the first trial. The experiment ended on D25pi. Daily clinical scores and temperature registration were kept. Blood samples were taken daily for first 2 weeks and thereafter every two days for PCR and weekly for serology.

On D4 all animals were found PCR positive. On D11pi two out of four sheep were seropositive. On D14pi all animals were seropositive. On D22pi one cow became PCR negative again.

A slight fever was seen during one day in one cow and in 3 out of 4 sheep during three to four days starting five days post inoculation. No seroconversion was detected in the calves.

The animal experiment leads to the following conclusions:

- Experimental infection in sheep and cattle can lead to PCR positive period of more than 38 days.
- Calves have a delayed or no serologic response.
- BTV6/net2008 gives nearly no clinical signs.

4.3 Molecular sequencing of BTV6/Net2008

The work done in the CRL for bluetongue in Pirbright and our national reference lab leads to the following conclusions:

- VP2 genome is 99.9% similar to VP2 of the South African MLV vaccine.
- Other genome segments are more than 99% related to the vaccine reference strain in the CRL from which the MLV strain is derived.
- Only genome segment 10 shows lower similarity to reference strain but may show very high similarity to the MLV strain.
- Full MLV strain sequence not available.

4.4 Conclusions of the research

The most important conclusions of all these experiments are:

- The BTV6/net2008 has a very strong resemblance with the vaccine reference strain of OBP and is most likely the South African MLV vaccine strain.
- BTV6/net2008 gives nearly no clinical signs.

5. General conclusions

Animals infected with BTV6/net2008 nearly show no clinical symptoms. In the animal experiment only fever was reported. According to the information of OBP fever is a normal reaction after vaccination. From the 22 animals detected BTV6/net2008 positive, 4 animals showed very little clinical signs.

BTV6 has spread over a limited area and with a low incidence which is very unusual for field viruses

The virus is very closely related to the vaccine strain used in South Africa for vaccine production

The main conclusion is that BTV6/net2008 is not a virulent strain and has strong resemblance with the original vaccine virus. Therefore it is not considered to be an outbreak and movement restrictions are not relevant.