Epidemiological analysis of the 2006 bluetongue virus serotype 8 epidemic in north-western Europe
Executive Summary

Bluetongue (BT) is an arthropod-borne viral disease of domestic and wild ruminants, affecting particularly certain breeds of sheep with severe clinical disease, including mortality. On 14 August 2006, a private veterinary practitioner in the southern province of Limburg, in The Netherlands, notified the veterinary authorities of BT-suspect cases on four different holdings in that Member State (MS). These were the first suspicions of a rapidly-spreading BT virus (BTV)-epidemic in north-western Europe, which has since affected cattle and sheep holdings in Belgium, Germany, France, Luxembourg, and The Netherlands. On 28 August 2006, the CRL in Pirbright announced that BTV-serotype 8 (BTV-8) was causing the outbreaks.

The veterinary authorities of the three initially-affected countries (Belgium, Germany, and the Netherlands) decided at the onset of the BTV-epidemic in north-western Europe to form a research group with epidemiologists from these countries in order to provide science-based decision support for future BTV monitoring and surveillance. The European Food Safety Authority (EFSA) was requested by the European Commission (EC) to carry out a global epidemiological analysis of the ongoing outbreak. Therefore a BTV-8 epidemiology working group (BTV-8WG) was established by EFSA on 6th of October 2006.

This report describes the results of the different tasks that were performed to investigate factors associated with introduction, establishment and spread of BTV-8 in north-western Europe. They concern the following aspects of the outbreak investigation:

- The introduction of the BTV-8 serotype (focussing on the place, time and possible routes for this introduction);
- A section on clinical aspects in which the nature and severity of the disease caused by this strain are described;
- The characterisation of within-herd spread to assess among others the ratio between sub-clinical and clinical cases. This has implications for detection of BTV-8-infected herds;
- The information on factors favouring virus establishment. This includes the results of vector surveillance in the affected countries and factors that can affect virus persistence;
- Elements that may influence short-distance spread. This included the study of observed speed of local spread and the characteristics and pertinence of the 20 km zones;
- Finally, in the section on factors affecting long-distance spread into new areas, the potential for vector spread through wind and restrictions on animal movements were considered.

The provisional results, to be delivered by 31 January 2007, were presented to the BTV-8WG on the 6th of February 2007 and to the European Commission and Chief Veterinary Officers (CVOs) at an EC meeting on the 7th of February 2007 in Brussels. Taking into account the feedback from the meetings on the 6th and the 7th of February 2007 the reports were then revised. For the final report additional data through 31 January 2007 were considered as well, where relevant.
A full epidemiological analysis of the relationships between disease incidence and possible risk factors requires a multivariable analysis that allows taking account of possible interactions and confounding between important risk factors. Due to time constraints it was not possible to conduct such an analysis. Thus, the main focus of this report is on the descriptive and exploratory analysis of the epidemic.

The main findings reported by the EFSA bluetongue working group are reported below.

1. Statistical modelling showed that the initial infection occurred in the area close to Maastricht. Difficulties in the initial diagnosis were due to the fact that the disease had never been detected in the area before.

The source of the introduction of BTV-8 could not be identified and the exact origin and route of the introduction of BTV-8 thus far remains unknown. However, the absence of legal import of ruminants from outside the EU into the Area of First Infection and the absence of BTV-8 from southern Europe suggest that the introduction of the BTV-8 infection into north-western Europe is likely to have occurred via a other than through import of infected ruminants. Hence, the potential for introduction via mechanisms other than those incriminated in previous introductions also needs to be considered. Specifically, the potential for Culicoides to be imported along with or independently of the import of animals, plants or other ‘materials’ merits further study.

2. In BTV-8 infected herds, cattle and sheep expressed different clinical signs. BTV-8 associated clinical signs were much more common in sheep than in cattle, although it was found that a small number of cattle within a herd can show distinct clinical signs.

3. Based on the (sparse) data from whole herd sampling there was a trend suggesting a high proportion of cattle to be PCR- and sero-positive in infected cattle herds and a small proportion of sheep to be PCR- and sero-positive in infected sheep herds.

Taking into account the findings in relation to the within-herd patterns of clinical signs and serology, it can therefore be suggested that:
- for sheep flocks a monitoring system based on clinical signs should be considered; whereas
- for cattle a monitoring system based on serological surveillance appears to be the more effective approach.

4. The BTV-8 virus was found to be present in vectors (Culicoides species) which are endemic to north-western Europe. C. imicola, which is thought to be responsible for at least 90% of BTV transmission in the Mediterranean Basin, was not found amongst a total of approximately 100,000 Culicoides collected in the infected MS. This demonstrates that species endemic to the palaearctic region are capable of transmitting BTV and – judging from the rapid spread of the
virus – no pre-adaptive phase was required in the indigenous *Culicoides*. Species found to be PCR-positive were *C. dewulfi* (a species breeding exclusively in the dung of cattle and horses) and *C. obsoletus / C. scoticus*.

Vectors of BTV in other parts of the world have been shown to transmit a range of other viral pathogens of livestock (African horse sickness virus, Akabane virus, epizootic haemorrhagic virus, equine encephalosis) suggesting that such pathogens might be transmitted if they were to be introduced into northern Europe during climatically favourable periods. However, overall, there is a paucity of information on the behavioural activities of vector species of *Culicoides*, especially in relation to their interactions with host animals and their biting activities. Therefore, detailed data are urgently required before clear and reliable recommendations can be provided to the veterinary authorities on the subject.

Studies in France and in the Netherlands showed that later in the season, when the temperatures began to drop, more *Culicoides* were captured inside rather than outside stables. This undermines the rationale of the recommendation to house livestock indoors to reduce the *Culicoides* attack rate and indicates a need to revisit the definition of vector-free period to be when “…<10 *Culicoides* are found in a light trap suspended outdoors for one night …”. More comprehensive data on the effectiveness of control measures to prevent influx of *Culicoides* into stables are needed to allow meaningful recommendations.

Low numbers of adult *Culicoides* principally of the Obsoletus Complex, including freshly blood fed individuals, were on occasion captured in light traps operated throughout the winter (January, February and March 2007) in various MSs in north-western Europe. In all likelihood this persistent activity of adult *Culicoides* owes much to the mild temperatures that have continued to prevail across northern Europe during the winter of 2006/2007. However, the numbers of midges remaining active appear to have been too few to sustain the BTV transmission cycle as no sero-conversions in sentinel cattle was reported during that period.

Within the infected area, the presence of BTV-8 was favoured in locations that were warmer on average, where temperatures varied less throughout the year and where temperatures rose quickly in spring reaching a peak earlier in the year. The daily variation of number of *Culicoides* captured in the Netherlands was found to be linked with prevailing temperatures, for all *Culicoides* species present. The prevailing high temperatures in the summer of 2006 resulted in high numbers of various *Culicoides* species. Comparing current meteorological data in Germany to historical long term weather records (from 1961 until 1990) it was found that the adjusted mean temperature were exceptionally high during the months of July and September – October 2006. Thus, warm climatic conditions may favour the establishment of these viruses after they have been adventitiously introduced in a new part of Europe. The number of new bluetongue cases over time was equally influenced by changes in temperatures. The lag time between a change in temperature and a correlated change in number of outbreaks was estimated to be about 4 weeks.
This resulted in an initial peak and then a second peak of new cases which were separated by a cooler period. Besides temperature, the number of observed bluetongue cases was also related to other environmental factors such as altitude and animal density.

5. Local spread was modelled and found to occur at a rate of about 2 km per day or approximately 15 km per week. This estimation is consistent with published data on active flight distances covered by Culicoides and observed spread of BTV-outbreaks in Sardinia.

6. It was demonstrated that wind may affect spread over long distances. In particular, the density of the observed wind events contributed to explaining, at least in part,
   - the predominant east-west spread of the epidemic,
   - the more limited spread towards the north and south, and
   - the absence of recorded outbreaks in the U.K.

The movement of cattle mainly occurred in a north-western direction and the extension of the epidemic to the east can therefore not be explained by these transports.

In conclusion, changes in climatic conditions coupled with increased worldwide traffic might increase the risk in the appearance and the establishment of diseases in parts of Europe that were thus far exotic to those regions.
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# Abbreviations and definitions

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<td>AHAW</td>
<td>Animal health and animal welfare</td>
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<tr>
<td>Avia-GIS</td>
<td>Agri-Veterinary Intelligence and Analysis</td>
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<td>ADNS</td>
<td>Animal Disease Notification System</td>
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<td>AFI</td>
<td>Area of First Infection</td>
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<td>ATKIS</td>
<td>Amtliches Topographisch-Kartophisches Informationssystem (topographic data system in Germany)</td>
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<td>BT</td>
<td>Bluetongue</td>
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<td>BTV</td>
<td>Bluetongue virus</td>
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<td>BTV-8</td>
<td>Bluetongue virus-serotype 8</td>
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<td>BTV-8WG</td>
<td>EFSA WG on the epidemiology of bluetongue virus serotype 8</td>
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<td>subgroup of the BTV-8WG</td>
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<td>CDSS</td>
<td>Clinical Decision Support System</td>
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<td>CIDC</td>
<td>Central Institute for Animal Disease Control, Wageningen UR</td>
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<td>CIRAD</td>
<td>Centre de coopération internationale en recherche agronomique pour le développement</td>
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<td>CLC</td>
<td>CORINE land-cover data</td>
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<td>CSF</td>
<td>Classic Swine Fever</td>
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<td>CVOs</td>
<td>Chief Veterinary Officers</td>
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<td>DWD</td>
<td>German Meteorological Service</td>
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<td>EC</td>
<td>European Commission</td>
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<td>ECMWF</td>
<td>European Centre for Medium Weather Forecast</td>
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<td>EDS</td>
<td>Early Detection System</td>
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<td>EFSA</td>
<td>European Food Safety Authority</td>
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<td>EU</td>
<td>European Union</td>
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<td>FMD</td>
<td>Foot and Mouth Disease</td>
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<td>HRP</td>
<td>High Risk Period</td>
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<td>FLI</td>
<td>Friedrich Loeffler Institute</td>
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<td>HI-Tier</td>
<td>German cattle database</td>
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<td>IAH</td>
<td>Institute for Animal Health (in the United Kingdom)</td>
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<td>IDW</td>
<td>Inverse Distance Weighting</td>
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<tr>
<td>IZS</td>
<td>Istituto Zooprofilattico Sperimentale (Animal and veterinary public health institution in Italy)</td>
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<td>KNMI</td>
<td>Royal Dutch Meteorological Institute</td>
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<tr>
<td>MS</td>
<td>Member State(s)</td>
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<tr>
<td>NASA</td>
<td>National Aeronautics and Space Administration (American administration)</td>
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<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
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<td>RS</td>
<td>Remotely-sensed</td>
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<tr>
<td>SANITEL</td>
<td>Belgian animal identification and registration system</td>
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<tr>
<td>SEEG</td>
<td>Spatial Ecology and Epidemiology Group in Oxford University</td>
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<tr>
<td>Acronym</td>
<td>Full Description</td>
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<td>SIGAL</td>
<td>French Animal Information System</td>
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<tr>
<td>SRTM</td>
<td>Shuttle Radar Topography Mission (altitude data from the NASA)</td>
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<td>TRACES</td>
<td>Trade Control and Expert System</td>
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<td>TSN</td>
<td>German animal disease notification system</td>
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<tr>
<td>VAR</td>
<td>Veterinary and Agrochemical Research Centre</td>
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<td>WG</td>
<td>Working Group</td>
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1 Introduction

Bluetongue (BT) is an arthropod-borne non-contagious viral disease of domestic and wild ruminants, affecting particularly certain breeds of sheep with severe clinical disease, including mortality.

At present, 24 BT virus (BTV) -serotypes have been identified. They are transmitted by biting midges (Culicoides). BTV has a worldwide distribution between approximate latitudes 35°S and 40°N, although in parts of western North America, China and Kazakhstan the virus may extend up to almost 50°N. Recently, there has been a succession of BTV incursions into certain southern Member States of the European Union (EU), including Italy, Greece, the French island of Corsica, the Spanish islands of Menorca and Mallorca, mainland Spain and Portugal. Several Balkan States and European and Anatolian Turkey have also been affected. Between 1998 and mid-2006 BTV-serotypes 1, 2, 4, 9 and 16 had been involved in epidemics in one or more of the above countries.

On 14 August 2006, a private veterinary practitioner in the province of Limburg in the south of the Netherlands, notified the veterinary authorities of BT-suspect cases on four different holdings in that country. These were the first indications of a rapidly spreading BTV-epidemic in north-western Europe, which subsequently affected cattle and sheep holdings in the Netherlands, Belgium, Germany, France, and Luxembourg. On 28 August 2006, the CRL in Pirbright reported that the outbreaks were caused by BTV-serotype 8 (BTV-8).

The epidemiological mechanisms underlying this BT epidemic were unclear, including its origin and the geographic, climatic, and husbandry factors influencing the establishment and the spread of this disease.

BTV-8 is new to the European region. BTV-8 has previously been identified in Pakistan/India, southern and West Africa, and the Caribbean regions. The occurrence of this virus in north-western Europe far from its endemic regions needed to be associated with some mechanism of virus entry. The course of the epidemic has shown that given the appropriate conditions, BTV can rapidly spread within north-western Europe. Even though incursion of BTV-8 might be a rare event this ability for BTV to spread made it essential to determine what these ‘appropriate conditions’ were. This is needed both for adequate assessment of future risk and for devising effective strategies for monitoring and surveillance.

This report describes the results of the different tasks that were performed to investigate the factors associated with introduction, establishment and spread of BTV-8 in north-western Europe.
2 Mandate from the European Commission

The veterinary authorities of the three initially-affected countries (Belgium, Germany, and the Netherlands) decided at the onset of the BTV-epidemic in north-western Europe to form a research group with epidemiologists from these countries in order to provide science-based decision support for future BTV monitoring and surveillance. The research group (BT51 group) was established in Liege on 8 September 2006.

The European Food Safety Authority (EFSA) was requested by the European Commission (EC) to carry out a global epidemiological analysis of the ongoing outbreak. The mandate from the EC was issued to EFSA on the 5th of October 2006 (Appendix 1). It consists of two components. The first pertains to regular reports on the disease situation along with an analysis of the epidemiological data in relation to the outgoing outbreak. The second task consists of a global epidemiological analysis of the outbreak.

The legal basis for the request was Article 31 of Regulation (EC) No 178/2002 relating to the provision by EFSA of scientific and technical assistance to the EC. Within EFSA the project has been handled by the Assessment Methodology Unit in its Scientific Cooperation and Assistance (SCA) department.

3 General approach

The BTV epidemiology working group was established on 6 October 2006. It included the members from the BT51 group. The 1st meeting of the EFSA BTV-8WG took place on the 6th of October. Subsequently, a subgroup (BTV-8 subgroup) met on a weekly basis.

The first task of this group was to initiate the weekly reporting. This task has been carried out through weekly updates on the EFSA website (http://www.efsa.europa.eu) and is not further addressed in the present report.

To be able to conduct an overall analysis of the outbreaks on the basis of available data generated during the epidemic in the different countries it was essential:

- to agree on the prioritisation of the objectives for the analysis;
- to make sure that the institutes involved were given access by the competent authorities to the data and had the authorisation to share them as needed;
- to collect, verify, share, standardise, and collate the data from the different countries; and
- to divide the epidemiological analysis tasks among the institutes involved.

A full epidemiological analysis of the relationships between disease incidence and possible risk factors requires a multivariable analysis that allows taking account of possible interactions and
confounding between important risk factors. Due to time constraints it was not possible to conduct such an analysis. Thus, the main focus of this report is on the descriptive and exploratory analysis of the epidemic.

These issues were addressed by the BTV-8 subgroup. A first (general) proposal was presented to the meeting of the CVOs at their October meeting, which was organized by the EC and the Finnish presidency. The BTV-8 WG met again on the 15th of December 2006 in Paris to review the detailed proposal.

In order to make sure that adequate resources were available to complete this project in this short time period, EFSA launched a tender through the negotiated procedure with the institutes that had the *de facto* monopoly of access to the outbreak data. The tender procedure with these four national research institutes (one in Belgium, one in France, one in Germany, and one in The Netherlands) was completed successfully by the end of December 2006.

Due to the fact that the epidemic was still on-going at the time the data were being gathered and the time limitations for the delivery of the report, it was decided that the 31 January report would be based on data that obtained until 30 November 2006. For the final report additional data through 31 January 2007 were considered, as appropriate.

The provisional results to be delivered by 31 January 2007 were presented to the BTV-8 WG on the 6th of February and to the CVOs on the 7th of February 2007 in Brussels. Experts from the EFSA AHAW panel working group on bluetongue were invited to join the former meeting. Using the feedback from the meetings on the 6th and the 7th of February 2007 the reports were revised.

In this final report, for each task the Introduction and Objectives are reported in the main document whereas the Materials and Methods and the Results and Discussion are attached as separate appendices as a result of their volume. This report concludes with a general discussion and conclusions based on the main findings from the different tasks.

4 Data needs

4.1 Introduction & objectives

The timeline for completion of the data acquisition and exchange was 15 January, 2007. Each of the tasks required data to be collected from all affected MS. This activity has required significant effort. Particularly aspects of authorisation to access data and the need for standardisation of data from different MS proved to be time-consuming.
4.2 Approach taken

It included the following steps: data collection, collation, verification, and standardisation, and the establishment of a server platform for access to common data. There is no single database of the data that have been exchanged. In the interest of time, two approaches were taken:

- Data common for all tasks were placed on a single ftp server with one of the four institutes. The access to this server was password-protected and access limited to the individuals that needed the data to carry out their specific task in the context of the mandate. The following data were considered necessary for all tasks: BTV-8 outbreak data, laboratory investigations, and data on location of animal populations;
- Data that were specific for a task were sent directly to the institute responsible for carrying out the respective task. This includes animal movement records, environmental and climatic, and vector survey data.

4.3 Data collection

- Belgium
  - Livestock density data were extracted from the Belgian animal identification and registration system (SANITEL) to provide estimates of the populations at risk at NUTS4 level (cattle and sheep);
  - National animal outbound movement data from the area of first infection (AFI) to other regions of the country were obtained from SANITEL for the period between 1 January and 30 November 2006;
  - Laboratory results and I&R data from confirmed clinical case data;
  - Clinical and tracing data obtained from questionnaires that were applied during visits of the official veterinary authorities to confirmed clinical case holdings;
  - Temperature, rainfall and humidity data obtained from the METAGRI database and provided by the Royal Meteorological Institute; and
  - Data on control measures in Belgium were obtained from the website of the Federal Agency for the Safety of the Food Chain.

- France
  - Livestock density data were extracted from the French animal information system (SIGAL) to provide estimates of the populations at risk at NUTS4 level (Commune). They included the commune name and INSEE code, the number of cattle and small ruminant farms, and the number of cattle per commune;
  - Laboratory results from confirmed clinical cases data were obtained from CIRAD and AFSSA. They included date of blood sampling and serological and virological results;
Clinical data were obtained from visits to confirmed clinically infected herds by the veterinary services. They included outbreak location, type of herd, animal species with clinical signs of BT, number of animals present, by species;
- Temperature and rainfall data were provided by Meteo France; and
- Data on control measures taken in France were obtained from the official journal.

Germany
- Livestock density data in relation to the number of cattle farms and animals on municipality level (NUTS 4) was extracted from the German cattle database (HI-Tier). The density of sheep farms and animals was compiled from animal census data on the spatial level of districts (NUTS 3; DESTATIS);
- National livestock movement information data for affected districts were generated using the German cattle database (HI-Tier) following approval of the affected Federal States;
- Results of the virological and serological tests from cross-sectional studies and surveillance programs were compiled and prepared by the FLI;
- Data on BTV-8 outbreaks were extracted from the German animal disease notification system (TSN);
- Climate data regarding the daily temperature, rainfall and humidity from weather stations of the German Meteorological Service (DWD) in the outbreak area since May 2006 and the ‘normative many years average’ meteorological data set from 1961 until 1990;
- Data on control measures that were taken in Germany from the official journal; and
- High resolution land survey data of Germany were also obtained (ATKIS).

The Netherlands
- Livestock density data were obtained on cattle, sheep and goats from the Dutch Animal Identification and Registration Service;
- Animal movement data for affected districts were obtained from Dutch Animal Identification and Registration Services;
- Results of the virological and serological tests from outbreak investigations and surveillance programs were compiled and prepared by the CIDC;
- Clinical data were obtained from visits to confirmed clinically infected herds by the veterinary services;
- Temperature, rainfall, humidity were obtained from the Royal Dutch Meteorological Institute (KNMI); and
- Data on control measures that were taken in The Netherlands were obtained from the official journal.
• Data collected at the European level
  ▪ BTV-8 case data at farm level were extracted from the EC’s Animal Disease Notification System (ADNS). These case data were based on the following disease reporting mechanisms:
    o Mostly they represent herds in which the veterinary practitioner who has been consulted by the animal owner identified suspicious cases and where one or more of those clinically suspected animals were subsequently confirmed positive using a laboratory test (Polymerase chain reaction (PCR) or serology) and notified to the veterinary authorities.
    In addition, herds without clinical signs but with sero-positive animals that were subsequently confirmed positive with PCR or virus isolation were also included. Such, animals that were exposed to the virus can also be detected when tested serologically for certification prior to trade between zones with different BTV-8 status within a Member State or prior to export. Similarly, subclinical cases can be detected through cross-sectional serological screening programmes and through sentinel studies that have been initiated. Again, these cases need to be reported to the ADNS. However, the latter cases are not normally included in this study as these screening programmes mostly started in January 2007.
    o Subsequently, the EU Member States’ veterinary authorities notified the Commission and the Member States of disease outbreaks, in accordance with Directive 82/894/EEC. These notifications were entered into the ADNS (http://ec.europa.eu/food/animal/diseases/adns/index_en.htm). The legal base making it compulsory for Member States to notify outbreaks of animal diseases on its territory to the Commission and to other Member States is Council Directive 82/894/EEC. In the Directive it is laid down that Member States shall notify within 24 hours primary outbreaks (outbreaks not epizootiologically linked with a previous outbreak in the same region of a Member State or the first outbreak in a different region of the same Member State) and on the first working day of each week, the secondary outbreaks. The procedures for the actual notification are also laid down in this Directive.
    o From the ADNS database, the following data fields were used for each case: the country name, the date of confirmation, the geographical coordinates, and the animal species.
  ▪ A cattle and sheep database consisting of the number of herds and livestock numbers per herd was collated at municipality level from the databases of the affected Member States. Polygon shape files (EuroGeographics; Marne-la-Vallée, France) with 2002 boundaries of municipalities (LAU2; NUTS4/5) were obtained from Eurostat;
  ▪ International in- and outbound movement data for animals of the mammal species, live ruminant products (embryos, ova, or semen) in relation to municipalities
included in the area of first infection (AFI) were obtained from the EC’s TRACES system for the period between 1 January and 30 November 2006;

- Inbound movements for animals of the ruminant species to Belgium, the Netherlands or Germany for the period between 1 January and 30 November 2006;
- Wind data for the affected area during the period of the outbreak were obtained from the European Centre for Medium Weather Forecast (ECMWF);
- Moderate-resolution Imaging Spectroradiometer (MODIS) data (1 km by 1 km) from the Terra sensor (SEEG TFA MODIS dataset version 1) included daytime land surface temperature, night-time land surface temperature, middle infrared reflectance, and enhanced vegetation index (SEEG, Oxford University); and
- CORINE land cover data (CLC data; European Environment Agency) and altitude data with a resolution of 90 m Shuttle Radar Topography Mission Digital Elevation Model (NASA SRTM data) (http://srtm.usgs.gov) in ESRI Grid format were also acquired for the outbreak region.

4.4 Verification, collation, and standardisation of the common data

- BTV-8 case data at farm level extracted from the EC’s Animal Disease Notification System (ADNS) were verified prior to their use by checking for obvious inaccuracies. Missing or implausible geo-references were excluded from the analysis. Complementary BTV-8 outbreak data from the national animal disease notification systems of the Member States were collated and integrated into the ADNS outbreak database, except for data missing only at the highest level of precision (seconds).
- The changes in the administrative structure of the Member States since 2002 and different identification keys of the municipalities in the livestock database required considerable updating of the municipality database. Using a commercial NUTS4/5 dataset (Macon; Waghäusel, Germany) the livestock databases were then linked to the EuroGeographics municipality dataset.

4.5 Verification, collation, and standardisation of the other data

Some of these data were collected using standardized spreadsheets or were of small size and required little subsequent standardization. For other data a considerable amount of standardization was necessary.

- Daily weather data (mean, minimum and maximum temperature; precipitation and humidity) from 247 weather stations from The Netherlands, Belgium, Germany and France was integrated into one space-time meteorological database including data from 1 May until 30 November 2006. Furthermore, a long-term averaged weather data series of each German weather station was established and used for comparative
analysis. Furthermore, high resolution temperature data from the weather station in Maastricht, Belgium were obtained for the year 2005 and 2006. 

- CORINE land cover data (CLC data; European Environment Agency) and altitude data with a resolution of 90 m (NASA SRTM data) in ESRI Grid format were collated, verified and processed for the outbreak region.
- The temperature data were spatially interpolated using a modified model of the German meteorological service for large scale maps (Müller-Westermeier 1995; Ahrens 2006). The raw temperature values of the weather stations were reduced to sea level (N.N.) using the estimated parameters of a linear regression model between the altitude of the weather stations and temperature.

\[ t_{N,N_i} = t_i - b \times alt_i \]

- Maps of the spatially interpolated mean daily temperature and BTV-8 cases using the earliest date available were generated at daily intervals and combined to a descriptive video sequence for the time period between 1 May and 30 November 2006.

5 Characterisation of clinical signs observed in cattle and sheep

Early detection of BTV infected livestock herds depends strongly on the performance characteristics of the clinical diagnostic procedures. The diagnosis of BTV includes early recognition of a suspect clinical case by the farmer and the veterinary practitioner, clinical inspection by veterinary specialists and laboratory tests of blood for detecting the virus or specific antibodies against it. Clinical signs associated with BTV can vary considerably between animal species and are mostly non-specific. Furthermore, farmers and veterinary practitioners in north-western Europe were unfamiliar with the disease. Nevertheless, accurate interpretation of clinical signs by the livestock owner and/or the veterinary practitioner, and subsequent notification to the veterinary authorities of a clinically suspect case are crucial elements in an early detection system (EDS), because they are the first step in the diagnostic process. The
sensitivity of this clinical diagnosis, therefore, determines whether an infection with BTV will be recognized shortly after infection of the flock.

There have been earlier reports of BTV-8 outbreaks in India (Prasad et al. 1992), Africa (Gerdes 2004), and the Dominican Republic in the Caribbean (Mo et al. 1994), but there have not been detailed accounts on the clinical signs associated with BTV-8. Although BTV may infect many different species of ruminants, clinical disease signs are generally associated with sheep and consequently most descriptions of the disease apply to sheep (Erasmus 1990). Although experienced farmers in BTV endemic areas in South Africa report to occasionally have observed clinical BT in their cattle, researchers believed that BT did not produce more than transient and mild, if any, clinical signs in cattle (Hourrigan and Klingsporn 1975).

The objective of this study was to describe the distribution of clinical signs observed in BTV-infected cattle and sheep herds during the BTV-8 epidemic in the affected countries. In addition, the study describes the patterns of clinical detection, morbidity, mortality, and case fatality in cattle and sheep herds during the epidemic. This information should help farmers and veterinary practitioners in north-western Europe and other countries to be better prepared for clinical recognition of the disease.

The full report on morbidity, mortality and case fatality can be found in Appendix 2, whereas the main findings are discussed in chapter 11.

6 Within-herd distribution of infection

For the development of surveillance programs in the aftermath of the epidemic it is important to understand what is to be expected with respect to the distribution of infection within livestock herds. Livestock herds are epidemiological units within geographical compartments in a country from which (sentinel) animals are sampled to determine the infection status. Sample size calculations to detect disease or estimate prevalence of disease are dependent on an assumed a priori prevalence of disease to be expected after its introduction into an animal herd.

The objective of this investigation was to describe the pattern of laboratory confirmed infection (serology and PCR) within cattle and sheep herds in the affected countries.

The full report on the pattern of PCR and serologically positive animals can be found in Appendix 3, whereas the main findings are discussed in chapter 11.
7 Time-space pattern of virus spread between herds

In a situation with a transboundary epidemic, such as with BTV in this case, an analysis at the national level is only of very limited use for understanding the patterns of spread and drawing conclusions about effective control methods. Moreover, analysing the epidemic in an aggregated fashion across the whole area masks essential elements of the spatial and temporal structure of such a phenomenon. It has therefore been decided to separate the data into clusters to be able to characterise local spread within these and to identify differences between the infected areas.

The aim of the space/time exploratory analysis was to draw inferences from the data collected since the beginning of the BTV-8 episode. Furthermore, these analyses are used to formally test for the presence of spatial and temporal patterns which in turn would be the basis for formulating hypotheses about factors associated with spread to be investigated in further analyses.

Time-space patterns were studied in two stages:
• identification of statistically significant clusters and characterisation of the disease within these clusters;
• production of relative risk maps.
A stochastic model was developed and fitted to the data in order to describe the local spread of infection and to enable the identification of the first place of infection.

Furthermore, a sensitivity and uncertainty analysis was done in order to draw a risk map for comparison with spatial and temporal data of observed occurrence.

The full report on the clustering approach, risk mapping, the model-based determination of first place of infection, and the local spread are in Appendix 4, whereas the main findings are discussed in chapter 11.

8 Role of environmental factors, including wind and wildlife infections, in the spread of the disease

8.1 Introduction & objectives

The timing and distribution of bluetongue outbreaks have been linked to climate and other environmental factors at a range of scales (Baylis et al. 1998; Baylis, Meiswinkel and Venter 1999; Baylis and Mellor 2001; Baylis, Mellor and Meiswinkel 1999; Purse et al. 2005; Walker 1977). Whilst temperature and moisture levels modulate key events in both the BTV transmission cycle and in the lifecycle of its Culicoides vectors (Mellor, Boorman and Baylis 2000; Wittmann
and Baylis 2000), the wind is responsible for the passive dispersal of midges and, in turn, for the rapid spread of the diseases they carry.

Landscape elements such as land-cover and topography also influence patterns in Culicoides-borne diseases, probably via their effects on habitat availability, both for Culicoides and their ruminant hosts.

The confirmation of infections in wildlife during the current outbreak indicates that these species could potentially act as reservoir hosts for BTV. The presence of wildlife species is therefore considered here, alongside that of other hosts, among the suite of environmental factors that could impact the distribution and spread of BTV. This project was divided into the following three areas of investigation: wind analysis, small- (including wildlife) and large-scale environmental analysis.

8.2 Wind analysis

Recent work has shown that wind events relevant to the spread of Culicoides and bluetongue can be quantified for a series of recent Mediterranean BTV outbreaks. This analysis conducted by Ducheyne et al. (in press) using historical outbreak data of BTV serotypes 1, 4, 9 and 16 from 1999 to 2001 in Bulgaria and Greece, has been applied and extended using data from the current BTV-8 outbreak.

The objective of this study was to test the hypothesis whether BTV-8 outbreaks have been spread by wind and more specifically to:

- Produce weekly wind density raster maps;
- To perform a statistical analysis comparing observed BTV-8 spread patterns with computed wind density maps taking also into consideration other factors such as livestock movement patterns, livestock densities and terrain features;
- To assess the operational value of the developed approach as part of a risk assessment tool.

The full report on the wind analysis is in Appendix 5, whereas the main findings are discussed in chapter 11.

8.3 Small-scale (outbreak region) environmental analysis including wildlife

The outbreak in Belgium, the Netherlands, Germany, France and Luxembourg were the first reports of BTV north of 51 degrees latitude in Europe. The conditions in the new affected regions are different in terms of climatic and topographical factors to the previous outbreaks in the Mediterranean areas. The enormous spread of the epidemic in recent affected areas confirmed that, under certain conditions, BTV can rapidly spread in Europe even beyond the 51st latitude.
This section of the study is a first attempt to identify and explore potential environmental factors including wildlife affecting the establishment and spread of BTV in the outbreak region with the overall aim of generating hypotheses regarding their impact.

The specific objectives were:

- To investigate time and spatial distribution of BT outbreaks in relation to meteorological factors (temperature, rainfall, and humidity) using multi-step comparative analysis. The meteorological factors are based on the daily records of a small scale grid of weather stations in the outbreak area.
- To examine the potential influence of land cover pattern and altitude in relation to outbreaks, corrected by farm and animal density.
- To compare current meteorological data with historical long term weather records to identify specific climatic conditions observed during the introduction and establishment phase of the outbreak.
- To study the potential impact of wildlife infections related to BTV spread based on data of a serological survey.

The full report on the small-scale environmental analysis is in Appendix 6, whereas the main findings are discussed in chapter 11.

8.4 Large-scale environmental analysis

This area of investigation also examines the environmental conditions favouring BTV transmission in north-western Europe in 2006 but, instead matches patterns in outbreaks with patterns in remotely-sensed (RS) correlates of temperature, vegetation and moisture available from satellites.

RS climate variables have been shown to be useful for characterising the habitats of *Culicoides* vector species in several regional studies (Baylis et al. 1998; Baylis, Meiswinkel and Venter, 1999; Baylis et al. 2001; Purse et al. 2004; Tatem et al. 2003) and are now available as monthly images that are continuous across Europe at a 1km by 1km resolution. Thus, we can investigate how much the conditions favouring BTV transmission in north-western Europe in 2006 differed from conditions normally experienced in this region, and (2) from conditions that have favoured transmission in southern Europe. To what extent must we re-adjust our picture of the range of conditions in which BTV transmission can occur, in light of these recent outbreaks?

Though the distribution of clinical outbreaks is likely to underestimate the entire distribution of transmission, the performance of these basic models should at least provide initial indications of whether outbreak distribution has been strongly determined by particular environmental variables.
and which other areas of northern Europe might be similar environmentally to the locations where outbreaks occurred this year. Topographical, land cover and host densities will hopefully be integrated within the same statistical analysis.

The specific objectives were:

- Can we identify consistent climatic different areas between areas where BTV-8 was present and areas where BTV-8 was absent in north-western Europe in 2006? Which climatic factors e.g. temperature versus vegetation activity or soil moisture are most important in separating these areas?
- Were the seasonal conditions that favoured BTV transmission in north-western Europe in 2006 unusual compared to average conditions experienced in the region?
- Which areas of north-western Europe are similar environmentally to those in which transmission has occurred in 2006?

The full report on the large scale environmental analysis is in Appendix 7, whereas the main findings are discussed in chapter 11.

9 Role of human interventions

The introduction, establishment, and spread of animal diseases can be heavily influenced by human interventions and therefore the role of human interventions needs to be included in an epidemiological outbreak analysis. Movement of infected ruminants or non-susceptible mammals carrying infected Culicoides can cause the introduction of BTV and can affect its subsequent spread into a new area. BTV introduction can also result from trade in infected live animal products such as semen, ova or embryos. Thus, information on animal movements or transfer of live animal products which occurred at the onset and during the course of the epidemic is of relevance for identifying factors associated with the introduction and spread of this virus.

Surveillance and control measures implemented in relation to animals, live animal products, or the vectors aim at preventing the dispersal of the virus or at eliminating the virus. Thus, it is of interest to evaluate the impact of the implemented surveillance and control measures on BTV-8 incidence throughout the course of the epidemic during 2006.

This part of the report describes the results of investigations that were conducted in relation to the potential role of the above-mentioned human interventions on the introduction and spread of BTV-8. The first objective of the study was to explore several potential routes of introduction of BTV-8 into the area. A second objective was to report on some human interventions and to determine whether they may have influenced the subsequent spread of the virus in either a positive or a negative way. The interventions that were considered included the legal movements
of some animal species, the legal transports of live animal products, and the disease control measures that were implemented during the epidemic.

The full report on the role of human intervention can be found in Appendix 8, whereas the main findings are discussed in chapter 11.

10 Distribution and dynamics of vector species

Various species of *Culicoides* may be responsible for the transmission of BTV. The prevalence of these *Culicoides* species has not been monitored in much detail in northern Europe.

The objective of this part of the report is to describe the distribution and dynamics of the vector in the affected countries. It considers documented studies on the distribution and dynamics of *Culicoides* spp. before the start of the BTV-8 epidemic in 2006. Besides, it reviews the results of the vector monitoring that was set up at different sites within affected countries during the BTV-8 epidemic.

The main findings are discussed in chapter 11, whereas Appendix 9 presents an overall summary of the findings with in annex the four studies on insect trappings:

- Study on the distribution and dynamics of the vector executed at several sites within the Netherlands over August 2005 – August 2006, before the start of the BTV-8 epidemic.
- Study on the preliminary vector monitoring results of the trappings performed in the vicinity of confirmed outbreaks in the Netherlands during the BTV-8 epidemic.
- Data on distribution and dynamics (abundance and parity) of *Culicoides* in Northern France (indoor and outdoor) from September to December 2006 both in BTV-8 free and in affected farms.
- Preliminary results of the trappings performed in the vicinity of confirmed outbreaks in Belgium since the onset of the epidemic, including data on a study in Luxembourg.
11 General Discussion and Conclusions

This study was a step towards improving our understanding of the mechanisms that have contributed to the introduction, establishment, and spread of BTV-8 in this north-western part of Europe. It is important to emphasize that it was not possible to come up with conclusive answers to all the questions due to limitations in data and time. Nevertheless, conclusions relevant to decision makers are available and are discussed according to specific questions:

a. Descriptive space-time characteristics of the BTV-8 epidemic

The first outbreak was notified on 17 August 2006. By 22 August, 40 outbreaks were reported and by 1 September 138. Areas on the eastern side of the Rhine river in Nordrhein-Westfalen, Germany (Köln area) reported outbreaks on 4 September without any clear continuity of the spatial pattern of cases in connection with the initially affected Maastricht area. On September 18, outbreaks were first notified around the Gent area in Belgium.

Mapping of the outbreaks indicates that infection spread in an east-west and southward direction but that only limited northward spread occurred.

Two peaks of case notifications were observed (Figure 1), i.e. during weeks 34 (21 to 28 August) and 42 (16 to 22 October 2006). Two main spatial clusters of outbreaks (Maastricht and Gent) were identified and by the end of 2006 these two clusters of cases had not merged into one large cluster i.e. the gap between the Maastricht and Gent clusters remained. In the Köln and Gent clusters infection seems to have started later such that the first peak of the epidemic was restricted to the Maastricht area.
The most probable explanation for this bi-modal shape is that the period of lower number of notifications reflects a decrease of transmission frequency at an earlier time, which in turn is probably due to a reduction in the vector population density linked to meteorological circumstances. The time lag between the reduction in transmission frequency and reduction in associated number of disease notifications following is a consequence of the incubation period in the insect, the delay between biting meals, the incubation period in the animals, and the delay between appearance of clinical signs and the disease notification, the sum of which is estimated to be about 4 weeks. When comparing the temporal patterns of the average temperature and the weekly number of BTV-8 cases, the strongest correlation was identified for a time lag of 4 weeks (Spearman correlation coefficient = 0.884, p<0.01).

During the first phase of the epidemic, the disease spread over a distance of around 150 km (from 17 August, date of first notification in the Netherlands, to 15 September 2007). This period of one month is based on date of first notification data rather than date of first infection.

Long distance spread must have occurred but with locally varying consequences. The Gent cluster and the cases in France appear to be the result of long-distance spread originating from the Maastricht cluster. However, the consequences of long distance exposure were different between the Gent cluster and the north of France. In Gent, the introduction through long-distance spread was followed by local spread, whereas in France the virus did not circulate after its introduction.

Taking into account the population at risk for each period of time, the cumulative incidence rate was calculated. A maximum of 1% of the cattle farms at risk were infected in the whole study area.
b. Introduction of the BTV-8 serotype into the affected area

Place and time for this BTV-8 introduction

The statistical analysis of the spread of infection indicates that the place of introduction is likely to be located in a circle with a radius of 20 km, in the Maastricht region, around a centre with coordinates (5.89°-50.84°). This circle includes the geographical location of the first officially reported outbreaks in Belgium, Germany and the Netherlands including the Belgium farm where the earliest clinical symptoms were reported.

Possible routes for introduction

The objective of this work was to explore some of the potential entry routes for the virus that resulted in the BTV-8 epidemic. As a consequence of data and time constraints the adopted approach is associated with some limitations but it is still possible to draw the following conclusions.

- A source for the introduction of BTV-8 could not be identified, and the exact origin and route of the introduction of BTV-8 therefore remains unknown.
- Historically, BTV serotypes present in EU-neighbouring countries were introduced into the southern part of the EU. Further data on occurrence of BTV-8 in countries along the routes of introduction of other serotypes would be useful to further ascertain the plausibility of BTV-8 introduction via these routes. However, the absence of legal imports of ruminants from outside the EU into the AFI and the absence of BTV-8 from southern Europe suggest that the introduction of the BTV-8 infection into the more northern part of Europe took place via a different route.
- Hence, the potential for introduction via mechanisms other than those that have been previously identified also needs to be considered. Specifically, the potential for infected Culicoides to be imported along with or independently of the import of animals, plants or other ‘materials’, and the effectiveness of measures to reduce such a possibility, merit further study.

c. Clinical aspects

Nature and severity of the disease caused by this strain

In contrast to previous experience that BTV does not produce more than transient and mild, if any, clinical signs in cattle our BTV-8 study indicates that a small number of cattle within a herd can show distinct clinical signs.
- Approximately 10% of the BTV-8 infected cattle herds did not show any clinical signs in cattle at clinical inspection.
• Seven % of the BTV-8 infected sheep flocks were without clinical signs in sheep at clinical inspection.
• In the large majority of cases, only 1 or 2 animals showed clinical signs at the time of clinical inspection of infected cattle herds or sheep flocks.

BTV-8 associated clinical signs were much more prominent in sheep than in cattle. Clinical signs in BTV-8 infected herds were expressed differently in cattle herds and sheep flocks.
• The most prominent BTV-8 associated clinical signs in cattle were: crusts/lesions of nasal mucosa, salivation, fever, conjunctivitis, dysphagia, serous nasal discharge, apathy and/or tiredness, hyperaemic/purple coloration, lesions of teats, lameness and coronitis.
• The most prominent BTV-8 associated clinical signs in sheep were: fever, salivation, erosions of the oral cavity, facial oedema, dysphagia, apathy and tiredness, congestion, erythema, redness of oral mucosa, and lameness.

No BTV-associated mortality was observed in 66% of the sheep flocks and 91% of the cattle herds. 23% of the sheep flocks showed a case fatality of 50% whereas 6% of the cattle herds showed a case fatality of 50%. Hence, morbidity, mortality and case fatality were much higher in sheep flocks compared to cattle herds.

There were no reports of clinical disease (morbidity or mortality) in goats in the affected countries. We do not know if this is because goats do not easily show clinical signs or because goat herds were not exposed to infected midges.

In the 2006 epidemic in north-western Europe there was a long interval between first clinical signs observed by the animal owners and reporting of a clinically suspect situation to the competent authorities (≥ 2 weeks). This is partly due to a lack of familiarity amongst farmers and veterinary practitioners in this part of Europe with BT symptoms during the early phase of the epidemic.

Only one or two animals with clinical signs were observed at clinical inspection in a cattle herd or sheep flock. At present there have been no follow-up investigations of the herds in this study. It is well possible that after the clinical inspection there were additional animal deaths and a further development of clinical signs. Consequently the data presented here may underestimate the true extent of the 2006 epidemic in north-western Europe.
d. Characterisation of within-herd spread

*Ratio of sub clinical to clinical cases and implications for detection of BTV-8-infected herds*

In almost all cases only clinically sick animals (a total of one to three animals per herd) were sampled, in the majority all these animals were positive on PCR and/or serology.

Based on the sparse data from whole herd sampling there was a trend suggesting a high proportion of cattle to be PCR- and sero-positive in infected cattle herds and a small proportion of sheep to be PCR- and sero-positive in infected sheep flocks. In addition, within herds (almost exclusively cattle herds) without clinical signs a high proportion of PCR and serology positive animals could still be found.

Hence, whereas infected sheep tended to show clear clinical signs, often only a few sheep within a flock were PCR or sero-positive. These findings therefore suggest that a monitoring system based on clinical signs could be considered for sheep flocks. In contrast, since in infected cattle herds only a small portion (if any) of animals tended to show clinical signs but a large proportion of cattle were PCR or sero-positive (even when no clinical signs were seen), a monitoring system based on serological screening of cattle seems to be the more effective option for surveillance in cattle herds.

As wild ruminant species are considered as potential reservoir for BTV, some important free ranging species and fenced game were included in the serological survey during the hunting season 2006/2007 in Germany. A total number of 1897 samples from different wildlife species were analysed. Positive serological samples were only recorded in the Federal State of North Rhine-Westphalia, in the area where livestock was most severely affected. A total of 18 animals tested positive. Although these preliminary results indicate that the sero-prevalence with regard to BTV-8 in wildlife ruminants was low in Germany, a potential role of these animals in the epidemiology of the disease cannot be excluded.

e. Factors favouring virus establishment

*Relevant vectors*

For its dissemination, bluetongue virus is reliant on various species of biting midges of the genus *Culicoides*, which are the only known biological vectors. To date four species of *Culicoides* have been incriminated as vectors in southern Europe; the most significant of these is the Afro-asiatic *C. imicola* responsible for at least 90% of BTV transmission in the Mediterranean Basin. It is regarded by some researchers to be a recent invader from Africa and to be spreading northwards. The three remaining vectors are endemic to the Palaearctic region but have till now played only a
minor role in the spread of BTV; however, their importance appears to be increasing as BTV is moving northwards at a pace that is outstripping the slow advance of *C. imicola*.

The principal entomological questions for this northern outbreak were: which *Culicoides* are involved as vectors and do they include *C. imicola*? In order to attempt to answer these questions vector surveys were quickly implemented in most of the affected countries. These commenced operation towards the end of August 2006 in France and were soon followed by similar studies in the remaining affected MSs. In all of these countries the collection of *Culicoides* continued well into December 2006, in parallel with occurrence of outbreaks. The results of the various entomological investigations are summarised below:

- Not a single specimen of *C. imicola* was detected amongst a total of approximately 100,000 *Culicoides* collected in France, Belgium, Luxembourg, Germany and The Netherlands. This demonstrates that other species endemic to the Palaearctic region are quite capable of transmitting BTV and — judging from the rapid spread of the virus — it required no ‘pre-adaptive’ phase in indigenous *Culicoides*.

- In The Netherlands a novel, potential vector of BTV was discovered when a pool of 50 parous, non-engorged *C. dewulfi* were found RT-PCR+ to BTV-8. Subsequent to the virological findings made in regard to *C. dewulfi* three pools of parous mixed *C. obsoletus/ C. scoticus* collected on the same farm in Crapoel in the south-eastern Netherlands, were found also to be RT-PCR+ to BTV-8. This is the clearest evidence we have that more than one species of *Culicoides* was involved in the outbreak of BT across north-western Europe.

- It is of concern to note that all except one (*C. imicola*) of the previously identified vectors of BTV in southern Europe are now known to occur also widely across northern Europe. In The Netherlands, a ‘snapshot’ survey revealed species of the Obsoletus Complex (including *C. obsoletus* and *C. scoticus*) to be exceptionally widespread occurring in 94% of the light trap collections made on 108 cattle farms sampled nationally.

- Additional studies made in The Netherlands and in the other affected MSs in north-western Europe have confirmed the widespread dominance of the Obsoletus Complex on livestock farms; in addition — and like *C. dewulfi* — this insect complex had a high parity rate of 40%, which increases the likelihood of it having played a significant role in the outbreak of BTV a supposition that is supported by the three RT-PCR+ results mentioned above.

- *C. pulicaris ss* comprised a mere 3-6% of all the *Culicoides* captured and its distribution was limited so that it occurred on only 14% of the 108 farms surveyed in The Netherlands. Taken as a whole therefore, the vector surveillance studies indicate *C.
pulicaris ss played no significant role in the north-western European BT outbreak possibly because of its low abundance and restricted distribution in the area.

The incrimination of C. dewulfi may explain the differing vectorial capacities reported amongst various populations of the Obsoletus Complex in the United Kingdom where the highest competency rates were linked to populations in which C. dewulfi occurred most abundantly. In turn this might explain — in part — why BTV did not spread in north-eastern France where the numbers of C. dewulfi were approximately 10 times lower than those encountered in the Gulpen area of the south-eastern Netherlands and which was close to the Maastricht area of first infection.

C. dewulfi breeds exclusively in the dung of cattle and horses; this irrevocable link with cattle and horses translates into added risk for livestock owners as C. dewulfi might be competent also in the transmission of some viral pathogens of horses. The OIE “important notifiable disease” African horse sickness which is caused by a virus related to BTV is probably the most important of these Culicoides-transmitted horse pathogens.

During a ‘snapshot’ survey of The Netherlands C. dewulfi was found to comprise >11% of the total Culicoides captured and occurred on 71% of the 108 farms surveyed nationally, which, after the Obsoletus Complex, made it the second-most prevalent taxon. Furthermore, light trap collections made nightly in the Gulpen area showed local populations of C. dewulfi to have a high parity rate i.e. 40% of the individuals captured comprised older females indicating that their survival rate was high and, also, that they were feeding repeatedly (these two elements in the life cycle of Culicoides being crucial to the successful replication and subsequent transmission of BTV). In Belgium there is some evidence to suggest that C. dewulfi has expanded its range in the last 50 years; however, this may be an artefact of the different sampling methods used over time or it may be due to the low emphasis placed on the collection of Culicoides in the vicinity of livestock in the past.

The belated discovery of C. dewulfi as the fifth species now suspected to be involved in the transmission of BTV in Europe owes much to the fact that very few researchers are able to identify it with confidence and instead lump it under the Obsoletus species complex. This illustrates the need to continuously develop the level of taxonomic expertise that currently exists within western Europe and where the number of active Culicoides taxonomists can be counted on one hand.

For this reason it is essential that the veterinary authorities in all affected and adjoining MSs initiate comprehensive vector surveillance programmes to identify which species of Culicoides occur abundantly in the vicinity of all major breeds of livestock and to determine their seasonal profiles. These surveys will help identify regions in which multiple vector species occur (as was found for 10% of the farms surveyed in The Netherlands) and may highlight areas that are vector-free and thus suitable for the “quarantining” of livestock to facilitate the development of a safe disease-free export trade in ruminants.
Presence of vectors indoors and vector survival in winter

In an effort to reduce the impact of outbreaks of BTV, the competent authorities recommended that livestock be housed at night in the belief that this would reduce significantly the *Culicoides* attack rate (and thereby lower the BTV transmission rate). All farmers within the 20 km infection zone were therefore required to keep their animals indoors each night and to treat the animals monthly with a pour-on insecticide. But in France and in The Netherlands it has been discovered that *Culicoides* enter animal housing quite freely. Of particular concern, is that >95% of these comprised the vector species *C. obsoletus* and *C. dewulfi*. Work in these two MSs showed that early in the season when night-time temperatures remained high larger numbers of *Culicoides* were captured in light traps operated outside stables. However, later in the season, when the temperatures began to drop to single digits, a reversal occurred and more *Culicoides* were captured both earlier in the evening and inside rather than outside stables.

These data suggest that during the cooler times of the year *Culicoides* emerge from their resting places sooner in the day (when it is still reasonably warm) probably to attack livestock while still at pasture. It is well known that species such as *C. obsoletus* will intensify their attacks on overcast days when low-light conditions prevail. In such situations it is possible that attacking *Culicoides* may follow the cattle returning to their milking sheds and accompany them indoors. Once inside the biting midges would then be able to complete their blood feeding activities, only to be captured subsequently in the light traps operated nearby. This sequence of events would explain why increased numbers of midges were captured inside animal houses late in the season and why a high percentage of them were freshly blood fed.

In order to protect housed animals from attack by *Culicoides*, it may be required that such housing be sealed to a level where the lack of circulation of air might become a welfare problem or which is economically not viable. But even if such well-sealed buildings were to be ventilated perhaps by screening with insect-proof mesh this would do little to prevent *Culicoides* from entering the housing along with cattle in the late afternoon (as described above). To control this possible influx by *Culicoides* would require the installation of walk-through insecticidal sprayers. Overall, there is a paucity of information on the behavioural activities of vector species of *Culicoides*, especially in relation to their interactions with host animals and their biting activities. Detailed data are urgently required before clear and reliable recommendations can be provided to the veterinary authorities on the subject.

The discovery of significant numbers of *Culicoides* in buildings towards the end of the season has raised two additional points of concern:

- Do some species of *Culicoides* breed indoors? and
- Can BTV-infected late-season adult *Culicoides* overwinter inside cattle sheds to emerge months later in spring to initiate a recrudescence of the BTV transmission cycle?

These questions have yet to be answered.
The discovery that larger numbers of *Culicoides* may be found indoors compared with outdoors, especially towards the end of the season, has discredited earlier attempts to declare the vector-free period to be when “…<10 *Culicoides* are found in a light trap suspended outdoors for one night”. Consequently, it will be necessary to amend this criterion possibly by using light traps suspended both inside and outside animal housing. Because of a lack of reliable data on vector competence rates, transmission rates and vector ecology in the northern European context, it is not yet possible to define the vector-free season with an acceptable degree of certainty.

The most recent entomological data to emerge is that low numbers of adult *Culicoides* principally of the Obsoletus Complex, and including freshly blood fed individuals, have on occasion been captured in light traps operated throughout the winter (January, February and March 2007) in various MSs in north-western Europe. In all likelihood this persistent activity of adult *Culicoides* owes much to the mild temperatures that have continued to prevail across northern Europe. However, the numbers of midges that have remained active appear to have been too few to sustain the BTV transmission cycle as no sero-conversions to the virus in sentinel cattle have been reported since January 2007. Nevertheless, at the time of writing, it would be premature to conclude that BTV will not overwinter in the region and therefore vigilance must remain high.

It is now evident that the *Culicoides* fauna endemic to northern Europe harbours multiple vectors of BTV. As vectors of BTV in other parts of the world have been shown to transmit a range of other viral pathogens of livestock (African horse sickness virus, Akabane virus, epizootic haemorrhagic disease virus, equine encephalosis) this suggests that such pathogens may be transmitted if they were to be introduced into northern Europe during climatically favourable periods. Veterinary authorities in the MSs should be aware of these threats in order to be able to respond quickly to possible incursions.

**f. Factors that can be used to predict virus persistence**

*Meteorological conditions*

The daily variation of the number of *Culicoides* captured in The Netherlands was found to be correlated with prevailing temperatures, for all species. The lag time between a change in temperature and a correlated change in reported number of outbreaks was estimated to be about 4 weeks. Also, the first farm officially reported with clinical symptoms and the two peaks of the epidemic shifted back by 4 weeks coincided with the peaks of the warmer time periods.

In June and particularly July, a high number of consecutive days with temperatures above 25°C up to 10 hours per day were observed. Furthermore, the temperature did not decrease below 20°C during the night on several days in mid July. The high temperatures on consecutive days by end of June until mid July 2006 may have favoured the establishment of BTV-8 in the *Culicoides* population following virus introduction.
In the following weeks the drop of temperature may have slowed down the replication of *Culicoides*, the biting rate and the virus replication in the midges until the second warm period generated a second peak of the epidemic. A much higher incidence was recorded during this period due to the larger number of infected animals, which may have served as a source for midges to become infected. However, increased awareness and intensified disease surveillance may have resulted in more accurate incidence estimates of the epidemic at this stage. With temperatures decreasing over the last weeks of the observation period, the number of cases per week also showed a falling trend.

The daily map series of precipitation and BTV-8 cases did not show evidence for a direct association between the spatial and temporal distribution of rainfall and the occurrence and dynamics of BTV-8 cases. Unfortunately, humidity which should better describe the influence of moisture on the vector population could not be investigated as this weather data set was only available in a scattered format in time and space.

The high accuracy with which locations could be assigned to BTV-8 presence or absence locations, using preliminary climate-driven models, indicates that the distribution of BTV-8 in north-western Europe in 2006 was at least partly determined by climate. Consistent climatic differences were identified between areas where BTV-8 was present in north-western Europe and areas relatively close by that did not record disease outbreaks in 2006.

Specifically, the presence of BTV-8 was favoured in locations that were warmer on average, where temperatures varied less throughout the year and where temperatures rose quickly in spring to peak earlier in the year. These locations were around 1.5 °C warmer in the winter preceding the outbreak. In 2006, in the affected countries temperatures rose unusually rapidly in summer and peaked around 1.5 °C higher than average both in the day and at night.

To characterize prevailing climatic conditions during the course of the epidemic meteorological data of the year 2006 in Germany were compared with historical long term (from 1961 until 1990) weather records on a monthly base. The altitude-adjusted mean temperature of 2006, averaged over the affected area of Germany, equalled the long-term average adjusted maximum temperature in July. The adjusted mean temperature in August 2006 was very close to long-term average adjusted temperature. During September and October 2006, the altitude-adjusted mean temperature again rose very close to the long-term adjusted maximum temperature. These exceptional high temperature events during July and September - October 2006 may have favourably influenced the establishment and dissemination of BTV-8.

In a first approach to develop a risk map for the epidemic potential of BTV in the Netherlands working with the basic reproduction number ($R_0$) the model showed that a few regions in the Netherlands are more at risk from BTV epidemics than others. These regions include the South of Limburg, a south western part of Brabant and the Flevopolder. This fits with the observations...
during the epidemic in 2006, when the virus showed a considerable spread in South Limburg, and scattered around from two main foci of the epidemic (South of Limburg and Gent in Belgium) into the southern half of the Netherlands. The region in Brabant became infected later in the season, which may explain the lower incidence there, and the infection never reached the third risk area, the Flevopolder, which remained free from infections.

These findings should be considered as providing a crude indication of the relevant levels of risk based on the limited information available at present. This modelling study should be refined especially based on more information on the midges, their preferential niche, dynamics and biting behaviour become available. This should lead to better parameter estimates and spatial variation can be assessed at a finer scale. When a real midge dynamics model is available, real time risk maps can be made, which also will allow the simulation of specific weather conditions.

**Animal densities, environmental factors**

Landscape elements such as altitude and land-cover also influence the spatial distribution of vector-borne diseases.

Analysis and spatio-temporal mapping showed a strong association between temperature and altitude. As expected, the hilly areas were always cooler compared to areas of lower elevation. Even during the summer months, the temperature differences can be several degrees centigrade. The elevation distribution of BTV-8 cases stratified by Member State showed an upper altitude limit of approximately 650 m. This finding may at least in part explain the role of mountains in limiting the spread of the disease at the moment. The lower cattle and sheep densities, as well as the higher proportion of forest areas or a different habitat composition at higher altitudes may also influence the spatial distribution of BTV-8 cases in the affected area.

In areas with a high proportion of urbanization, the limitation in the spread of BTV-8 was apparently associated with the much lower density of farm ruminants in such areas. Therefore, lower cattle and sheep densities may have interrupted the spatial continuity of outbreaks of BTV-8 in the area and may have been responsible for the separation of local disease clusters. Areas of northern Europe predicted to be similar environmentally to those where BTV-8 transmission has occurred in 2006 do not extend far outside the currently affected area. These are however conclusions based on preliminary models that need to be checked for over-fitting.

A more detailed analysis of the environmental features shows that each country presents with a different environmental pattern possibly linked to the disease distribution. Some of the case municipalities are significantly clustered in areas with a high percentage of forest especially in Germany and in the Ardennes area in Belgium, while clusters of case municipalities with lower values of forest cover percentage are located in North-West Belgium (Ghent cluster) and in the initially infected area in Maastricht. This highlights a significant difference in the landscape pattern (as far as forest is concerned) and it seems possible that different vectors are involved in the disease transmission in different areas (e.g. members of the *C. obsoletus* complex vs. *C.*
For example, the case municipalities in the Ghent cluster in North-West Belgium are characterized by high values of cattle density and one can speculate that this increases the probability that \textit{C. dewulfi} plays a more important role in transmission in that area.

Over land, due to the roughness of terrain, wind spread occurred mainly in a hopping pattern where large distances may be covered between the start and the end of the epidemic. This pattern of disease spread is described in the ecological literature as “stratified dispersal” and is typical of dispersal events resulting from processes taking place at different spatial scales, short-distance contagion, and long-distance jump spread. Terrain roughness may be a factor important for preventing spread of infected midges.

g. Factors affecting short distance spread

\textit{Observed speed of local spread}

The observed speed of local spread was about 2 km per day or approximately 15 km per week. This estimation is consistent with published data on active flight distances covered by \textit{Culicoides} and observed spread of BTV-outbreaks in Sardinia.

\textit{Characteristics of 20 km zone}

Although the majority of new infections occurred within the 20 km restricted zones from an early stage of the epidemic in Belgium, cases continued to appear outside these zones until week 43 by which time these zones covered almost all of Belgium. Each new case outside the 20 km restriction zone may contribute to a new episode of local spread. The time lag between suspicion of a case and its inclusion in a 20 km zone is not only influenced by the geographical area covered by the 20 km zone but also by the time between suspicion and notification of the case. From the available data it was not possible to separate these two time periods, especially since the accuracy of the earliest date of suspicion is unclear. In addition, detection of the presence of BTV was based entirely upon observation of clinically affected animals, which means that time may have elapsed between occurrence of infected and infectious animals and appearance of animals suspected to show clinical signs.

\textit{Control measures}

The analysis of the association in time between the implementation of the first control measures and incidence of new infections of BTV-8 did not show a significant statistical association of the control measures with the slope of the epidemic curve. The incidence graphs for the different affected MS continued to show a large increase in incidence after the first control measures were implemented. The subsequent relaxations of the control measures also did not coincide with a significant change in the incidence curve. The single statistically significant association between
the decrease of the incidence and the last amendment of the control measures in all MS is contradictory to intuition since it concerned an amendment resulting in less restrictive measures. Overall, these findings should be interpreted with care. Firstly, this finding is not surprising because it concerns a vector-transmitted disease and it is difficult to limit local vector movement. Secondly, we could not assess the consequences of not taking control measures at all but, it is possible if not likely, that this would have resulted in even wider spread. The efficiency of a 20 km restriction zone depends on whether its size is adequate and whether the control measures to prevent spread are effective and sufficient.

h. Factors affecting long-distance-spread into new areas

Potential for vector spread through wind

One particular feature of the BTV8 epidemic under discussion is its predominantly horizontal (east-west) spread. Density of wind events contributes to explain at least part of the horizontal spread pattern of the epidemic. Wind is not the sole variable to explain the disease spread but to date no other variable has been identified which describes the observed spatial spread of outbreaks in such a comprehensive way, including limited spread south- and very limited spread northwards.

Density of observed wind events also contributes to explain, at least in part, the absence of recorded outbreaks in the U.K. A relationship between wind density and case density was established but more work is needed to relate weekly disease spread and wind density patterns. Model outputs may become an important risk management tool and could enable the implementation of more cost efficient monitoring and prevention activities.

Controlling density dependent factors affecting longer range spread may play an important role in preventing the spread of an epidemic at an early stage. Preventing the establishment of primary outbreak clusters that are sufficiently dense may inhibit the long range spread of the epidemic in the absence of other virus carrier movements.

Animal movements

Outbound movement of infected domestic or wild ruminants or infected ruminant-live products from an infected area may contribute to the spread of BTV-8 within and among affected MS and beyond. Data on ruminant transports leaving the AFI were obtained from the three initially affected MS. The geographical dispersion of the movements was mapped for two time periods:

- 1 April - 18 August: during which free movement of ruminants was allowed;
- 18 August - 30 November: during which movements of ruminants were restricted.
A negative binomial model was used to model the cumulative number of cases that occurred in a municipality outside the AFI as a function of the number of movements or the number of animals moved to that municipality. The best fitting statistical model included the following municipality-level covariates: number of animals, municipality surface, and number of farms. The parameter estimates resulting from the model show a significant positive relationship between the total number of cases that occurred in a municipality by the end of the epidemic and the total number of ruminants that were introduced in the municipality between 1 April and 30 November 2006, while correcting for the other covariates in the model. However, little overlap was seen between the observed number of cases and the numbers predicted by the resulting model. Only a small proportion (23%) of the variability in the data was explained by the model which suggests a lack of model fit. The extension of the epidemic to the east can not be explained by the movement of animals, which mainly occurred in a north-western direction. This indicates that it is important to consider the inclusion of other influential covariates into the model such as wind direction, temperature, and local spatial correlation between cases.
12 Acknowledgements

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13 Working Group and its Subgroup

The EFSA BTV-8WG consists of a group of experts from all Member States, which have been invited to join. It was chaired by D. Pfeiffer (RVC, UK). Specific analytical tasks were carried out by the BTV-8subgroup which currently includes the following experts: A. Brouw (EC, Belgium), A. Conte (OIE Ref Lab., Italy), H. Deluyker (EFSA, Italy), A. R.W. Elbers (CIDC-Lelystad, Wageningen UR, the Netherlands), G. Gerbier (CIRAD, France), G. Hendrickx (Avia-GIS), R. Meiswinkel (CIDC-Lelystad, Wageningen UR, The Netherlands), Ph. Mellor (IAH, UK), K. Mintiens (VAR, Belgium), B. Purse (Oxford, U.K.), D. Pfeiffer (RVC, U.K.), C. Staubach (FLI, Germany), and S. Zientara (AFSSA, France).
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