Epidemiological analysis of the 2006 bluetongue virus serotype 8 epidemic in north-western Europe: provisional findings through 31 January 2007
Executive Summary

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. 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. These were the first suspicions of a rapidly-spreading BT virus (BTV)-epidemic in northern 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 northern 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 through 31 January 2007 to determine the conditions for introduction, establishment and spread of BTV-8 in northern Europe. They concern the following aspects of the outbreak investigation:

- The introduction of the BTV-8 serotype; which focused 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 which is of relevance to assess the ratio of sub clinical versus clinical cases. This has implications for detection of BTV-8-infected herds;
- The information on factors favouring virus establishment includes the results of vector surveillance in the affected countries and factors that can affect virus persistence such as animal densities, environmental factors, and meteorological conditions;
- Elements that may influence short-distance spread that were studied include 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.

These provisional results, to be delivered by 31 January, were presented to the BTV-8WG on the 6th of February and to the Chief Veterinary Officers (CVOs) at an EC meeting on the 7th of February in Brussels. Using the feedback from the meetings on the 6th and the 7th of February the reports were revised and finalised.
The final outcome of a full epidemiological analysis on the identification of associations or causality between disease incidence and possible risk factors requires a multiple variable analysis assessing all possible risk factors simultaneously. Such an analysis was not achievable in the time frame given to generate this first report. Thus, the focus of this report is more of a descriptive and exploratory nature. The initial 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 was thus far not known (exotic) in the area. An obvious source for the introduction of BTV-8, such as import of infected ruminants, 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 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 another route.

Hence, the potential for introduction via mechanisms other than those that have been previously incriminated also need to be considered. Specifically, the potential for 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.

2. Clinical signs in BTV-8 infected herds were expressed differently in cattle herds and sheep flocks. BTV-8 associated clinical signs were much more prominent in sheep than in cattle.

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

Taken together with the results on clinical signs, these findings therefore suggest that
   - for sheep flocks a monitoring system based on clinical signs could be considered; and
   - 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 once amongst a total of 100,000 Culicoides collected in the infected MS. This demonstrates that species endemic to the Palaearctic region are quite capable of transmitting BTV and – judging from the rapid spread of the virus – no pre-adaptive phase was required in the indigenous Culicoides. One of the species found to be PCR-positive was C. dewulfi a species which breeds exclusively in the dung of cattle and sheep.
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 encephalitis virus), this suggests that such pathogens may 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. Detailed data are urgently required before clear and reliable recommendations can be provided to the veterinary authorities on the subject.

The discovery that larger numbers of *Culicoides* may be found indoors than outdoors, and especially towards the end of the season, demands that the earlier attempt to declare the vector-free period to be when “…<10 *Culicoides* are found in a light trap suspended outdoors for one night” be refined.

The daily variation of number of *Culicoides* captured in The Netherlands were found to be correlated with prevailing temperatures, for all species. The prevailing high temperatures in the summer of 2006 resulted in high numbers of various *Culicoides* species. Thus, the warm(ing) 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 15 km per week. The timing of implementation of the first restriction measures and the timing of the implementation of subsequent changes to these restriction measures did not coincide with subsequent changes in the pattern of the epidemic curve.

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

In conclusion, changes in climatic conditions coupled with frequent travel might increase the risk in the appearance and the establishment of diseases in parts of Europe that were thus far exotic for those regions.
A final report based on data obtained until 1 February 2007 and on analyses conducted through 31 March 2007 is under review.
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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>CDSS</td>
<td>Clinical Decision Support System</td>
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<tr>
<td>CIRC</td>
<td>Central Institute for Animal Disease Control</td>
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<td>CIRAD</td>
<td>Centre de coopération internationale en recherche agronomique pour le développement</td>
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<tr>
<td>CLC</td>
<td>CORINE land-cover data</td>
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<tr>
<td>CSF</td>
<td>Classic Swine Fever</td>
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<tr>
<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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<tr>
<td>HI-Tier</td>
<td>German cattle database</td>
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<tr>
<td>IAH</td>
<td>Institute for Animal Health (in the United Kingdom)</td>
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<tr>
<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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<tr>
<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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<tr>
<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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<td>SIGAL</td>
<td>French Animal Information System</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>SRTM</td>
<td>Shuttle Radar Topography Mission (altitude data from the NASA)</td>
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<tr>
<td>TRACES</td>
<td>Trade Control and Expert System</td>
</tr>
<tr>
<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 EU Member States.

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 country. These were the first indications of a rapidly spreading BTV-epidemic in northern Europe, which has since affected cattle and sheep holdings in the Netherlands, Belgium, Germany, France, and Luxembourg. On 28 August 2006, the CRL in Pirbright announced that BTV-serotype 8 (BTV-8) was causing the outbreaks.

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 precise source of the BTV incursion into Europe and the route of entry are at present unknown. The occurrence of this virus in northern Europe far from its endemic regions suggests that it may have gained entry via the importation of infected wild animals like e.g. giraffe or antelope, importation of infected germ plasms or biologicals and movement of infected vectors in planes or trucks. Typing of the virus is important in tracking where BTV-8 came from.

The course of the epidemic has shown that under the right conditions, BTV can rapidly spread in northern Europe. Even though incursion of BTV-8 might turn out to be a rare or unique event this ability for BTV to spread made it essential to determine what these ‘right conditions’ were. This is needed both for adequate assessment of future risk and to devise protocols for monitoring and surveillance.
This report describes the results through 31 January 2007 of the different tasks that were performed to determine the conditions for introduction, establishment and spread of BTV-8 in northern 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 northern 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 ongoing 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. As a consequence, within EFSA the project has been handled by the Assessment Methodology Unit in its Scientific Cooperation and Assistance (SCA) department.

The BTV epidemiology working group was established on 6 October 2006. It includes 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-8subgroup) met on a weekly basis.

3 General approach

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/en/in_focus/bluetongue/outbreak_overview.html). This task 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.

In addition, due to the fact that the epidemic was still on-going at the time the data were being gathered to the time limitations for the delivery of the report, a decision had to be made on the
data to be used for the study: It was decided that the 31 January report would be based on data that obtained until 30 November 2006.

The final outcome of a full epidemiological analysis is the identification of associations or causality between disease incidence and possible risk factors. This requires a multiple variable analysis assessing all possible risk factors simultaneously. It was decided that such an analysis was not achievable in the time frame given to generate this report. Thus the focus of this report is more of a descriptive and exploratory nature.

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 in Paris to review the detailed proposal.

In order to make sure that adequate resources were available to complete this project in short order, EFSA launched a tender through the negotiated procedure with the institutes that have 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) were completed successfully by the end of December.

The provisional results to be delivered by 31 January were presented to the BTV-8 WG on the 6th of February and to the CVOs on the 7th of February 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 the reports were revised and finalised.

For each task, the following is reported:
- Introduction and Objectives in the main document; and
- Materials and Methods and the Results and Discussion in separate appendices.
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 quite an effort. Particularly aspects of authorisation to access data and the need for standardisation of data from different MS proved to be quite 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 at this moment 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 is password-protected and was limited to the individuals that need access to the data to carry out their specific task in the framework 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 have been sent directly to the institute to carry out the task. This includes animal movement records, environmental and climatic data, 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 (AFI) to other regions of the country were sought from SANITEL for the period between 1 January and 30 November 2006;
  - Laboratory results and I&R data from confirmed clinical cases data;
  - Clinical data and tracing data obtained from questionnaires that were taken during visits of the official veterinary authorities to confirmed clinical cases;
  - Temperature, rainfall and humidity data obtained from the METAGRI database and provided by the Royal Meteorological Institute; and
Data on control measures taken in Belgium 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.
  - Data on control measures taken in France were obtained from the official journal.

- Germany
  - Livestock density data regarding 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;
  - 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 cross-sectional studies 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);
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 on farm level were compiled from the EC’s Animal Disease Notification System (ADNS). These case data used were obtained as follows:
  - Mostly they represent herds in which the veterinary practitioner who has been consulted by the animal owner identifies 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 are 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 studies mostly started in January 2007.
  - 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 are entered in 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.
  - 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 on 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 inbound 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 on farm level compiled 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 in 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 was 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 magnitude and required little subsequent standardization. For other data a considerable amount of standardization was needed.

- Daily weather data (mean, minimum and maximum temperature; precipitation and humidity) from 247 weather stations from The Netherlands, Belgium, Germany and France was integrated in one space-time meteorological database starting retrospective
at the 1 May until 30 November 2006 for this report. Furthermore, a long-term averaged weather data series of each weather station was established and used for comparative analysis.

- 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). 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.

\[
t_{N.N,i} = t_i - b \cdot \text{alt}_i
\]

- \( t_{N.N,i} \) = temperature at the weather station \( i \) reduced to sea level (N.N.)
- \( t_i \) = temperature at the weather station \( i \)
- \( b \) = parameter estimate for altitude of the linear regression model
- \( \text{alt}_i \) = altitude at the weather station \( i \)

These reduced values are interpolated by Inversed Distance Weighting on a grid cell size of 250 m and subsequently retransformed to values in the actual topography using altitude data with an original resolution of 90 m (NASA SRTM data) resampled to 250 m grid cell size.

- 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.
II. DESCRIPTIVE EPIDEMIOLOGY OF THE BTV-8 EPIDEMIC

5 Characterisation of clinical signs observed in cattle and sheep

5.1 Introduction & objectives

The performance of the clinical diagnostic procedure to detect BTV infected livestock herds is crucial for early detection. The diagnosis of BTV includes early recognition of a suspect clinical situation by the farmer and the veterinary practitioner, clinical inspection by veterinary specialists and laboratory tests on blood to detect 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 Northern Europe are unfamiliar with the disease. Nevertheless it is beyond any doubt that an accurate interpretation of clinical signs by the livestock owner or the veterinary practitioner, and subsequent notification to the veterinary authorities of a clinically suspect situation are crucial elements in an early detection system (EDS) because they are at the frontline of the diagnostic process. The quality 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 on 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 of South Africa believed that from time to time they had 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 clinical detection, morbidity, mortality, and case fatality in cattle and sheep herds during the epidemic. This will help farmers and veterinary practitioners in Northern Europe and other countries to be better prepared for clinical recognition of the disease.

5.2 Report
The report on the clinical signs is in Appendix 2a.

The report on morbidity, mortality and case fatality can be found in Appendix 2b.
6 Within herd distribution of infection

6.1 Introduction & objectives

For the development of surveillance programs in the aftermath of the epidemic it is important to know what is to be expected on 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 to disease or estimate prevalence of disease are dependent on the \textit{a priori} prevalence of disease to be expected after its introduction into an animal herd.

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

6.2 Report

The report on the distribution of PCR and serologically positive animals can be found in Appendix 3
7 Time-space distribution of virus spread between herds

7.1 Introduction & objectives
With such a transboundary epizootic, it is obvious that analysis at the national level only has limited performance. Moreover, analysing the whole epizootic does not make sense as there is a spatial and temporal structure of such a phenomenon. It has therefore been decided to divide the data in clusters to be able to characterise local spread and to identify contrasts between the different infected areas.

The aim of the space/time exploratory analysis was to derive assumptions 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 leads to the formulation of hypotheses.

Time-space distribution has been studied in two stages:
• identification of statistically significant clusters and characterisation of the disease within these clusters;
• drawing of relative risk maps.

Finally a stochastic model was built and fitted to the data in order to describe the local spread of contamination and to enable the determination of the place of infection.

7.2 Report

The report on the clustering approach, risk mapping, the model-based determination of first place of infection, and the local spread are in Appendix 44
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 & Venter, 1999; Baylis & Mellor, 2001; Baylis, Mellor & 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 & Baylis, 2000; Wittmann & 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.

Due to a limited study period and for practical reasons the project was divided into the following three areas of investigation: wind analysis, small scale environmental analysis (including wildlife) and large scale environmental analysis.

8.1.1 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. (submitted) using historical outbreak data of BTV serotypes 1, 4, 9 and 16 from 1999 to 2001 in Bulgaria and Greece, has recently been applied and extended using data from the ongoing BTV-8 Belgian outbreak.

The objective of this study section 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.
8.1.2 Small scale (outbreak region) environmental analysis including wildlife

The current outbreak in Belgium, the Netherlands, Germany, France and Luxembourg were the first reports of BTV northern 51st latitude in Europe. The conditions in the new affected regions are not comparable in terms of climatic and topographical factors to the previous outbreaks in the Mediterranean areas. However, the enormous spread of the epidemic in recent affected areas confirmed that, under the certain conditions, BTV can rapidly spread in Europe even beyond the 51st latitude.

The mechanisms underlying the current bluetongue epidemic are unclear, particularly the relative role of geographic, climatic and wildlife factors influencing the establishment and the spread of the disease.

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.

In the final report this will be supplemented with the following:

- A comparison of 2006 weather data with historical long term weather records to identify specific climatic conditions observed during the introduction and establishment phase of the outbreak.
- A study on the potential impact of wildlife infections related to BTV spread based on data of a serological survey.

8.1.3 Large scale environmental analysis

This area of investigation also examines the environmental conditions favouring BTV transmission in northern 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 & 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 northern Europe in 2006 differed from conditions normally experienced in this region, and (2) from conditions that have favourered 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 Northern 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 northern Europe in 2006 unusual compared to average conditions experienced in the region?
- Which areas of Northern Europe are similar environmentally to those in which transmission has occurred in 2006?

8.2 **Report on wind analysis**

The report on the wind analysis is in Appendix 55

8.3 **Report on small-scale environmental analysis**

The report on the large scale environmental analysis is in Appendix 66

8.4 **Report on large-scale environmental analysis**

The report on the large scale environmental analysis is in Appendix 77
9 Role of human interventions

9.1 Introduction & objectives

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 in 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 during the onset and the course of the epidemic is of relevance to try to identify conditions for the introduction and spread of this virus.

Surveillance and control measures implemented on animals, live animal products, or the vectors aim at preventing the dispersion 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 on 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 whether they may have influenced the subsequent spread of the virus in either a positive or a negative way. The interventions that were considered were: 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.

9.2 Report

The report on the role of human intervention can be found in Appendix 88
10 Distribution and dynamics of vector

10.1 Introduction & objectives
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 was to describe the distribution and dynamics of the vector in the affected countries. It considered 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.

10.2 Reports
Appendix 99 includes results of insect trappings from the following studies:
- Study on the distribution and dynamics of the vector executed at several sites within the Netherlands in the period August 2005 – August 2006, so before the start of the BTV-8 epidemic.
- Study on the preliminary vector monitoring results of the trappings that have been 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 that have been performed in the vicinity of confirmed outbreaks in Belgium since the onset of the epidemic. Data on a study in Luxembourg were also provided.
- An overall summary of the findings in these four studies.
11 General Discussion and Conclusions

This study was a first step towards improving our understanding of the mechanisms that have contributed to the introduction, establishment, and spread of BTV-8 in this part of Europe. It is important to repeat that all results discussed here are preliminary. Nevertheless, at this stage some initial conclusions relevant to decision makers are available. The discussion below summarises the findings and is structured according to a number of specific questions.

Descriptive space-time characteristics of the BTV-8 epidemic

From the description of the spatial and temporal distribution of cases, the following major conclusions can be drawn:

- 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;
- within the Maastricht cluster spread occurred in two separate periods;
- as measured using the data from the early stages, the rate of local spread was approximately 15 km/week;
- in addition, long distance spread must have occurred as well. Its consequences varied, depending on the location;
- at the fringe of the epizootic, virus circulation was very limited,

Introduction of the BTV-8 serotype in 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 was 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 outbreak that was officially reported with clinical signs.

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. Even though, because of time constraints, there are many limitations to the approach that was taken some conclusions can be drawn.

- An obvious source for the introduction of BTV-8, such as import of infected ruminants, could not be identified and the exact origin and route of the introduction of BTV-8 thus far 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 via these routes. However,
  o the absence of legal import of ruminants from outside the EU into the AFI; and;
  o 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 another route.

Hence, the potential for introduction via mechanisms other than those that have been previously incriminated also need to be considered. Specifically, the potential for 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.

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.
• Less than 2% 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.

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

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 mucous membrane, 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 mucous membrane, and lameness.
No BTV-associated mortality was observed in 66% of the sheep flocks and 91% of the cattle herds. Hence, morbidity, mortality and case fatality were much higher in sheep flocks compared to cattle herds.

One has to take into consideration that no follow-up investigations were conducted following the first clinical investigation to confirm infection. Therefore, it is possible that total mortality, morbidity and case fatality in an affected herd may be higher than what is reported here.

**Characterisation of within-herd spread**

**Ratio of sub clinical versus 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 majority all these animals turned up positive by 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 seropositive in infected cattle herds and a small proportion of sheep to be PCR and seropositive in infected sheep flocks. In addition, within herds (almost exclusively cattle herds) without clinical signs a high proportion of PCR and-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 seropositive. 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 seropositive (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.
Factors favouring virus establishment

The principal entomological questions posed were: which Culicoides are involved as vectors and do they include C. imicola?

For its dissemination bluetongue virus (BTV) 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 rapidly northwards threatening the livestock industry there. The three remaining vectors are endemic to the Palaeartic 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.

In order to try and answer the above questions vector surveys were quickly implemented in most of the affected countries. These commenced operation towards the end of August 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 in parallel with outbreaks of the disease. 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 species endemic to the Palaeartic 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. 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.

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 10x lower than those encountered in the Gulpen area of the south-eastern Netherlands and which was close to the Maastricht area of first infection (AFI).
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.

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 northern Europe. Thus the particular climatic conditions that prevailed in the area at the time of the outbreak, which made it significantly warmer than at any time in recorded history, appear to have been such as to promote the successful replication of BTV in and transmission by more than one species of vector. A lesson to be learned here is that in the future the continuation of climate warming is likely to enhance the vector potential of still other species of *Culicoides* endemic to the region.

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.

In this context 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, for example, 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 northern 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.

The most recent entomological data to emerge is that low numbers of adult Culicoides principally of the Obsoletus Complex, and including freshly bloodfed individuals, have on occasion been captured in light traps operated throughout the winter (January, February and March 2007) in various MSs in northern 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 seroconversions to the virus in sentinel cattle have been reported since January 2007. Nevertheless, at the time of writing in March 2007, it would be premature to conclude that BTV will not overwinter in the region and therefore vigilance must remain high.

In regard to midge abundance levels it is notable that >1 000 Culicoides were found in fewer than 5% of the approximately 500 light trap collections made to date throughout northern Europe and when using mostly the ‘golden standard’ Onderstepoort-type blacklight trap. In The Netherlands, during the ‘snapshot’ survey conducted in September 2006, an average of 333 Culicoides was captured/trap night. Of these only half belonged to vector species, which, on average, is 40-180x lower than the average vector densities encountered during the same month in parts of the Mediterranean Basin where BTV was also circulating. This finding can be interpreted as follows:

- It may indicate that comparatively low mean Culicoides densities were able to maintain a very efficient level of BTV circulation across northern Europe, and/or
- It may indicate that vector densities as estimated from light trap collections are a gross underestimate of the actual numbers of Culicoides attacking livestock, possibly because midges may bite also during the daytime particularly on days when it is overcast and when light intensity levels are low at which times light traps are not usually operated.

The proportion of the midge populations involved in such daytime attacks is not known but if it is large this could have important implications for the diurnal transmission of BTV. For obvious reasons it is not possible to monitor diurnal Culicoides activity using light traps; different capture methodologies will have to be implemented to obtain this kind of information in the future (e.g. bait animals, suction traps, CO₂ traps).

Various attempts (both in France and in The Netherlands) failed to incriminate species of the Pulicaris Complex in the transmission of BTV. In northern Europe the Pulicaris Complex comprises at least six species and when these are counted together this can result in the complex being recorded as the dominant taxon in some areas. However, previous work, elsewhere, has incriminated only one of the constituent species of the Pulicaris Complex i.e. C. pulicaris sensu stricto (ss) as being involved in the transmission of BTV and in northern Europe seems less
abundant than some of the other members of the complex such as *C. punctatus* and *C. newsteadi*. Indeed, according to the data collected thus far *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 northern European BT outbreaks possibly because of its low abundance and restricted distribution in the area.

It is worth examining also, and in greater detail, additional data collected on *C. pulicaris ss* in Crapoel (The Netherlands) and in north-eastern France. In Crapoel it comprised 15% of the *Culicoides* captured outside cattle sheds and in France 11%. However, inside cattle sheds it comprised, respectively, only 0.2% and 0.3% of the thousands of *Culicoides* captured. These data may mean that:

- *C. pulicaris ss* is strongly exophilic and preferentially feeds only on cattle that are maintained outdoors;
- *C. pulicaris ss* is exophilic and rarely feeds on cattle preferring some unknown alternative host such as birds; or
- *C. pulicaris ss* feeds regularly on cattle but does so mostly diurnally and for this reason it is not being captured in significant numbers in light traps which operate efficiently only in the dark.

These various possible interpretations demonstrate how little knowledge exists on the daily life cycle and biting habits of the vector *Culicoides* of northern Europe and they require detailed ecological investigations to resolve.

As noted above at least six species of the Pulicaris Complex occur in The Netherlands and it is likely that a similar situation applies also in the other MSs in the region. During the recent ‘snapshot’ survey in The Netherlands the geographic ranges of all these species were mapped and showed most to have distinct patterns of occurrence, which are a reflection of their specific biologies. For example, *C. halophilus* was found in association with saline coastal habitats whereas *C. impunctatus* — represented by a single specimen — was found only in the vicinity of peat bogs. This preference for bog-lands was confirmed in a separate study conducted in The Netherlands in which it was found that *C. impunctatus* occurred also in wetlands and that it was on the wing only in the first half of summer. This finding suggests that *C. impunctatus* played no significant role in the epidemiology of the 2006 BT outbreak which occurred in the latter part of the year at a time when this species was no longer active. These observations highlight the fact that each taxon within the Pulicaris Complex inhabits an almost exclusive biotope, which will influence greatly its distribution both in space and time and hence its potential involvement in any BTV outbreak. This makes it imperative to be able to identify each of the taxa accurately in order to be able to interpret field data correctly and provide appropriate advice to the veterinary authorities.
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 compelled 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 require 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 find answers.

The discovery that larger numbers of Culicoides may be found indoors than outdoors, and 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 certainty.

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 encephalitis virus) 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.

**Factors that can predict virus persistence: animal densities, environmental factors, and 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 number of outbreaks was estimated to be about 4 weeks. Even the first officially reported farm with clinical symptoms and the two peaks of the epidemic shifted back by 4 weeks coincided with the peaks of the warmer time periods, which may indicate that in June and July some farms might have been infected and that the transmission rate of BTV-8 by Culicoides increased with increasing temperature between weeks 26 and 29. 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 showed also a falling trend.

Consistent differences in meteorological conditions were identified between areas where BTV-8 was present and areas where BTV-8 was absent in the affected counties 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 also 2 or 3 °C warmer in the winter preceding the outbreak. In 2006, in the affected countries temperatures rose unusually rapidly in summer and peaked 3 to 5 °C higher than average both in the day and at night. Whether conditions in 2006 represent a significant (or more importantly a biologically relevant) departure from the longer term climatology experienced in Northern Europe is a question best tackled using long time –series of ground-based meteorological data.
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. 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.

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 to the training set data.
Factors affecting short distance spread

*Observed speed of local spread*

As indicated above, the observed speed of local spread was estimated to be 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 in Sardinia.

*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.

Factors affecting long-distance-spread into new areas

*Potential for vector spread through wind*

Density of observed wind events contribute to explain, at least in part,
- the predominant horizontal (east-west) spread,
- the more limited spread north and south spread, and
- the absence of recorded outbreaks in the U.K.

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 installation of primary outbreak clusters that are sufficiently dense may inhibit the long range spread of the epidemic in the absence of virus carrier movement.

Terrain roughness may be an important factor preventing spread of infected midges.
Restrictions on animal movements

The analysis of the association in time between the implementation of control measures and incidence of new infections of BTV-8 did not indicate a significant impact of the control measures. 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 since it concerned an amendment resulting in less restrictive measures. This may suggest that control measures had only a limited or even no effect on the dispersion of the virus. But it should be noted that the analysis did not consider the possible impact of covariates which may also have had an influence on the spread of the disease, such as daily temperature or humidity.
12 Acknowledgements

The data utilised by the EFSA Bluetongue Epidemiology Working Group are the result of the work of a large number of dedicated individuals, including animal owners, veterinary practitioners, experts in diagnostic and other aspects of bluetongue disease, national and European-level veterinary services, and EFSA staff.

In addition, the following individuals also contributed to various sections of the epidemiological analysis: M. Aerts (Hasselt University, Belgium), B. Amzal (EFSA, Italy), A. Backx (CIDC-Lelystad, the Netherlands), T. Baldet (CIRAD, France), D. Benz (Oxford University, U.K.), A. Besch (Ministry of Agriculture, Luxembourg), J. Bortels (Gembloux Agricultural University, Belgium), D. Carton (EC, Belgium), M. Cavelier (Wallon Agricultural Research Center Gembloux, Belgium), B. Codina (Avia-GIS consultant, Belgium), F.J. Conraths (FLI, Germany), R. Cors (Wallon Agricultural Research Center Gembloux, Belgium), J. Cortinas (Hasselt University, Belgium), R. De Deken (ITMA, Belgium), T. DeFrance (Wallon Agricultural Research Center Gembloux, Belgium), B. De Groot (Avia-GIS, Belgium), J.-C. Delecolle (University Louis Pasteur France), E. Ducheyne (Avia-GIS, Belgium), H.M. Ekker (Food and Consumer Product Safety Authority, the Netherlands), C. Faes (Hasselt University, Belgium), C. Fassotte (Wallon Agricultural Research Center Gembloux, Belgium), F. Francis (Gembloux Agricultural University, Belgium), A. Froehlich (FLI, Germany), J. Gethmann (FLI, Germany), M. Gilbert (Avia-GIS consultant, Belgium), J. Gloster (IAH, U.K.), M. Goffredo (IZS Teramo, Italy), E. Haubruge (Gembloux Agricultural University, Belgium), P. Leis (CUR, The Netherlands), B. Losson (University of Liège, Belgium), M. Madder (ITMA, Belgium) E. Meroc (VAR, Belgium), N.V. Nguyen (EC), D. Rogers (Oxford University, U.K.), J. Scharlemann (Oxford University, U.K.), F. Unger (FLI, Germany), A.N. van der Spek (Food and Consumer Product Safety Authority, the Netherlands), P.A. van Rijn (CIDC-Lelystad, the Netherlands), D. Verloo (EFSA, Italy).

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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-8 subgroup which currently includes the following experts: A. Brouw (EC, Belgium), A. Conte (OIE Ref Lab., Italy), H. Deluyker (EFSA, Italy), A. Elbers (CIDC-Lelystad, the Netherlands), G. Gerbier (CIRAD, France), G. Hendrickx (Avia-GIS), R. Meiswinkel (CIDC-Lelystad, The Netherlands), Ph. Mellor (IAH, UK), K.
Appendix 1

Mandate from the European Commission
Dear Ms. Grelier-Lautelle,

Bluetongue is a non-contagious, insect-transmitted, viral disease of domestic and wild ruminants. A serious outbreak of this disease, caused by bluetongue serotype 8 (BTV8), is currently affecting the Netherlands, Belgium, Germany and France.

At the meeting of the Chief Veterinary Officers of all Member States organised by the Commission on 22 September 2006, in which EFSA representatives participated, the type of scientific assistance that might be required in relation to the ongoing outbreak was discussed. The preparation of reports on the disease situation and epidemiological analysis of surveillance data at EU level were identified as areas where EFSA could assist the Commission and the Member States and support their decision-making process in relation to the measures to be taken against this disease.

Therefore, in the context of Article 31 of Regulation (EC) No 178/2002, I would request EFSA to provide scientific assistance to the Commission by preparing regular reports on the disease situation, as well as an analysis of epidemiological data in relation to the ongoing outbreak, which, taking into account the seasonal pattern of this disease, is expected to fade out within the next two months. This work should start as soon as possible.

In addition, I would like EFSA to carry out a global epidemiological analysis of the ongoing outbreak which should be finalized by 31 January 2007.

I understand that EFSA has already planned work on these issues, and I will be happy if EFSA could ensure full coordination and synergy, when carrying out the tasks mentioned above, with the Commission and the other veterinary authorities responsible for taking action and measures in relation to this disease.

I propose that we keep this in mind during our review with the aim of adapting it in the light of the evolution of the disease and look forward to our future cooperation on this topic.

Mr. Catherine Grelier-Lautelle
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Brussels, 05 Oct. 2006
SANCO 0 b. 15. 2006
05 OCT. 2006

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Appendix 2a
Characterisation of clinical signs

Appendix 2b
Characterization of morbidity, mortality and case fatality

Appendix 3
Within herd distribution of infection

Appendix 4
Time space distribution of virus spread between herds

Appendix 5
Role of environmental factors – wind analysis

Appendix 6
Role of environmental factors – small scale environmental analysis

Appendix 7
Role of environmental factors – large scale environmental analysis

Appendix 8
Role of human intervention

Appendix 9
Vectors