Preliminary Risk Analysis of introduction of Bluetongue virus and West Nile virus into the Netherlands

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Summary

Outbreaks of Bluetongue virus (BTV) were recently reported on Sardinia in Italy (17 September 2003) and on the Spanish island of Minorca (28 October 2003). In addition, an article suggesting that BTV might reach Northern Europe as well appeared in the British press (The Guardian) only a short time earlier (10 September 2003).
On 21 October 2003, two people were reported to be infected with West Nile virus (WNV) in Southeastern France. It is assumed that this infection was contracted locally. In addition, antibodies against WNV were recently found in birds in the United Kingdom. Although the latter reports must still be further confirmed, a meeting of the working group "Arboviruses" of the European Committee in Brussels concluded that the introduction of WNV into (Northern) Europe is possible.

This series of events induced the Veterinary and Food Policy Department (VVA) of the Dutch Ministry of Agriculture, Nature and Food Quality (LNV) to ask for advice regarding a Risk Analysis of the possible introduction of BTV and WNV in the Netherlands.
This report is an initial step in conducting a Risk Analysis of the introduction of BTV and WNV in the Netherlands.

Bluetongue
Based on the BTV outbreaks described in recent literature and the related distribution of *Culicoides spp*. (midges), which are an important vector for BTV, the following can be stated:

1) The fact that a tendency towards a warmer climate in Northern Europe has set in over the last decades has to be taken into account. Within a larger framework of time (centuries) this does not necessarily mean a permanent change, but it is likely that Northern Europe will have a warmer climate for the next decades.

2) In combination with the previously described changing climatic conditions, the area where the *Culicoides spp.*, the vectors associated with BTV, can survive will expand from Southern Europe to the North.

3) As a result of the suppositions stated under 1) and 2) there is an actual possibility that BTV might be introduced in Northern Europe, and thus also in the Netherlands.

4) Animal transport in general and in the North-South direction in particular has intensified in recent years. This also enables the vector and/or BTV to expand its range of distribution.

5) In order to conduct a genuine Risk Analysis, the Netherlands will have to collect information or make information available regarding:

a) The frequency and presence of *Culicoides spp*. in the Netherlands and surrounding countries such as Belgium and Germany (entomological knowledge centres in the Netherlands such as WUR - Alterra).
We know that about 50 *Culicoides spp*. occur in the Netherlands, but little or no information is available about distribution, density and seasonal cycles.

b) The vector competence for BTV transmission of *Culicoides spp.* that are already present in the Netherlands.
No information on this is currently available.

Recomendation:
Commission an entomological knowledge centre (WUR - Alterra) to conduct a preliminary study in collaboration with CIDC-Lelystad, making an inventory of the knowledge with respect to:
- **the frequency and the presence of *Culicoides spp*. in the Netherlands and surrounding countries such as Belgium and Germany;**
- **the vector competence for BTV transmission of *Culicoides spp.* that are already present in the Netherlands.**

And to indicate the requirements for filling in any knowledge gaps

6) Serological and virological BTV tests that meet the OIE standards are currently available and performed at CIDC-Lelystad. However, the expertise is limited, as is the immediately available stock. A supply of about 10,000 serological test samples is available that can be replenished within four weeks. However, in case of an international outbreak in - say - Northern Europe, it may be expected that the international supply of test kits will stagnate. The virological test (to identify BTV as such) is a labour-intensive process and the results may take a while (up to 2 weeks). Moreover, the test is very specialised and consequently the number of people that know how to handle it cannot easily be increased. Currently research is being conducted to develop a fast test to identify BTV (PCR diagnostics). An initial concept is expected to become available over the course of 2004.

7) In case of introduction to the Netherlands, the control of a BTV outbreak will initially have to focus on disrupting the major transmission routes of the virus (eliminating the vector, reducing animal transport, etc.). Vaccination is not attractive from a trade economy point of view; moreover, it is extremely complex. BTV includes 24 different serotypes, so characterizing the virus involved in the outbreak (which of the 24 serotypes is involved) is a prerequisite. Currently, CIDC-Lelystad cannot perform this characterization. It has been decided that the OIE reference laboratory will do the characterization. No emergency vaccine is currently available for any BTV serotype.

8) It is recommended to develop crisis strategies for BTV control so that considerations and choices regarding the possible use of vaccines and vector control methods can be dealt with now.

Recommendation: Develop crisis strategies for BTV control

9) If the threat increases, major investments are required in the development of tools to adequately and effectively fight a BTV outbreak.

Recommendation:
Keep a watchful eye on the BTV situation in Southern Europe and the possible advance to Northern Europe. Should outbreaks be reported from Southwest England and/or from France, it will be time to develop tools to adequately and effectively fight a possible BTV outbreak in the Netherlands

The conclusion is that the Netherlands should currently advocate an incremental response with regard to BTV. This should include developing a crisis strategy for BTV control. Furthermore, the frequency and the presence of *Culicoides spp*. in the Netherlands and the surrounding countries such as Belgium and Germany should be investigated; the vector competence of *Culicoides spp.* that are already present in the Netherlands should also be investigated. Last but not least the BTV situation in Southern Europe and the possible advance to Northern Europe should be carefully monitored.
Should outbreaks be reported from Southwest England and/or from France it will be time to develop tools, in combination with the answers to the previously described research questions regarding the vector, to adequately and effectively fight a possible BTV outbreak in the Netherlands.

West Nile virus
The presence of West Nile virus (WNV) has been proven in Northern Europe, in any case in wild birds. Until recently there were no indications for and/or observations of human West Nile Fever or encephalitis in Northern Europe (with the exception of infections contracted while visiting abroad, for example in the USA). However, very recently, on 21 October 2003, two people with a West Nile virus (WNV) infection were reported in Southeastern France. It is assumed that this infection was locally contracted.
This would indicate that infected vectors have reached Southern France. Indications suggesting this were also observed about a week earlier (11 October 2003), when a horse in the same region in Southeastern France was suspected of having been infected with WNV. Currently no reports have been received of excessive numbers of deaths among wild birds in Northern Europe, which could indicate a WNV introduction.
Consequently, the introduction of a well-functioning surveillance programme (focused on possible infection of people, horses, birds and midges) is now a good measure.

Recommendation:
Extend the surveillance programme for detecting possible WNV infections in humans, horses (in collaboration with the Faculty of Veterinary Medicine) and birds, as developed by the RIVM (the Dutch National Institute for Public Health and the Environment), with a surveillance programme for midges in the Netherlands that may be infected with WNV .

At the RIVM, people are working on extending the flavivirus diagnostics for WNV to include RT-PCR diagnostics. Then at least the acute problems can be diagnosed, though the capacity will be limited and insufficient for major outbreaks.
CIDC-Lelystad is considering maintaining its own diagnostic capacity (PCR, commercially available serological assays), thus creating diagnostics capacity in combination with the RIVM.

For a genuine Risk Analysis regarding WNV in the Netherlands, information will have to be collected or made available regarding:

a) the presence and the density of types of midges that are capable of acting as a vector in the Netherlands and in surrounding countries such as Belgium and Germany (entomological knowledge centres in the Netherlands, such as WUR - Alterra);

b) the vector competence for WNV transmission of the types of midges present in the Netherlands;

c) what native bird types are susceptible to WNV infection and may play a role in the epidemiology of WNV. These birds might possibly be a suitable target group in surveillance activities, as well.

d) the diagnostics capacity required in case of WNV outbreaks in the Netherlands.

Recommendation:
Commission Entomological knowledge centre (WUR - Alterra) to conduct a preliminary study in collaboration with CIDC-Lelystad, making an inventory of the knowledge with respect to:
- **the presence and the density of types of midges that are capable of acting as a vector, in the Netherlands and surrounding countries such as Belgium and Germany;**
- **the vector competence for WNV transmission among the types of midges present in the Netherlands.**

And to indicate the requirements for filling in any knowledge gaps.

Recommendation:
Commission Ornithological knowledge centre (WUR - Alterra) to conduct a preliminary study in collaboration with CIDC-Lelystad, making an inventory of the knowledge with respect to:
- **which native bird types are susceptible to WNV infection and may as such play a role in the epidemiology of WNV**

And to indicate the requirements for filling in any knowledge gaps.

Recommendation:
Commission CIDC-Lelystad and RIVM to make a joint assessment of the required diagnostics capacity in case of WNV outbreaks in the Netherlands based on experience gained in the United States of America.

Moreover, developing crisis strategies for WNV control is recommended so that considerations and choices regarding vector control methods can be dealt with now.

Recommendation: Develop crisis strategies for WNV control

It has recently become clear that WNV can be transmitted via breast feeding, blood transfusion, organ transplant and trans-placental infection. These aspects have not yet been fully studied and require further research that is being conducted abroad. It is essential to keep a careful watch on the developments in this respect.

Recommendation:
Keep a watchful eye on developments in the research (conducted abroad) into the transmission of WNV via breast feeding, blood transfusion, organ transplant and trans-placental infection.

In view of the recent report regarding a local WNV infection in people in Southern France, it is recommended that the possible risks of a WNV infection be pointed out and that the related precautionary measures be explained to Dutch holidaymakers (for themselves as well as for pets they bring along) prior to the upcoming tourist season by launching an information campaign.

Recommendation:
Prepare an information campaign prior to the summer season of 2004 in order to point out the possible risks of a WNV infection and the related precautionary measures to holydaymakers heading for Southern France.

The conclusion is that the Netherlands should currently advocate an incremental response with regard to WNV. This should include developing a crisis strategy for WNV control. Moreover, the presence and the density of types of midges that are capable of acting as a vector in the Netherlands and the surrounding countries must be investigated, as well as the vector competence for WNV transmission of the types of midges present in the Netherlands. Furthermore, what native bird types are susceptible to WNV infection and may play a role in the epidemiology of WNV must also be investigated. Last but not least, the WNV situation in Southern Europe and the possible advance to Northern Europe should be carefully monitored.

1. Background
Outbreaks of Bluetongue virus (BTV) were recently reported on Sardinia in Italy (17 September 2003) and on the Spanish island of Minorca (28 October 2003). In addition, an article suggesting that BTV might reach Northern Europe as well appeared in the British press (The Guardian) only a short time earlier (10 September 2003).
On 21 October 2003, two people were reported with a West Nile virus (WNV) infection in Southeastern France. It is assumed that this infection has been locally contracted. In addition, antibodies against WNV were recently found in birds in the United Kingdom. Although the latter reports still have to be further confirmed, a meeting of the "Arboviruses" working group of the European Committee in Brussels concluded that introduction of WNV in (Northern) Europe is possible.

This series of events induced the Veterinary and Food Policy Department (VVA) of the Dutch Ministry of Agriculture, Nature and Food Quality (LNV) to ask for advice regarding a Risk Analysis of the possible introduction of BTV and WNV in the Netherlands.

2. Bluetongue (BT)

2.1 Hazard Identification

2.1.1 *Virus*
BTV is an RNA virus (genus: Orbivirus, family: Reoviridae) of which 24 different serotypes are known. BTV can infect all ruminants, but serious clinical disease specifically occurs in some types of sheep and some types of deer.

2.1.2 *Vector*
BTV primarily spreads via a vector (certain types of midges) and is not contagious. The most important BTV vector in the 'old world' is the midge *Culicoides imicola*. This is an African-Asian type and one of the widely spread types of *Culicoides spp.*.
C. imicola was first observed in Southern Europe in 1982 and is currently found in large parts of Portugal and Southwest Spain. Moreover, *C. imicola* has also been reported on various Greek islands. *C. imicola* reproduces in humid and wet soil that is enriched with fresh and composted manure and feeds on the blood of large vertebrate hosts. Consequently the situation on and around large cattle breeding farms is ideal for *C. imicola* since reproduction sites and food (blood from cattle, sheep and horses) are abundantly available. The number of *C. imicola* midges in the environment strongly decreases as the distance from a cattle breeding farm increases.

The following *Culicoides* types of midges have been found in relation to BT (sources: Meiswinkel et al., 1994; Mellor and Wittmann, 2002; Savini et al., 2003; Caracappa et al., 2003; Monteys and Saiz-Ardanaz, 2003; Miranda et al., 2003) :

Culicoides spp.	**Countries**
C. tororoensis, C. imicola	Africa, Asia, Southern Europe
C. bolitinos, C. gulbenkiani	Africa, Asia
C. obsoletus	North America, Italy, Cyprus, Spain including the Balearic Islands
C. insignis	Central & South America
C. milnei	Africa, Asia
C. pycnosticus	Africa, Asia
C. variipennis (sonorensis)	North America
C. pulicaris	Southern Europe
C. brevitarsis, C. actoni, C. fulvus, C. oxystoma, C. peregrinus, C. wadai	Australia

The vector capacity of a *Culicoides* population (and thus the virus transmission potential) is influenced by:
a) the number of fully grown midges in the population. The development rate of *Culicoides* from egg to fully grown is directly related to the temperature.
b) the percentage of fully grown midges that is capable of virus transmission depends on:
- the vector competence (the suitability of the vector to act as a virus replicator);
- the development time of the virus in the vector;
- the suitability of the vector to act as a virus multiplier;
- the survival of fully grown midges;
- the length of the blood meal interval.

The vector competence is influenced in part by the temperature. For example, no Orbivirus (to which BTV belongs) replication is possible in *Culicoides* at temperatures lower than 10-15 degrees Celsius (depending on Orbivirus spp. and serotype). Generally there is a linear increase in vector competence as the temperature increases within the temperature range in which Orbiviruses can develop.
Not much vector research has been conducted into the differences between the types of midges within the *Culicoides* group, but the studies that have been conducted indicate that differences in vector competence between the various types of *Culicoides* midges are to be expected. For example, *C. imicola* is very competent; in comparison *C. obsoletus* is expected to be less competent, though still effective enough for virus transmission

2.1.3 *Clinical signs*
Clinical signs associated with a BTV infections that may be observed, are: fever, depression, excessive salivation, a drippy nose, facial edema, hyperaemia and infection of the mucous membranes of the mouth, coronitis, lameness, amyosthenia, abortion and death.
The percentage of deaths and the seriousness of the clinical signals may vary by race and by the age of the infected animals (older animals are more susceptible), the virulence of the virus and environmental factors. Native sheep, goats and cattle in third world countries generally have a higher resistance (because of the presence of antibodies and/or a genetic resistance) to the clinical effects of infection; as a result, the majority of BTV outbreaks worldwide are not apparent. This hidden presence of BTV (the presence of (sub-)clinical infections with negligible clinical signs) has a large, negative influence on the international trade in cattle, sheep and their semenand egg-cells since countries want to preserve their BT-free status.

2.1.4 *Diagnostics*
Various isolation systems are suitable for BTV, including embryonated chicken eggs, sheep and cell culture (BHK-21, Vero or AA cells). After incubation the virus can be identified using antigenic detection methods. Moreover, the virus can be identified using PCR techniques, immunological methods (Immunofluorescence, antigenic-ELISA and immunoperoxidase tests) which make serotyping possible. Since cross-reactivity with Epizootic haemorrhagic disease virus (EHDV) may occur, BTV group-specific monoclonal antibodies must be used. Furthermore, serological methods are used : Complement fixation test, agar gel immunodiffusion and competitive ELISA (OIE Diagnostics Manual).

2.2 Risk Assessment

2.2.1 *Epidemiology*
BTV primarily spreads via a vector (certain types of midges) and is not contagious. Practically no virus is found in the secretion and excretion products of infected animals. Moreover, BTV can also spread without mediation of this vector (via semen, egg-cells and through contamination of the fetus). However, where the spread of BTV is involved, the route via the vector is by far the most important.
In the field, BTV is virtually exclusively transmitted from one vertebrate host to another by various types of stinging arthropods. The most important of these vectors are various types of *Culicoides* midges.
BTV enters the vector when the midges feed on blood and the virus replicates in the salivary glands. When the vector then feeds on other animals, the virus spreads to new hosts. Consequently, the BTV distribution is primarily correlated to the presence of these types of vectors. The vector activities, in

turn, depend on the parts of the year with the most favourable climatic conditions. Indeed, BTV outbreak zones show a peak in *Culicoides* populations in late summer and in autumn and this corresponds to the time of year in which BTV is most often observed.
If the vector cannot survive a period of one year, annual outbreaks either may occur because of new introductions from infected areas in the vicinity or may be proof of a low level of perseverance from year to year. Annual reintroduction from enzootic foci of the virus is possible over a range of more than 100 km because of transport of infected *Culicoides* midges by the wind (evidence has been found suggesting it is possible to bridge distances of up to 700 km through the air).
Conventional models for the life cycle and the transmission of BTV assume that bad (vector free) winter conditions for a period of more than 100 days (the maximum viraemic period in cattle, in sheep this is about 50 days), should prevent the virus from surviving from one year to the next. However, in some parts of the world it has been demonstrated that BTV has survived during the winter despite the absence of the vector, for example in Turkey between 1977 and 1981. In Corsica and Sardinia and in Calabria on the Italian mainland between 2000 and 2002, and in Serbia and Kosovo.
Protracted virus perseverance in the mammal host could be the mechanism at work here. This hypothesis is supported by the finding that BTV can cause persistent *in-vitro* infection of sheep T-cells (Takamatsu et al., 2003).

The spatial and temporal incidence of BTV is influenced by the distribution of the *Culicoides* vector and its capacity to transmit the virus to a host. Distribution and transmission capacity are influenced strongly by climatic factors.
As stated earlier, the vector competence is influenced by the temperature. For example, no Orbivirus replication is possible in *Culicoides* at temperatures lower than 10-15 degrees Celsius (depending on Orbivirus spp. and serotype). Generally there is a linear increase in vector competence as the temperature increases within the temperature range in which Orbiviruses can develop. Consequently, it is plausible that changes in weather and in climate will result in changes in the extent to which BTV will occur:

1) Climatic changes scenario: global warming, increased frequency in the number of warm days; spring sets in earlier and autumn starts later; the average growing season in Europe has increased by eleven days since the 1960’s because of higher temperatures.

2) It is likely that warmer temperatures will result in an extension of the distribution range of vector types (in the direction of the poles or to higher situated parts). This could cause *C.imicola* to settle for longer periods of time or even permanently in Eastern Spain, Southern France, Northern Italy, Northern and Southern Greece and the coastal areas of Albania, Montenegro, Bosnia-Herzegovina and Croatia.

The implications are far-reaching: the expansion of the *C.imicola* range enlarges the area in Europe in which there is a risk of BTV. Moreover, the wider distribution of *C. imicola* could bring BTV within reach of *C. obsoletus/pulicaris* midges more often, which could lead to larger areas in Europe being stricken with BTV.

3) Changes in vector capacity: higher temperatures will increase the number of fully grown *Culicoides* as well as the number of generations per year and the hibernation capacity (shorter and warmer winters). Furthermore, it is likely that the proportion of fully grown *Culicoides* capable of transmitting BTV will become larger, which leads to an increased chance that BTV will develop in the vector.

2.2.2 *Worldwide distribution*

The distribution of the vector, and thus of BTV, is generally limited to the area between 35° south latitude and 40° north latitude. Within this area the vector is distributed virtually worldwide. It has been observed in North, Central and South America, Africa, the Middle East, the Indian subcontinent, China, South-East Asia and Australia. In addition, the distribution area appears to extent to almost 50° north latitude in some parts of Western North America and in China (Mellor et al., 2000).
BTV has also hit Europe a number of times. In the 1956-1960 BTV epidemic in Spain and Portugal (serotype 10) 180,000 sheep died. In 1979-1980 an epidemic occurred on the Greek islands of Rhodes and Lesbos. BTV appears to have established itself in Southern Europe.

2.2.3 *Epidemiological situation 1998-2001*

Between 1998 and 2001, BTV outbreaks developed in at least 10 countries in the Mediterranean where the disease does not usually occur (Mellor and Wittmann, 2002).

2.2.3.1 *Greece, Bulgaria and Turkey (Southeastern Europe)*

After having been absent for nearly 20 years, BTV was detected at four different Greek islands in October of 1998 (serotype 9). In June 1999 BTV outbreaks were observed in Southeast Bulgaria (500 km from the Greek outbreaks), and subsequently BTV spread rapidly to the West via Southern Bulgaria (also serotype 9). The outbreaks in Southern Bulgaria continued up to October 1999. In July of 1999, BTV outbreaks were reported from Turkey, in two provinces bordering Bulgaria and the Greek mainland. In reaction to this, 60,000 sheep were vaccinated with a live vaccine (serotype 4) at a point in time at which it was not yet clear that serotype 9 also circulated in Turkey. In August 2000, BTV was again back in Turkey, but now serotype 16 was isolated. In August 1999, BTV was reported on the Greek mainland, initially in the Northeast near the Turkish border and from there it spread to the West until December 1999. After the winter period, some sporadic outbreaks occurred in the summer of 2000. In addition there was a separate introduction of BTV to the Island of Lesbos, which spread to various other small Greek islands.

One complicating factor in the Greek outbreaks was that various types of serotypes were detected, which is an indication of different, independent introductions.

In September 2001, BTV was again reported from Greece, making that the fourth year in a row. This time the outbreak was in the Northwest, near the Albanian border. At the same time BTV outbreaks were reported from Serbia, Montenegro, Kosovo, Macedonia, and Bulgaria and there were suspicions in Croatia. BTV had never been reported before in these areas.

With the outbreaks in Serbia at 44.30°N, and the detection of antibodies against BTV in ruminants in Kazakhstan at 49.30°N (Lundervold et al., 2003), BTV has reached its most northern outpost in (Eastern) Europe so far.

With regard to the vectors found in this region, the following could be concluded:
1) *C.imicola* is currently spreading to the North and to the West;
2) In Northern Greece and Southern Bulgaria a new, not yet identified vector has probably been added, most likely *C. obsoletus* and/or *C. pulicaris*.

2.2.3.2 *North Africa, Italy, France and Spain (Southwestern Europe)*

BTV outbreaks in North Tunisia were first reported in January 2000 (serotype 2). In July 2000 BTV outbreaks were reported from East Algeria, which borders Tunisia, and by the end of 2000 antibodies were found in animals in 18 different provinces as far as Northern Morocco.

A dramatic development occurred in August 2000, when BTV (serotype 2) first appeared in Italy and spread over the islands of Sardinia (4000 outbreaks and 90,000 sheep died or were killed) and, to a lesser extent, Sicily. BTV subsequently spread to the Southern regions (Calabria) of the Italian mainland (4).

The winter of 2000-2001 was relatively quiet, probably because of the lower temperatures that reduced the vector or were too unfavourable for an effective biological cycle. However, in October 2000 BTV outbreaks were reported on the French island of Corsica and on the Spanish islands of Minorca and Majorca.

There, too, the outbreaks lasted until November and December of 2000. No outbreaks have been reported since then. However, in September of 2001 BTV outbreaks were reported at various locations on Corsica (serotype 2), at more than 200 locations on Sardinia (serotype 2) and in Calabria (serotype 9) (possibly introduced from Greece) and various parts of Italy north of Rome. This wide spread indicates that the virus had few problems hibernating, despite the rather northerly geographical position of these areas. This new ‘hibernation’ may be quite significant since it represents a dramatic expansion of the virus risk area northward.

2.2.4 *Epidemiological situation 2002 - present*

In 2002 BTV outbreaks were reported in Bosnia-Herzegovina and in Bulgaria and in 2003 BTV outbreaks were reported in Tunisia, Yugoslavia and very recently in Italy (Sardinia) where both serotype 2 and serotype 4 were detected. Italy has started a vaccination campaign, distinguishing three types of vaccination areas: a) vaccination with a monovalent vaccine, b) vaccination with a bivalent vaccine, c) vaccination with a pentavalent vaccine. At a meeting of the EU Standing Committee on the Food Chain and Animal Health on eight and nine October 2003 it was reported that

the vaccination level varies considerably per region (0 to 90% of the animals), which is anything but ideal. In areas with a high level of vaccination the infection could be eliminated.
France feared that BTV would cross from Sardinia (in view of the recent outbreaks there) to Corsica, and was planning to vaccinate Corsica's entire sheep stock with a bivalent vaccine. Unfortunately they appeared to be too late. An outbreak of BTV in sheep on Corsica was reported on 31 October 2003. Furthermore, an outbreak of BTV in sheep was also reported on the Spanish island of Minorca on 29 October 2003.

2.3 Risk Management

2.3.1 *Control measures to prevent introduction*

Most countries in the EU, including the Netherlands, demand testing for BTV antibodies or virus isolation of cattle and sheep and animal products such as semen, originating from (third) countries that are not free of BTV. This is necessary because infected sheep can be viremic for about 50 days and cattle for up to as much as 100 days. Because of these imported and infected sheep, cattle and semen could be hazardous to the importing country.

2.3.2 *Control measures in case of outbreaks*

BTV is not contagious and spreads primarily via vector species of *Culicoides* midges. Incidental spread via blood, semen or egg cells may occur. Control and prevention of BTV outbreaks after introduction could thus be achieved in the following ways:

1) Restrict animal transportation to prevent infected animals from becoming a new source of infection;
2) Ban transportation and/or use of semen/egg cells to prevent the virus from spreading along this route;
3) Slaughter viremic animals to prevent them from becoming a virus source for the vector;
4) Slaughter serologically positive animals in due time;
5) Vector control;
6) Adapt housing;
7) Vaccination.

The first four measures require no explanation; the other measures will be further elaborated.

ad 5) It is practically impossible to fully destroy the vector populations. Thus the primary objective is to reduce the number of potentially infecting midge bites on the host to such a level that the epidemic will peter out (Reproduction number $R < 1$).

This can be achieved in various ways:
a) destroy the vector's breeding sites (places with manure, wet for 7-10 days) by irrigation, plugging leaks, etc;
b) use insecticide (synthetic pyrethroid) with low mammal toxicity in and around animal stables and on the hosts themselves;
c) apply larvicide to breeding places to stop larval states.

ad 6) This measure focuses on shielding susceptible animals and reducing vector access. This could involve stabling susceptible animals during periods during which the vector is active, which strongly reduces the number of bites and thus also the risk of infection; fit the stable windows with fine-meshed netting.

ad 7) vaccination is an important weapon; however, there are a few problems:
a) the only commercially available BTV vaccines (with the correct serotypes) are manufactured in South Africa and have so far not been admitted to the EU;
b) Manufacturing a vaccine that specifically applies to the local situation in Europe, for example, will lead to at least a 2 to 3-month delay in delivery, which means that such a vaccine can never be used as an emergency vaccination;
c) These vaccines are based on the use of live attenuated virus strains (standard composition of 5 different serotypes) which involves the following risks:
- excretion of vaccine virus in semen;
- possible feto-toxic effects of these living vaccines, thus not to be used on pregnant animals;

- risk of re-assortment between living vaccine viruses and field viruses, which may lead to new virus strains;
- possible circulation of vaccine virus in midge populations;
- return from vaccine virus to virulent virus;

d) The developed vaccines protect against clinical disease, but have not been tested to see if they prevent or dramatically reduce transmission;

e) The strains used in these vaccines originate from South Africa and Pakistan; it remains to be seen whether these are applicable in the European context.

Because of the vaccination issues listed above, various countries in Southern Europe have not used these vaccines. However, in a situation in which the vector expands dramatically, in which new vectors may play a role and the virus enters new geographical areas, the expectation that future BTV introductions can be effectively combated are low in view of the lack of a solid control programme plus vaccination.

2.3.3 *Conclusions*

Based on the BTV outbreaks described in recent literature and the related distribution of the vector *Culicoides spp.* the following can be stated:

1) The fact that a tendency towards a warmer climate in Northern Europe has set in over the last decades has to be taken into account. Within a larger framework of time (centuries) this does not necessarily mean a permanent change, but it is likely that Northern Europe will have a warmer climate for the next decades.

2) In combination with the previously described changing climatic conditions, the area where the *Culicoides spp.*, the vectors associated with BTV, can survive will expand from Southern Europe to the North.

3) As a result of the suppositions stated under 1) and 2) there is an actual possibility that BTV will be introduced in Northern Europe, and thus also in the Netherlands.

4) Animal transport in general and in the North-South direction in particular has intensified in recent years. This also enables the vector and/or BTV to expand its range of distribution.

5) In order to conduct a genuine Risk Analysis, the Netherlands will have to collect information or make information available regarding:

a) The frequency and presence of *Culicoides spp*. in the Netherlands and surrounding countries such as Belgium and Germany (entomological knowledge centres in the Netherlands such as WUR - Alterra).
We know that about 50 *Culicoides spp*. occur in the Netherlands, but little or no information is available about distribution, density and seasonal cycles.

b) The vector competence for BTV transmission of *Culicoides spp.* that are already present in the Netherlands. No information on this is currently available.

6) In case of introduction in the Netherlands:

- Diagnostics: Serological and virological BTV tests that meet the OIE standards are currently available and performed at CIDC-Lelystad. However, the expertise is limited, as is the immediately available stock. A supply of about 10,000 serological test samples is available that can be replenished within four weeks. However, in case of an international outbreak in - say - Northern Europe, it may be expected that the international supply of test kits will stagnate. The virological test (to identify BTV as such) is a labour-intensive process and the results may take a while (up to 2 weeks). Moreover, the test is very specialised and consequently the number of people that know how to handle it cannot easily be increased. Currently research is being conducted to develop a fast test to identify BTV (PCR diagnostics). An initial concept is expected to become available over the course of 2004.

- Intervention possibilities. the control of a BTV outbreak will initially have to focus on disrupting the major transmission routes of the virus (eliminating the vector, reducing animal transport, etc.). Vaccination is not attractive from a trade economy point of view; moreover, it is extremely complex. BTV includes 24 different serotypes, so characterizing the virus involved in the outbreak (which of the 24 serotypes is involved) is a prerequisite. Currently, CIDC-Lelystad cannot perform this characterization. It has been decided that the OIE reference laboratory will do the characterization.
- No emergency vaccine is currently available for any BTV serotype.

If the threat continues to increase, major investments will be required in the development of tools to adequately and effectively fight a BTV outbreak.
Furthermore, the recommendation is to develop crisis strategies for BTV control so that considerations and choices regarding the possible use of vaccines and vector control methods can be dealt with now.

The conclusion is that the Netherlands should currently advocate an incremental response with regard to BTV. This should include developing a crisis strategy for BTV control. Furthermore, the frequency and the presence of *Culicoides spp*. in the Netherlands and the surrounding countries such as Belgium and Germany should be investigated; the vector competence of *Culicoides spp.* that are already present in the Netherlands should also be investigated. Last but not least the BTV situation in Southern Europe and the possible advance to Northern Europe should be carefully monitored.
Should outbreaks be reported from Southwest England and/or from France, it will be time to develop tools, in combination with the answers to the previously described research questions regarding the vector, to adequately and effectively fight a possible BTV outbreak in the Netherlands.

3. West Nile virus (WNV)

3.1 Hazard identification

3.1.1 *Virus*
WNV is one of the Flaviviruses (family: Flaviviridae) and is primarily transmitted to people, birds, horses and a number of other animal species, including dogs, cats, squirrels, seals, bats, reindeer, alligators and frogs, by a vector (certain types of arthropods).

3.1.2 *Vector*
WNV is transmitted by midges of the genus *Culex*, which includes a large number of species. In addition, WNV is sometimes found in *Aedes*, *Anophele*s and *Mimomyia* spp. and in a number of types of ticks. In the moderate climate zones the house midge *Culex pipiens* is considered to be the most important virus transmitter to human beings. This midge feeds on people as well as on birds. Fully grown females can hibernate; in spring they lay their eggs on the surface of water containing a lot of organic material, such as trenches and ditches containing stagnant water.
It has been proven that the virus transmission efficiency is influenced by the temperature and that this might well be one of the most important factors in the establishment and the spread of WNV. The density of the midge population depends strongly on warm weather and a lot of precipitation. 1999 was particularly warm in North America (just like 2003 in Northern Europe) and this may have substantially contributed to the successful establishment of the virus in the wild animal life in North America.

3.1.3 *Clinical signs*

3.1.3.1 *Clinical signs in humans*
Although the virus is endemic in Africa, virtually no epidemics occur with clinical signs in people and animals. Serological research has shown that people, birds and horses have antibodies against WNV, which indicates that sub-clinical infections have a high prevalence (about 80%).
Generally speaking this is also true for major parts of Central and Western Europe and Asia, although recent outbreaks have occurred in Israel, Romania and the Russian Federation. Possible explanations for this recent increase in human outbreaks are the impact of climatic changes or changes in the genetic characteristics of the virus.
Only about 20% of the WNV infections result in West Nile Fever, which is characterized by high fever, complete malaise, headache, backache, and a retro-orbital pain that is worsened by eye movements. Moreover, non-specific clinical signs are reported, such as anorexia, nausea, diarrhoea, coughing and a sore throat. In some patients, conjunctivitis, lymphadenopathy and a red facial coloration occur.
The symptoms of West Nile encephalitis, which are observed in less than 0.1% of the infections, consist of meningitis, encephalitis or myelitis or a combination of the three.
After 1-7 days of headache, weakness and gastro-intestinal phenomena patients become disoriented, dazed and forgetful. Symptoms of a meningitis infection are sometimes observed as well, such as a stiff neck. In some cases WNV may cause paralysis of the limbs and the respiratory muscles, so that artificial respiration is required.
Most patients in the United States with a WN encephalitis or meningitis (WNME) were older people (> 50 years). The median age of 142 reported cases of WNME between 1999-2001 in the United States was 68 years and in 2002, the median age of 2942 reported cases of WNME was 59. Only 4% of the reported cases involved people younger than 18.

3.1.3.2 C*linical signs in horses*
Experience shows that horses are among the most important sentinels for WNV infection and human risk in some parts of the USA. The clinical phenomena in horses may vary strongly, but specifically involve dysfunction of the central and peripheral nervous systems: ataxia (including stumbling, a swaying gait or a lack of coordination) and walking in circles, weakness in the hind legs, difficulty in remaining upright, paralysis of various limbs, blindness, paralysis of the lip, grinding teeth, differences in level of consciousness and facial muscle contractions. Fever was not observed in all cases.

3.1.3.3 C*linical signs in birds*The WNV epidemic in the USA became particularly apparent because of the mass mortality of birds belonging to the Corvidae family (American crow). In Southern regions of the USA mass mortality occurred among infected Blue Jays. On the other hand, virtually no deaths have been observed among chickens, pigeons and pheasants after infection. As a result, these birds are considered to be good sentinels (susceptible to infection but practically no deaths, poor virus replicators so little risk of transmission is involved, but they provide multiple sampling possibilities (blood)).
Experimental infection studies of wild birds in the USA indicated that especially birds belonging to the Corvidae (including several types of crows and Blue Jay) and gulls, exhibit high mortality rates after infection.

3.1.3.4 *Clinical signs in dogs*
Epidemiological studies in the Middle East and in Africa frequently found dogs infected with WNV in serological surveys. Also in the United States10-20% of the dogs tested in serological surveys after 1999 were seropositive. Serious illness (neurological symptoms) and death due to WNV rarely occurs among dogs, though recently a case of fatal WNV encephalitis-myocarditis in a dog in the United States was reported, with kidney and neurological problems,.

3.1.4 *Diagnostics*
The most reliable indication of a WNV infection is virus isolation and subsequent identification with the help of monoclonal antibodies or reverse transcription PCR(RT-PCR) sequencing. Both tests are highly sensitive and very specific.
The use of serological tests (especially ELISA and haemagglutination inhibition tests) has disadvantages because these tests cross react with antibodies for related Flaviviruses.
At the RIVM, people are working on extending the flavivirus diagnostics for WNV to include RT-PCR diagnostics. Then at least the acute problems can be diagnosed, though the capacity will be limited and insufficient for major outbreaks.
CIDC-Lelystad is considering maintaining its own diagnostic capacity (PCR, commercially available serological assays), thus creating diagnostics capacity in combination with the RIVM.

3.2 Risk Assessment

3.2.1 *Epidemiology*
A total of 62 people were diagnosed with encephalitis WNV in New York in the United States in 1999, 7 of whom died of the consequences. At the same time mass bird mortality was observed in New York. The rapid spread of WNV through North America and the impact of the disease on people, birds, horses and a number of other animal species has put WNV high on the agenda of Western authorities and the media (Gould, 2003; van der Poel, 2000). In the entire Eastern part of the US over 4100 people became seriously ill and 284 of them died. In addition, some hundreds of Canadians appeared to be infected. It is very likely that these registered cases represent only a fraction of the actual number of infected people.
Some thousands of people are temporarily down with the flu due to a WNV infection, but the young and the fit usually escape with flu-like symptoms.
In a recent report the Chief Medical Officer (CMO, 2002) in the United Kingdom (UK) has called for attention to the development of a WNV scenario because of the possibility that WNV will be introduced in the UK. The report also indicated that changing environmental conditions because of climatic changes may lead to a temperature increase that will stimulate the population density of the vector midges. Among the 32 types of midges in the UK, at least 7 species (including *Culex pipiens* and *Aedes cantans*) are potentially capable of transmitting WNV.

A potentially important, and not yet fully investigated aspect of the spread of WNV among humans is the observation of WNV transmission via blood transfusion, organ transplant, breast feeding and trans-placental infection.

WNV infections in horses have only been reported a few times. So far, no areas have been discovered where WNV is endemic among horses and so far there are no indications that horses could play an important role as a source of infection for humans. Wild birds are the major reservoir for WNV. The virus has been isolated in a large number of bird types and birds may develop a long lasting viraemia.

Experimental infection studies among wild birds made clear that birds of the Corvidae family as well as the house sparrow and the house finch are among the birds that can readily transmit the virus. Migratory birds are associated with the introduction of WNV to the USA and Europe. In New York the infection was especially fatal for crows. Striking bird mortality was not observed at earlier outbreaks in Europe (in France and in Romania in the 1970's). WNV may be present in healthy birds as a persistent or latent infection.
Germany recently launched an investigation into the presence of WNV in birds and midges because Israel found WNV in storks originating from Germany.

Epidemiological studies in the Middle East and in Africa frequently found dogs infected with WNV in serological surveys. Also in the United States 10-20% of the dogs tested in serological surveys after 1999 were seropositive; one of these appeared to have contracted fatal WNV encephalitis.

It is generally assumed that the virus originally turned up in Africa some hundreds of years ago and was subsequently widely spread in the Mediterranean (the Camarque in France and the Pisa region in Italy), West and Central Europe and Asia and eventually also to Australia, where the virus evolved into a WNV sub-type, named Kunjin virus. Furthermore, the impression exists that in warm weather midges feed more on people and less on birds.

3.2.2 *Surveillance in North and Central America*
Since the first outbreaks in New York in 1999, WVN surveillance began in North America and later extended to include Central America, monitoring infections in people, horses, birds and midges.

A recent update of the WNV surveillance in **Mexico** shows that in the year 2003 up to 6 October:
- 4 of the 481 examined people were WNV positive (PCR);
- 1453 of the 4472 horses examined were seropositive, all but one without clear clinical signs. The horse with clinical signs died.
- 63 of the 17168 birds examined were seropositive (and 2 dead birds), virtually all without clear clinical signs.

A recent update of WNV surveillance in **Canada** shows that in the year 2003 up to 6 October:
- 1130 possible or confirmed human infections were found, 7 people died. Most cases were observed in the prairie states in the Mid-West (Saskatchewan, Alberta, and Manitoba) but also in the Yukon and in British Columbia. In 2002 only 416 possible or confirmed human infections were found and 21 people died (mainly in Ontario and in Quebec). This indicated how strongly the disease is spreading Westward.
- 406 possible or confirmed infections in horses were found, mainly in Quebec, Ontario, Manitoba, Saskatchewan and Alberta; indications are that the risk area is getting larger.
- 1519 of the 11038 wild birds examined were seropositive.

A recent update of WNV surveillance in the **United States (US)** shows that in the year 2003 up to 6 October:
- about 2000 human cases were found in Colorado, about 1000 human cases were found in Nebraska and more than 800 human cases were found in South Dakota;

It is expected that the number of infections in 2003 will be similar to the number in 2002, when 4156 human cases were diagnosed and 284 people died.

The development of WNV in 2003 differs from the development in 2002 because it mainly affects rural areas. Previous outbreaks were caused by midges living near houses and buildings, but in 2003 WNV is primarily spread throughout the Western states of the US by midges of the type *Culex tarsalis*. These midges live near farms, can move over large distances and have been identified as the most efficient WNV vectors so far.

The 2002 WNV outbreaks cost the US a total of US$ 200 million for medical facilities.
The epicentre for WNV infections in the human population in 2004 is predicted to be in California, one of the most densely populated states in the US.

3.2.3 *Surveillance in Northern Europe*
Both in France and in the United Kingdom surveillance of people, birds, horses and midges is conducted; Sweden conducts such a system exclusively for people. Within the European MedVetNet project, in which CIDC-Lelystad participates, diagnostic knowledge regarding WNV is mutually exchanged, as well as samples, primers, antigen and test protocols. Furthermore, a data bank of WNV strains is being created.

3.2.3.1 *Early-Warning System (EWS) in the Netherlands*
Recently antibodies against WNV were found in birds in the United Kingdom. Although the reports still have to be further confirmed, a meeting of the “Arboviruses” working group of the European Committee in Brussels concluded that introduction of WNV in (Northern) Europe is possible. Consequently, the Dutch Ministry of Health Welfare and Sport (VWS) commissioned the Dutch National Institute for Public Health and the Environment (RIVM) to develop an EWS for WNV. Research into the presence of viruses and/or antibodies against WNV in human patients, horses and possibly in birds that may be suspected of being infected with WNV based on their clinical symptoms, is considered to be one of the components of the EWS. Thus, a copy of what has been done in North and Central America.
The examination of horses will be conducted on animals with (neurological) phenomena that suggest WNV, which are presented at the clinic for Internal Diseases of the Department of Equine Sciences of the Faculty of Veterinary Medicine at the University of Utrecht. Moreover, equine practitioners in the Netherlands are requested to participate in the EWS. The clinical symptoms of WNV in horses may vary widely: ataxia, dysmetry, changes in the level of consciousness, hyperesthesia and facial contortions. In case of suspect cases in the field, people are requested to send in a serum sample or paired sera.
The RIVM has conducted a retrospective investigation of human encephalitis patients with virological indications but no diagnosis. Some hundreds of sera have been screened but no WNV infections turned up. Meanwhile, WNV infections as a result of a visit to the US have been diagnosed in three people in the Netherlands. There are plans for conducting a retrospective examination of people with viral encephalitis phenomena.

3.3 Risk Management

3.3.1 *Control measures to prevent introduction*
No possibilities are available.

3.3.2 *Control measures in case of outbreaks*
No specific anti-viral treatment is available for WNV infections. High quality medication to reduce and control the side effects of the disease is effective. Currently various anti-viral medicines are being tested that might be useful over the long term, but it will take many years before these will be effective. Furthermore, research is being conducted into a chimaeric vaccine (based on the very successful Yellow Fever (YFV) 17D vaccine, in which the immunological important component (envelope) of YFV has been replaced by a WNV component); this has shown good results in recent trials.
If the public accepts it, this might well be the precautionary method to prevent future WNV infections. However, it will be several more years before such a vaccine can be marketed.
The most effective and economical precautionary method is changing and eliminating midges’ breeding sites. This involves such activities as removing discarded car tyres, cleaning water butts, birdbaths and unused swimming pools at private homes. Moreover, regional water management by midge control organizations may reduce the breeding sites and pesticides can be used for chemical control (spread via spraying from moving vehicles, etc).
The simplest and most practical control measure is to ensure that you are not bitten by an infected midge. This can be accomplished by staying indoors during those times of day when midges feed (at daybreak and at nightfall).

Moreover, houses will have to be equipped with mosquito nets so that midges cannot get in. It is also recommended that people use anti-midge remedies and wear clothes that cover most of the skin in areas where midges are known to occur.

It has recently become clear that WNV can be transmitted via breast-feeding, blood transfusion, organ transplant and trans-placental infection. These aspects have not yet been fully studied and require further research.

The precautionary methods mentioned above are also important from a veterinary perspective, for example changing and eliminating midge breeding sites. Bites by infected midges can be prevented by keeping livestock indoors during those times of day when midges feed (at daybreak and at nightfall). Furthermore, houses and stables will have to be equipped with mosquito nets so that midges cannot get into buildings where animals are housed.
Fairly recently a USDA approved inactivated vaccine has been marketed for vaccinating horses. This could prevent clinical phenomena and mortality among horses.

3.3.3 *Conclusions*

The presence of West Nile virus (WNV) has been proven in Northern Europe, in any case in wild birds. Until recently there were no indications for and/or observations of human West Nile Fever or encephalitis in Northern Europe (with the exception of infections contracted while visiting abroad, for example in the USA). However, over the last weeks at least 20 human WNV infections were reported in Tunisia and very recently, on 21 October, two people with a West Nile virus (WNV) infection were reported in Southeastern France. It is assumed that this infection was locally contracted.
This would indicate that infected vectors have reached Southern France. Indications suggesting this were also observed about a week earlier (11 October), when a horse in the same region in Southeastern France was suspected of having been infected with WNV. Currently no reports have been received of excessive numbers of deaths among wild birds in Northern Europe, which could indicate a WNV introduction.
Consequently, the introduction of a well-functioning surveillance programme (focused on possible infection of people, horses, birds and midges) is now a good measure.

At the RIVM, people are working on extending the flavivirus diagnostics for WNV to include RT-PCR diagnostics. Then at least the acute problems can be diagnosed, though the capacity will be limited and insufficient for major outbreaks.
CIDC-Lelystad is considering maintaining its own diagnostic capacity (PCR, commercially available serological assays), thus creating diagnostics capacity in combination with the RIVM.

For a genuine Risk Analysis regarding WNV in the Netherlands, information will have to be collected or made available regarding :

a) the presence and the density of types of midges that are capable of acting as a vector in the Netherlands and in surrounding countries such as Belgium and Germany (entomological knowledge centres in the Netherlands, such as WUR - Alterra);

b) the vector competence for WNV transmission of the types of midges present in the Netherlands;

c) what native bird types are susceptible to WNV infection and may play a role in the epidemiology of WNV. These birds might possibly be a suitable target group in surveillance activities, as well.

d) the diagnostics capacity required in case of WNV outbreaks in the Netherlands.

It has recently become clear that WNV can be transmitted via breast-feeding, blood transfusion, organ transplant and trans-placental infection. These aspects have not yet been fully studied and require further research that is being conducted abroad. It is essential to keep a careful watch on the developments in this respect.

In view of the recent report regarding a local WNV infection in people in Southern France, it is recommended that the possible risks of a WNV infection be pointed out and that the related precautionary measures be explained to Dutch holidaymakers (for themselves as well as for pets they bring along) prior to the upcoming tourist season by launching an information campaign.

Moreover, it is recommended that crisis strategies for WNV control be developed so that considerations and choices regarding vector control methods can be dealt with now.

The conclusion is that the Netherlands should currently advocate an incremental response with regard to WNV. This should include developing a crisis strategy for WNV control. Moreover, the presence and the density of types of midges that are capable of acting as a vector in the Netherlands and the surrounding countries must be investigated, as well as the vector competence for WNV transmission of the types of midges present in the Netherlands. Furthermore, what native bird types are susceptible to WNV infection and may play a role in the epidemiology of WNV must also be investigated. Last but not least, the WNV situation in Southern Europe and the possible advance to Northern Europe should be carefully monitored.

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