MEETING REPORT

Expert consultation on West Nile virus infection

Thessaloniki, 25–26 January 2011

1 Executive summary

During the summer of 2010, there were several reports of human outbreaks of West Nile virus (WNV) infection in and around Europe (e.g. Greece, Romania, Russian Federation and Turkey). To obtain a comprehensive overview of the unusual epidemiological situation relating to WNV in Europe in 2010; to establish the lessons learnt by each implicated country and to share and discuss best practices for preparedness, response and operational research, on 24–25 January 2010 the Hellenic Centre for Disease Control (KEELPNO), the European Centre for Disease Prevention and Control (ECDC) and WHO Regional Office for Europe organised a multi-sectoral consultation of WNV experts in Thessaloniki, Greece. This second expert consultation meeting addressed epidemiology, laboratory diagnostics, veterinary aspects, entomology and blood safety.

The epidemiological picture of WNV infection in humans during the 2010 transmission season in Europe indicated an increased intensity of viral circulation. Moreover, the 2010 transmission season was the first time lineage 2 WNV was detected in humans in an EU Member State, previous outbreaks in humans having been linked to lineage 1 WNV. Both the increased viral activity and the emergence of a viral strain may indicate that the epidemiology of WNV in Europe is changing.

The meeting identified priorities for strengthening operational responses to WNV infection outbreaks in humans. It also highlighted the need to develop and share common plans and protocols in the fields of preparedness, diagnosis, epidemiology, blood safety, risk communication, veterinary aspects and entomology.

The dynamics of transmission of WNV are complex and it is therefore difficult to predict the epidemiological situation for WNV in Europe in the coming years. Vigilance in countries/areas with recorded, historical WNV circulation and those at risk of WNV circulation is therefore encouraged.

Stockholm, June 2011

2 Background

Since 1996, there has been an increase in reports of both sporadic cases and outbreaks of West Nile virus (WNV) infection in Europe. The first large outbreak was reported in Bucharest, Romania in 1996–1997. Since then, infection has been reported in humans and horses in the Czech Republic (1997), France (2000, 2003, 2004, 2006), Italy (1998, 2008, 2009), Hungary (2000-2009), Romania (1997-2001, 2003-2009), Spain (2004) and Portugal (2004).

In order to identify and address the gaps in knowledge and preparedness in EU Member States, the European Centre for Disease Prevention and Control organised a first EU-level WNV expert consultation in April 2009.[1] This meeting resulted in a recommendation to develop a tool for use by public health authorities to conduct risk assessments on the transmission of WNV to humans, using information from multi-sectoral surveillance systems.

In 2010, the ecological parameters in central European and Mediterranean countries were very favourable for WNV transmission to humans. In the EU, a probable case of WNV infection was reported from the 'Lisboa e Vale do Tejo' region in Portugal in July 2010. A significant human outbreak was subsequently reported from the Region of Central Macedonia in northern Greece and human cases were also reported from Romania, Italy and Hungary in August 2010 and in Spain in September 2010. The outbreak in Greece was the first reported outbreak of WNV in humans from this country. Additionally, lineage 2 WNV was identified in both humans (blood donors) and mosquito vectors, which was the first time this virus strain had been implicated in the infection of humans in an EU Member State. A large outbreak in humans was also reported in the Volgograd region of the Russian Federation, and infections were confirmed in humans in Turkey and Israel, and in horses in Morocco.

In order to have a comprehensive overview of the unusual epidemiological WNV situation in and around Europe in 2010, to establish the lessons learnt by each implicated country, to share and discuss best practices for preparedness, response and operational research relating to WNV, the Hellenic Centre for Disease Control (KEELPNO), the ECDC and WHO Regional Office for Europe organised a multi-country and multi-sectoral consultation meeting of WNV experts on 25–26 January 2010 in Thessaloniki, Greece. This second expert consultation meeting addressed epidemiology, laboratory diagnostics, risk communication, veterinary aspects, entomology and blood safety. The European Commission (DG SANCO), together with the Greek authorities, also hosted a satellite meeting on blood safety and WNV.

3 Objective of the meeting

The objective of this second consultation meeting was to obtain a comprehensive overview of the changing WNV epidemiology in Europe in 2010 in order to propose recommendations for improving public health preparedness and local response during WNV outbreaks.

Specific objectives were:

- To review the most recent advances in understanding WNV infection in Europe, with specific regard to its epidemiology, surveillance and control of the vector, clinical findings and therapeutics;
- To establish lessons learnt from the response to outbreaks in 2009 and 2010 at the local and national levels, in order to strengthen preparedness at the European level for the 2011 season;
- To identify pertinent areas for further research to improve understanding of the WNV epidemiology in those countries currently affected.

[1] European Centre for Disease Prevention and Control. Expert consultation on West Nile virus infection. Stockholm: ECDC;2009. Available at: http://ecdc.europa.eu/en/publications/Publications/0909_MER_Expert_consultation_on_WNV.pdf

4 Epidemiological overview of WNV in the European region, 2010

During the first session, presentations were made by four countries that had experienced WNV infection outbreaks in humans during the 2010 transmission season (Greece, Romania, Turkey and the Russian Federation) and by Israel. During 2010, the public health authorities in Hungary identified 19 confirmed cases of WNV infection. In Spain, following an outbreak of WNV in horses, two human cases of WNV infection were identified and in Italy, three human cases of neuroinvasive WNV infection were reported as a result of enhanced seasonal WNV surveillance systems. Lineage 2 virus was identified in humans in three countries where outbreaks had occurred (Romania, Greece and the Russian Federation). Table 1 summarises the main findings for each of the four countries that experienced outbreaks in humans in 2010, while Table 2 gives an overview of the veterinary and entomological findings and the control measures implemented in these countries.

In Israel, WNV was first identified in the 1950s but no large outbreaks were recorded between 1974 and 2000. In 2000, 450 confirmed human cases of WNV infection were reported, with 29 deaths. Since 2001, WNV infection in humans has been a recurring and endemic public health problem. Each year, between June and November, 5–65 confirmed cases per month are reported, with a peak between August and September. There is no geographical epicentre for human WNV infections in the country. In 2001, an integrated surveillance and prevention network was set up for WNV, which included a coordinated preparedness plan involving the Ministry of Health, the Ministry of Agriculture and the Ministry of Environment and Protection. . As WNV is considered an endemic problem, there are no special restrictions placed on blood safety (i.e. no Nucleic Acid Testing [NAT] of blood donations).

Table 1 Outbreaks of WNV infection in humans in Greece, Romania, Turkey and the Russian Federation during 2010 transmission season

Country	Affected regions	Cases				Epidemic period	Viral strain identified[d]
		No. of reported cases	No. of neuro-invasive cases (%)[(a)]	No. of deaths[(b)]	Most affected age groups[(c)]		
Greece	Central Macedonia and Larissa prefecture and three sporadic cases in the districts of East and West Macedonia and the Region of Western Greece (one case each)	262 probable or confirmed	191 (72.9%)	35	>70 years (8.2) 60–69 years (2.4)	July–October, peak in third week of August	Lineage 2, similar to lineage 2 isolated from wild birds in Hungary in 2004 and in Austria in 2008
Romania	South-eastern part of the country, central Transylvania and the Moldavian Plateau	49 confirmed	46 (93.9%)	5	60–69 years (0.8) >70 years (0.5)	July–October, peak in third week of August	Lineage 2, 99.3% sequence identical to lineage 2 from Volgograd, Russian Federation in 2007
Turkey	15 provinces throughout Turkey	12 confirmed 35 probable	40 (85.1%)	10	>80 years (1.63) 70–79 years (1.29)	July–November, peak in early September	n/a
Volgograd region, Russian Federation	Volgograd Oblast	413 confirmed	21 (5.1%)	5	50–59 years (3.3) >70 years (3.0) Also, in the age group 20–29 years (1.7), compared to 0.3 in 2007 in this same age group.	July–October, peak in last week of August	Lineage 2, 99.6% homology to virus from 2007 outbreak.

(a) In most countries surveillance and detection is directed towards neuroinvasive cases.

(b) The Case Fatality Ratio (CFR) has not been calculated as surveillance systems in the reporting countries might not be comparable and the CFR would therefore not accurately reflect the epidemiological situation.

(c) Age-specific incidence per 100 000 population

(d) Virus identified from humans, mosquitoes and/or birds

n/a = Not available

NB: The tables only reflect what was presented during the meeting and might not provide a comprehensive overview of WNV outbreaks in these countries during 2010.

Table 2 Summary of veterinary and entomological findings and control measures implemented in Greece, Romania, Turkey and the Russian Federation during 2010 transmission season

Country	Veterinary and entomological findings	Control measures
Greece	• 46% of 220 horses tested in Central Macedonia during the outbreak had antibodies against WNV (IgM antibodies were detected in 19.5%) • Evidence of recent WNV infection also identified in domestic pigeons • Three pools of *Culex* mosquitoes identified with WNV lineage 2.	• Enhanced surveillance for human cases • Adulticiding (ultra-low volume spraying) of mosquitoes in villages with human cases • Public education campaigns for personal protection • Education and guidance to school teachers and health care personnel • Blood safety measures.
Romania	• Seroprevalence studies indicated viral circulation in horse populations, but no clinical disease has ever been observed in horses.	• Seasonal surveillance for human cases • Blood safety measures.
Turkey	• Two confirmed cases of horses with WNV infection in the province of Izmir.	• Improved surveillance (active and passive) for human cases • Inclusion of WNV as a notifiable disease for 2011 season.
The Russian Federation (Volgograd)	• Three-fold increase in density of *Culex* mosquitoes compared to annual mean during August 2010 • Evidence of WNV infection in *Culex*, *Aedes* and *Anopheles* mosquitoes • No evidence of increased bird mortality observed during transmission.	• Strengthening active and passive surveillance systems for human cases • Improving vector control strategies • Strengthening veterinarian and public health collaboration and improving risk assessment for blood safety.

5 Clinical manifestations of WNV infection

Since the first WNV outbreaks reported in humans in 1999, the United States has developed and documented experience in the clinical management and long-term monitoring of confirmed human cases with WNV infection. The follow-up of NAT-positive blood donors provided valuable information on the development of clinical signs and risk factors for WNV infection. Clearly demonstrated risk factors for the development of neuroinvasive disease include advanced age, gender (male) and immune suppression. The clinical manifestation for persons with West Nile fever in the US indicates that the duration of disease can be prolonged. The symptoms most commonly attributed to the prolonged presentation include fatigue, memory impairment, weakness and balance problems. WNV poliomyelitis with complete or near-complete recovery has only occurred in about one-third of patients. Long-term neurological sequelae are common in persons who have had WNV encephalitis in more than 50% of cases for more than three years following the acute illness. These people also have a documented higher rate of all-cause mortality at more than one year post infection. Details of specific aspects of WNV infection can be found in Annex 3.

6 Diagnostic tools for WNV infection

Greece

During the outbreak in Central Macedonia in 2010 suspected human cases of WNV were confirmed by detecting WNV IgM and IgG antibodies in cerebrospinal fluid and paired serum samples using ELISA,[2] in addition to an in-house indirect immunofluorescence assay. Additional tests were conducted to ensure the absence of cross reactivity with other flaviviruses. Molecular amplification was not used to test human samples because of the short and low viremia.

WNV RNA was detected in pools of *Culex* mosquitoes trapped in areas where human cases had occurred and in NAT-positive blood donors. A viral strain was isolated from a blood donor. Complete genome analysis of the WNV detected in the mosquitoes revealed closest genetic relationship to the lineage 2 Hungarian strain. The presence of a mutation P249 locus (H249P) might be responsible for the increased pathogenecity in humans infected with this virus. Due to the persistence of IgM antibodies, for the next transmission season it was suggested that paired samples should be tested for all suspected cases combined with IgG avidity tests to distinguish between recent and past WNV infections.

[2] ELISA – Enzyme-linked immunosorbent assay (ELISA) (Focus Diagnostics Inc., Cypress, California, USA)

Europe

Table 3 lists the diagnostic tools available for WNV detection. The sensitivity and specificity of the various tests depend on the clinical presentation, duration of infection, samples tested and previous flavivirus exposure. It is important to note that there is a high degree of cross-reactivity between the different flaviviruses when testing with immunofluorescence or ELISA (tick-borne encephalitis virus, Japanese encephalitis virus, yellow fever virus, WNV, dengue viruses and Usutu virus).

Table 3 Diagnostic possibilities for West Nile virus

	Duration of diagnosis	Sensitivity	Specificity
Virus detection			
Virus isolation	1–7 days	High[(a)]	High[(b)]
Hybridisation	3–4 hours	High[(d)]	Good
PCR	3–4 hours	High[(e)]	High
Electron microscopy	30 min	Low[(f)]	High
Capture ELISA	3–5 hours	Good[(g)]	High
Serology			
ELISA	3–4 hours	High	Good[(c)]
Immunofluorescence	2–4 hours	Good	Low/Good[(c)]
Immunoblot	2–4 hours	Good	Good
Neutralisation	4–7 days	Good	High
HIA	2–4 hours	Low	Good

(a) Depending on cultivation system;
(b) Depending on detection system;
(c) Depending on tests;
(d) Ca. 10^4 particle/ml;
(e) Ca. 200 genome equivalent/ml;
(f) ≥ 10^6 particle/ml;
(g) Ca. 0.01 µg antigen/ml

Between 1999 and 2009, the European Network for Diagnostics of Imported Viral Diseases (ENIVD) conducted various External Quality Assessments (EQA) of laboratories in Europe. The results from these assessments for WNV indicate that the capacity for detection of lineage 1 WNV using PCR is relatively good in the participating laboratories. However, only one third were able to correctly diagnose lineage 2 WNV infection. Commercial real-time PCR tests have proved to be better than in-house conventional and real-time PCR set-ups. With regard to the serology EQAs conducted for WNV, more laboratories were able to correctly diagnose anti-WNV IgG (range = 89.5%–100% of participating laboratories) than anti-WNV IgM (range = 57.9%–94.7% of 19 laboratories). Both the PCR and serology EQAs indicate the need to strengthen capacity in EU laboratories for the detection of anti-WNV antibodies and viral RNA using PCR methods.[3]

7 Blood safety issues

Once an outbreak of WNV infection in humans is confirmed, ensuring safe blood supplies is a major concern. According to EU Directive 2004/23/EC (Annex III), the deferral period for blood donors after leaving an area with ongoing transmission of WNV to humans is 28 days.[4] Challenges to the implementation of this directive include differing interpretations of 'ongoing WNV transmission to humans', the impact that such deferrals can have on blood supplies and the difficulty of establishing geographic limits around affected areas. With regard to control measures for ensuring blood safety during WNV infection outbreaks in humans, individual NAT screening of blood donations was found to be useful as a means of increasing vigilance against contaminated blood supplies. In addition, blood safety authorities may be able to pinpoint affected areas more accurately (and reduce the number of blood donation deferrals) while providing WNV screening for blood collected close to the affected areas.

[3] Niedrig M, Donoso Mantke O, Altmann D, Zeller H. First international diagnostic accuracy study for the serological detection of West Nile virus infection. BMC Infect Dis. 2007 Jul 3;7:72. and Niedrig M, Linke S, Zeller H, Drosten C. First international proficiency study on West Nile virus molecular detection. Clin Chem. 2006 Oct;52(10):1851-4.

[4] Commission Directive 2004/33/EC of 22 March 2004 implementing Directive 2002/98/EC of the European Parliament and of the Council as regards certain technical requirements for blood and blood components.

However, the introduction of NAT screening technology is potentially costly and requires significant human resources during outbreaks and therefore might not be appropriate in all settings.
Lessons learnt by the Greek blood safety authorities during the outbreak in 2010 included:

- Surveillance of blood donors provides information about asymptomatic prevalence of diseases in newly affected areas;
- Non-neuroinvasive WNV infection might escape detection during regular blood donor screening interviews;
- Post-donation information and post-transfusion haemovigilance in an outbreak context have proved valuable;
- Preparedness planning for blood safety during the next transmission season is essential.

Country-specific experiences were shared on how blood safety authorities handled blood safety issues during outbreaks of WNV infection in humans.

8 Integrated surveillance for WNV

Two examples of integrated surveillance for WNV were presented from the United States (ArboNET) and Italy's Emilia-Romagna region.

ArboNET combines information from veterinary, mosquito and human surveillance. Since its initiation in 1999, the added value from veterinary indicators (equine and bird surveillance systems) for the early warning and monitoring of WNV activity has decreased. Since the introduction of equine vaccinations, horses are no longer early indicators of viral activity. Similarly, in the early years when WNV became a public health problem bird mortality was an excellent early warning indicator of its geographic spread, however, dead bird reporting has decreased dramatically in recent years. In contrast, entomological surveillance indicators have remained consistently useful since they provide a more quantifiable assessment of human infection risk and can be used to monitor vector control operations.

In Emilia-Romagna, surveillance and risk assessment of WNV transmission to humans combines data from surveillance systems targeting mosquitoes, birds, horses and humans. The analysis of the reported information is combined into a single, routine WNV bulletin which is shared with all relevant stakeholders. This multi-sectoral approach has enabled the early detection of viral circulation in mosquitoes, horses and birds. It also offers the benefit of bringing together all the stakeholders in WNV surveillance on a regular basis with a common goal. The added value of horse surveillance is now less evident as vaccinations for horses against WNV increase in Italy. In 2010, surveillance systems for mosquitoes, birds and horses in Emilia-Romagna did identify WNV viral circulation, but at a much lower intensity than in 2009, whereas Usutu virus activity was much higher in 2010 than in 2009.

In Greece, apart from an Epidemiological Investigation Programme for Selected Equidae Diseases in 2001–2004 (which included serological testing of equidae for WNV), there was no official integrated surveillance system for WNV prior to 2010. However, there was evidence in serological studies of equids and other animals that WNV had previously circulated in these populations in Central Macedonia. Following the identification of WNV infected humans in Central Macedonia in August 2010, passive and active surveillance systems were improved for WNV infection in birds and horses. Additionally, serological studies conducted on horses in the affected areas indicated an overall 19.5% anti-WNV IgM seropositivity. In the months following the outbreak in humans, serological studies of one-year-old domestic pigeons in Central Macedonia demonstrated that in the epicentre of the outbreak area the seroprevalence ranged from 69%–82%.

9 Entomological aspects of WNV

Entomological surveillance is very resource-intensive and its value for risk assessment is still disputed. On the other hand, results from such surveillance systems can be useful to:

- Generate knowledge of potential WNV vectors;
- Offer early detection of WNV circulation 3–4 weeks before the onset of human cases;
- Gain a comprehensive understanding of the transmission dynamics of WNV;
- Confirm WNV circulation during an outbreak in humans by isolating it in local mosquitoes;
- Facilitate WNV isolation from infected mosquitoes during an outbreak in humans (for genetic sequencing);
- Assist vector control operations to monitor the impact of the methods used.

Examples of the above were provided in presentations from Portugal, Italy and Greece.

In Portugal, extensive entomological surveillance systems have been in place since 2005 ('Rede Vigilancia Vectores', REVIVE) and this has resulted in a thorough understanding of the most common mosquito species and potential disease vectors in the country (*Culex pipiens, Culex theileri* and *Aedes caspius* on the mainland and *Aedes aegypti* on the island of Madeira). However, so far not a single mosquito has been identified as being infected with WNV.

In Emilia-Romagna (Italy), after three years of experience with entomological surveillance, the following observations have been made:

- There is no evidence of other species besides *Culex pipiens* being implicated in the transmission of WNV;
- The relative abundance of *Culex pipiens* mosquitoes in 2008, 2009 and 2010 does not explain the WNV transmission to humans as increased abundance is not associated with higher transmission rates to humans;
- The density of *Culex pipiens* in Emilia-Romagna has remained stable for the last 20 years and therefore does not explain the emergence of WNV in this part of the country (significant changes in the local bird fauna might be more indicative of the disease emergence).

In Central Macedonia (Greece), prior to the human WNV infection outbreak of 2010, there was a mosquito control and monitoring system established by various private companies to address the nuisance of biting mosquitoes in the summer periods. As soon as human cases were identified in August 2010 this existing system was strengthened. Data suggests that *Culex pipiens* is the most abundant species, breeding predominantly in villages and urban areas, whereas the second most abundant species, *Culex modestus* , breeds mostly in rice fields and rural areas. *Culex pipiens* was also hypothesised to be the principle WNV vector in the outbreak area. During the outbreak in 2010 the objective of vector control activities was to reduce the density of *Culex pipiens* in villages where human cases of WNV infection had been identified. Both larviciding and ultra-low volume (ULV) adulticiding activities were implemented as vector control throughout the outbreak area. On average, ULV ground applications were able to reduce *Culex pipiens* densities in targeted locations by 61% (comparison of mosquito densities pre and post ground application), however the impact on WNV transmission could not be determined.

10 WG 1: Epidemiology for blood safety risk assessment

The objective of this working group was to identify the type and key sources of information that blood safety authorities require during WNV outbreaks in order to make qualitative and quantitative risk assessments for blood safety.

Definition of an affected area

An affected area is an area of ongoing significant transmission to humans within defined geopolitical boundaries for which a population denominator is available. Operational considerations when defining the limits of the affected area are:

- The affected area should be defined based on a documented appearance of at least one neuroinvasive case of confirmed infection;
- The population size should be not too large or small (problem with low numbers: overestimate the risk) and not too large ('dilution' of the risk);
- Once an area is defined as affected, it remains so until the end of the WNV transmission season;
- A previously designated area will only be considered to be an affected area during the next transmission season if a new neuroinvasive case of WNV infection is detected in a human.

In this way, the detection of a single autochthonous neuroinvasive case of lab-confirmed WNV infection in a human would be the trigger to establish blood safety measures for WNV in an area defined as affected by the Member State implicated.

Operational considerations for risk assessment

In order to take timely and appropriate decisions regarding the risk assessment for blood safety, it is important to have surveillance data for neuroinvasive disease in humans (including the date of onset and place of exposure) available as soon as possible. Therefore, for each reported case of WNV neuroinvasive disease (WNND) in humans a thorough epidemiological investigation and laboratory confirmation of infection is required. This type of human

surveillance data should be provided at frequent, regular intervals once an outbreak has been detected in order for blood safety decision-makers to have access to the most recent information.

Further information from veterinary and entomological surveillance systems is useful in order to:

- determine whether the affected areas need to be extended to include adjacent areas where viral circulation has been documented;
- inform blood safety authorities of the importance of blood donation screening and post-donation surveillance for WNV in areas where viral circulation has been documented;
- inform persons living in areas with any form of established WNV circulation of the need to start using preventive measures against mosquito bites.

Furthermore, both epidemiologists and blood safety experts need to be familiar with mathematical models to conduct quantitative risk assessments for blood safety. Risk assessments need to be done at regular intervals (i.e. weekly) during an outbreak, and retrospectively once the last confirmed human neuroinvasive case has been reported. The assessment will provide a general overview of the blood safety risk for the duration of the outbreak in any given area and this can be compared to national reference values.

Several issues related to blood safety that still need further investigation were identified, including:

- Risk of becoming infected with WNV after visiting an affected area, and documented travel to a WNV-affected area within Europe resulting in the identification of a travel-related WNV infection;
- Feasibility and impact of implementing deferral policies, in the affected country as well as in other countries for potential travel-related infections;
- Best practice for screening blood donations (i.e. NAT, using an evaluated PCR method detecting WNV lineage 1 and 2);
- Impact of blood safety measures taken during WNV outbreaks on the country's blood supply.

It was recommended to:

- Ensure the availability of regularly updated epidemiological data about WNV affected areas on the ECDC website during the transmission season;
- Study the incidence of WNV in travellers from EU Member States returning from 'affected areas';
- Provide EU Member States with guidance on how to conduct quantitative risk assessments for blood safety in relation to WNV in order to include these in national WNV preparedness plans;
- Collect data for 2010 on: (1) deferral criteria and other measures (NAT, inactivation) applied in different Member States, (2) impact on blood supply (3) number of blood donor deferrals due to WNV.

11 WG 2: WNV research to support operational responses in European countries

The objective of this working group was to identify gaps in knowledge of WNV epidemiology in the European context and establish appropriate research objectives, with the focus on public health preparedness. The list below summarises the findings of three parallel working groups identifying research needs.

Human health aspects

- Risk factors for human infection with WNV during outbreaks (related to occupation, susceptibility, residential environment, etc.);
- Thorough understanding of the clinical presentation and long-term sequelae of WNV lineage 1 and 2 infections (i.e. in Greece and Romania 2010); a methodology protocol could be beneficial.

Virological aspects

- Genetic diversity of circulating viral strains of WNV throughout the European region, both historically and during all future outbreaks;
- Potential impact of different climatic conditions on viral replication of WNV in mosquitoes and bird reservoirs;
- Varying levels of pathogenecity in viral strains using different animal models.

Entomological aspects

- Vector competence of mosquito species in affected European countries. Although *Culex pipiens* is the most probable vector in the majority of identified human outbreaks so far, the vector role of other mosquito species is uncertain;
- Mapping the presence/absence of hypothesised vectors in European countries;
- Role played by hibernating mosquitoes infected with virus in Europe in WNV survival and further transmission;
- Impact of vector control measures on WNV transmission during the amplification phase and outbreaks;
- Potential role of other arthropod vectors, such as ticks, in WNV transmission dynamics.

Veterinary aspects

- Comprehensive overview of bird migration patterns as well as WNV seroprevalence data of strains infecting migratory birds that pass through European countries:
- Understanding of the animal and bird species considered to be the most appropriate sentinels for early detection of WNV circulation in an affected country and other at-risk countries;
- Role of migratory and domestic birds in maintaining transmission cycles of WNV in any given area.

Risk assessment

- Understanding how the early detection of WNV circulation in birds, mosquitoes, horses or humans changes the course of an outbreak – i.e. how early detection of WNV can improve response and prevent further human cases;
- Where integrated multi-sectoral surveillance systems exist which can incorporate data from human, horse, bird, entomological and climatic surveillance, there is a need to research the feasibility of such integrated systems in WNV-affected and at-risk countries; construct integrated surveillance databases; use this information to provide more comprehensive insight into viral circulation and early detection of human outbreaks and analyse the cost benefit of such systems in countries where they have already been established.

General aspects

- Role of climate change on the patterns of WNV circulation and WNV epidemiology in humans across Europe;
- The cost-benefit of any new systems should always be taken into consideration during the evaluation phase.

Additional recommendations for future activities:

- Management & diagnostic algorithms for human cases, development and testing of a syndromic approach to case identification;
- Standardised communication kits for health professionals and veterinarians;
- Explore the use of reliable meteorological data for risk assessment;
- Use output from existing integrated surveillance systems to validate findings in future WNV transmission seasons.

12 Priorities and next steps

The following priorities were identified for further work on WNV preparedness at the EU level:

Preparedness and risk assessment

- ECDC-coordinated risk assessment tool for WNV outbreaks will be submitted for expert review when finalised;
- Need for a single repository for existing WNV preparedness plans in the European region, to serve as an information source for all EU Member States and neighbouring countries;
- Results of the current ECDC tender, which investigates the statistical and geographic links between climate/environmental and social determinants and WNV outbreaks in Europe, should be shared.

Diagnosis and case management in humans

- Develop clinical ascertainment and diagnosis algorithms that can be adapted at national level in affected countries;
- Encourage the thorough documentation of neuroinvasive cases, including long-term sequelae; possibly set up a European-level register of such cases;
- Continue to strengthen laboratory capacity in European countries at high risk of WNV transmission.

Epidemiology and virology

- Develop a comprehensive description of the overall epidemiology of WNV in the European Union. ECDC will coordinate the production of a scientific article offering this perspective for submission to a peer-reviewed journal;
- EU Member States to encourage the operational use of the proposed definition of WNV-affected area in future WNV transmission seasons;
- Explore the possibility of developing a common protocol for conducting sero-epidemiological studies of WNV in humans and horses;
- ECDC to explore the possibility of posting regular epidemiological updates on the WNV situation in EU and neighbouring countries on its website between May and October each year;
- The European Network for Diagnostics of Imported Viral Diseases (ENIVD) will continue providing laboratory diagnostics support for WNV and identifying the sequences of circulating WNV strains;
- Conduct additional virological studies under existing research projects to identify potential punctual mutations that might contribute to different virulence and pathogenic patterns.

Veterinary health aspects

- Promotion of sero-epidemiological surveys in horses and birds to understand WNV circulation.

Entomological aspects

- Conduct a thorough evidence-based literature search on the most appropriate and effective vector control measures for WNV outbreaks;
- Investigate the feasibility of detecting possible hot spots of WNV amplification in order to target preventive mosquito control operations;
- Identify and compare different approaches for the surveillance and monitoring of WNV mosquito vectors;
- Harmonise approaches to the surveillance and monitoring of WNV mosquito vectors;
- Increase opportunities for training medical entomologists and public health experts in aspects of entomology relevant to WNV;
- Establish vector mapping for mosquito vectors of WNV in the EU to assist countries in conducting risk assessments for WNV transmission to humans.

Blood safety aspects

- An ECDC-coordinated tool for assessing the contamination risk to blood products from infectious diseases will be available by summer 2011 for pilot testing;
- ECDC will provide a list of affected areas, as indicated by countries experiencing transmission;
- A scientific evaluation will be conducted to assess the risk of WNV in travellers returning from WNV endemic areas in relation to blood safety for the last 10 years;
- EU Member States that experienced WNV outbreaks in 2010 will attempt to document the impact on blood supplies, blood safety and cost effectiveness/feasibility of blood safety measures adopted in the public domain (i.e. peer-reviewed scientific journals);
- Investigate the possibility of setting up an expert working group on blood safety and the impact of vector-borne diseases in humans at EU level. Diseases to be addressed could include: tick-borne encephalitis, dengue, chikungunya, WNV, Sindbis. etc.

13 Conclusions and recommendations

The epidemiological picture of WNV infection in humans during the 2010 transmission season in and around Europe appears to indicate an increased intensity of viral circulation. This observation is based on the findings of research into two WNV infection outbreaks in humans in the Russian Federation and Greece; an increase in confirmed human infections in Romania; a larger number of confirmed human cases reported from Hungary and the first confirmed cases of WNV infection in humans reported from several provinces in Turkey. Moreover, during the 2010 WNV transmission season, lineage 2 WNV was isolated from human cases in Europe for the first time, whereas previous outbreaks in humans had been linked to lineage 1 WNV. Both the increased viral activity and the emergence of a new viral strain might be indicators that the epidemiology of WNV in Europe is changing.

This second expert consultation provided a comprehensive overview of the WNV situation in Europe in 2010. It also highlighted the challenges associated with implementing, monitoring and coordinating integrated surveillance systems for WNV and multi-sectoral responses to WNV outbreaks in humans in different country settings. Although the importance of early warning systems for WNV transmission to humans based on equine, bird and mosquito surveillance systems will be less critical in the future, it is widely recognised that a multi-sectoral approach to WNV preparedness and response is still crucial.

The consultation meeting and the satellite working group on blood safety proposed a clear definition of an affected area necessary for the implementation of a blood deferral policy during an outbreak. Furthermore, the meeting addressed the need to develop and share common plans and protocols in the areas of preparedness, diagnosis, epidemiology, veterinary medicine and entomology in order to follow up on WNV outbreaks and gain a better understanding of this complex vector-borne disease.

The dynamics of WNV transmission are complex and for this reason it is hard to predict the epidemiological situation for this disease in Europe and neighbouring countries during the 2011 season. Vigilance is encouraged in countries with recorded historical WNV circulation and those at risk.

Annex 1: Expert consultation meeting agenda

Tuesday, 25 January 2011

09:30–10:00	**Welcome and introduction – Day 1** *Prof. G. Sarogolou*
10:00–11:15	**Epidemiology of WNV in 'new reporting countries'** Chair: *Denis Coulombier and Takis Panagiotopoulos* **Outbreak of WNV in Greece in 2010** *Konstantinos Danis* **Outbreak of WNV in Romania in 2010** *Florin Popvici* **West Nile fever outbreak in humans in Volgograd province, Russian Federation, July–October 2010** *Dmitrij Viktorov and Nikolaj Sheenkov* **West Nile virus infection epidemiology in Turkey** *Gulay Korukluoglu and Handan Kalaycioglu* **The re-emergence of West Nile virus in Israel since 2000: epidemiological and clinical aspects and preventive measures** *Ella Mendelson*
11:15–11:45	**Coffee**
11:45–12:30	**WNV and the clinician** Chair: *Prof. G. Sarogolou* **Diagnostics of West Nile virus infection** *Anna Papa and Mathias Niedrig* **Clinical management of WNV in the United States** *Lyle Petersen*
12:30–13:30	**Lunch**
13:30–14:00	**WNV and blood safety** Chair: *Olga Solomon and Brita Kaltenbrunner-Bernitz* **WNV outbreak in Greece and implementation of blood safety measures – blood safety and WNV, an overview of issues** *Constantina Politis*
14:30–15:30	**Parallel sessions** **Working Group 1: Epidemiology for blood safety risk assessment** Working Group 1A: Facilitator – *Constantina Politis*, Rapporteur – *Annick Lenglet* Working Group 1B: Facilitator – *Henriette de Valk*, Rapporteur – *Sybille Rehmet* **Working Group 2: WNV research to support operational responses in European countries** Working Group 2A: Facilitator – *Ana Papa*, Rapporteur – *Konstantinos Danis* Working Group 2B: Facilitator – *Wim van Bortel*, Rapporteur – *Agoritsa Baka*
15:30–15:50	**Coffee**
15:50–16:30	**Parallel sessions (continued)** Working Group 1: Epidemiology for blood safety risk assessment Working Group 2: WNV research needs to support operational responses in European countries
16:30–17:00	**Feedback from Working Groups 1 and 2**

Wednesday, 26 January 2011

09:00 – 09:30	**Welcome and introduction – Day 2** *Prof. Saroglou and Denis Coulombier*
09:30 – 10:50	**Veterinary surveillance as a tool for improving risk assessments on transmission to humans** Chair: *Prof. O Papadopoulos and Ana Afonso* **West Nile virus surveillance in Italy: veterinary and entomological monitoring** *Michele Dottori* **Changing veterinary epidemiology of WNV in the United States and adapted strategies** *Lyle Petersen* **WNV infection in animals in Greece: Surveillance and disease reporting activities, studies in equines and domestic pigeons** *Dimitrios Dilaveris, Nikolaos Diakakis and Chrisostomos Dovas*
10:50–11:15	**Coffee break**
11:15–12:30	**Entomology and vector control: needs and experience in relation to risk assessment of WNV transmission to humans** Chair: *Romeo Bellini and Nikos Vakalis* **The mosquito surveillance programme in Portugal** *Hugo Costa Osorio* **The experience of entomological surveillance for vector control in Greece during the 2010 WNV outbreak** *N. Vakalis*
12:30 – 13:30	**Lunch**
13:30 – 14:00	**Multi-sector collaboration – introduction of issues** Chair: *Denis Coulombier* **Comparing the sensitivity and early detection ability of different surveillance methods in Italy** *Romeo Bellini* **Data analysis for risk assessment during the 2010 WNV outbreak in Greece** *Spiros Mourelatos* **Crisis management during a WNV outbreak – the experience from Greece** *Assimoula Economopoulou*
14:00 – 15:30	**Multi-sector cooperation in response to WNV outbreaks** Facilitator – *Denis Coulombier*
15:30 – 15:50	**Coffee break**
15:50–16:15	**ECDC presentation on the risk assessment tool, followed by discussion**
16:15–16:30	**Feedback from Working Group 3**
16:30–17:00	**Summary, conclusions and next steps**.

Annex 2: List of participants

External participants (in alphabetical order of country)

Name	Organisation	Country
Silvia Bino	Institute of Public Health, Ministry of Health	Albania
Gilles Follea	European Blood Alliance	Belgium
Zarema Obradovic	Public Health Institute, Ministry of Health	Bosnia and Herzegovina
Radosveta Filipova	Directorate of Communicable Diseases Surveillance, Ministry of Health	Bulgaria
Iva Christova	National Centre of Infectious and Parasitic Diseases	Bulgaria
Henrik Ullum	Copenhagen University Hospital	Denmark
Henriette de Valk	Institut Veille Sanitaire	France
Pierre Gaillian	Etablissement Français du Sang	France
Paul Reiter	Institut Pasteur	France
Gregory L'Ambert	EID-Méditerranée	France
Imad Sandid	Agence Française de Sécurité Sanitaire des Produits de Santé	France
Vladimir Kendrovski	Institute for Public Health, Ministry of Health	The former Yugoslav Republic of Macedonia
Mathias Niedrig	Robert Koch Institute and ENIVD	Germany
Agnes Csohan	National Centre for Epidemiology	Hungary
Viktor Zoldi	National Centre for Epidemiology	Hungary
Ella Mendelson	Chaim Sheba Medical Center	Israel
Dan Gandacu	Ministry of Health	Israel
Michele Dottori	Istituto Zooprofilattico Sperimentale Lombardia ed Emilia-Romagna	Italy
Giuliano Grazzini	National Blood Centre	Italy
Romeo Bellini	Centro Agricoltura Ambiente 'G.Nicoli'	Italy
Stela Gheorghita	National Center of Public Health	Moldova
Aleksandra Nikolić	Centre for Disease Control and Prevention,Institut of Public Health of Montenegro	Montenegro
Mart Jansen	Utrecht Medical Centre	The Netherlands
Sissel Dyrness	Directorate of Health	Norway
Maria João Alves	National Institute of Health	Portugal
Hugo Costa Osorio	Centre for Vectors and infectious Diseases Research/ National Institute of Health	Portugal
Florin Popovici	Institutul National de Sanatate Publica, Ministry of Health	Romania
Cornelia Ceianu	National Institute for Research and Development in Microbiology and Immunology CANTACUZINO	Romania
Doru Hristescu	National Veterinary and Food Safety Authority	Romania
Corina Posea	Blood Bank of the University Hospital in Bucharest	Romania
Dmitrij Viktorov	WNV Reference Centre, Research Institute for Plague Control Volgograd	Russia
Nikolay Sheenkov	Federal Service for Surveillance on Consumer Rights Protection and Human Well-being (Rospotrebnadzor) Moscow	Russia
Jelenu Obrenovic	Communicable diseases department, Institute of Public Health of Serbia	Serbia
Antonio Tenorio	National Centre for Microbiology Microbiology. Instituto de Salud Carlos III	Spain
J. Antonio Jaén	Junta de Andalucia	Spain
Gülay Korukluoglu	Refik Saydam National Public Health Agency	Turkey
Handan Kalaycioglu	Refik Saydam National Public Health Agency	Turkey
Lyle Petersen	Centers for Disease Control and Prevention	United States of America

Participants from Greece

Name	Organisation
Georgios Saroglou	Hellenic Centre for Disease Control - KEELPNO
Theodoros Papadimitriou	Hellenic Centre for Disease Control - KEELPNO
Maria Athanasiou	Hellenic Centre for Disease Control - KEELPNO
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Maria Vareli	Hellenic Centre for Disease Control - KEELPNO
Fani Goma	Hellenic Centre for Disease Control - KEELPNO
Marios Detsis	Hellenic Centre for Disease Control - KEELPNO
Georgios Dougas	Hellenic Centre for Disease Control - KEELPNO
Georgios Theocharopoulos	Hellenic Centre for Disease Control - KEELPNO
Ioanna Ignatiadi	Hellenic Centre for Disease Control - KEELPNO
Chrisovalantou Kefaloudi	Hellenic Centre for Disease Control - KEELPNO
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Agoritsa Baka	Hellenic Centre for Disease Control - KEELPNO
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Maria Papanastasopoulou	Laboratory of Microbiology, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Greece
Chrisostomos Dovas	Laboratory of Microbiology, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Greece

Name	Organisation
Antonios Bakas	'Limodon' Public Hospital of Special Diseases, Thessaloniki
Emmanouil Christodoulou	'Limodon' Public Hospital of Special Diseases, Thessaloniki
Elpiniki Vasileiadou	'Limodon' Public Hospital of Special Diseases, Thessaloniki
Athina Tsoukala	AHEPA University Hospital, Thessaloniki
Eleni Matami – Chasapopoulou	AHEPA University Hospital, Thessaloniki
Vassiliki Bakaloudi	AHEPA University Hospital, Thessaloniki
Maria Chatzitaki	General University Hospital, LARISSA
Konstantina Politi	Hellenic Coordinating Haemovigilance Centre (SKAE)
Antonios Michaelakis	BPI
Aggelos Ilarides	MOSQIUTO.ES
Elissavet Koumleli	MOSQIUTO.ES
Dimitris Kikopoulos	MOSQIUTO.ES
Ioanna Voutsina	MOSQIUTO.ES
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Konstantinos Zisioglou	D.Y. IMATHIAS
Christos Papadopoulos	D.Y. KAVALAS
Antonios Papadopoulos	D.Y. CHALKIDIKI
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Maria Papagianni	D.Y. LARISSA
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Alexandra Chaskopoulou	University of Florida
Spiros Mourelatos	ECODEV
Sandra Gewehr	ECODEV
Georgios Iatrou	ECODEV
Stella Kalaitzopoulou	ECODEV
Panagiotis Pergantas	BIOAPPLICATIONS
Javid Kashefi	USDA – ARS
Anastasia Pantazopoulou	Ministry of Health
Georgia Sanida	3rd YPE

World Health Organization

Name	Organisation	Country
Ana Paula Coutinho	WHO Regional Office for Europe	Denmark
Pierre Formenty	WHO Headquarters	Switzerland

European Commission and other agencies

Name	Organisation	Country
Olga Solomon	DG SANCO	Belgium
Brita Kaltenbrunner-Bernitz	DG SANCO	Belgium
Ramunas Freigofas	SANCO	Belgium
Ana Afonso	European Food Safety Authority (EFSA)	Italy

ECDC

Name	Unit
Denis Coulombier	Surveillance and Response Support
Herve Zeller	Office of the Chief Scientist
Wim van Bortel	Surveillance and Response Support
Sybille Rehmet	Public Health Capacity and Communication
Annick Lenglet	Surveillance and Response Support

Annex 3: Clinical presentation of WNV in the United States

Some valuable insights were provided into the clinical management of WNV infection in the United States. In summary, the follow-up of NAT-positive blood donors provided valuable information on the development of clinical signs and risk factors for WNV infection. Clearly demonstrated risk factors for the development of neuroinvasive disease include advanced age, gender (male) and immunosuppression. The clinical manifestation for persons with WN fever in the US indicates that the duration of disease can be prolonged and that fatigue is a commonly reported, prolonged symptom. Other symptoms include memory impairment, weakness and balance problems. WNV poliomyelitis has been described and long-term outcomes are common in persons who have had WNV encephalitis. These include disabling neurological sequelae, movement disorders and subjective cognitive problems in over 50% of cases and for more than three years following the acute illness. In these persons there is also a documented higher rate of all-cause mortality after one year post infection.

- **Blood donor follow-up**: A clinical follow-up of 576 blood donors who tested NAT positive (but were IgM negative at the time of donation) revealed that 26% became symptomatic with WNV-like fever. Risk factors for developing symptoms were being female and having a higher viral load.
- **Clinical management of WN fever**: in the US routine diagnostic testing is not recommended for WN fever and treatment is mostly supportive. Patient counselling is important because the duration of symptoms may be prolonged and for pregnant women who might have associated anxiety related to the disease. Studies of the clinical prognosis for persons with WN fever in the US indicate that the duration of disease can be prolonged (in more than 50% of patients and for up to 60 days), that the acute disease is severe enough for people to miss school or work. Persistent symptoms of fatigue, memory impairment, weakness and balance problems are commonly reported.
- **Pregnancy and WNV infection**: one case of intrauterine transmission was reported in 2002. Between 2003 and 2005 three infections shortly after birth were reported and hypothesised to be due to transplacental transmission.
- **Risk factors and clinical management of neuroinvasive disease**: Risk factors for the development of neuroinvasive disease include advanced age, gender (male) and immunosuppression. Other suggested risk factors for severe clinical disease include underlying diseases (hypertension, cardiovascular disease and diabetes) and alcohol and drug abuse. Risk factors for death include advanced age, immunosuppression and mechanical ventilation. Clinical management in neuroinvasive disease is based on supportive care such as mechanical ventilation (with special attention to bulbar signs), investigational therapies and counselling.
- **WNV poliomyelitis**: approximately 15% of persons with neuroinvasive disease develop paralysis (85% due to poliomyelitis-like syndrome), the majority are between 40–60 years of age. The clinical prognosis for persons affected by WNV poliomyelitis is that one third reach near-baseline recovery, one third show significant improvement in affected limbs and one third show little or no recovery. There is a documented 28% fatality within 5 years of paralysis onset.
- **WNV encephalitis:** overall mortality of persons with WNV encephalitis in the United States is 10–15%, with increased fatality recorded in the elderly or immunocompromised persons. Long-term neurological and functional sequelae are common in these persons when discharged from hospital. So far the only independent predictor of sequelae appears to be age.
- **WNV long-term outcomes**: long-term outcomes common in persons who have had WNV encephalitis include disabling neurological sequelae, tremors, movement disorders and subjective cognitive problems (in over 50% of cases and for more than three years following the acute illness). There is also a documented higher rate of all-cause mortality in these persons after one year post infection.
- **Investigational treatments – placebo controlled trials**: one placebo-controlled trial investigating the intravenous use of IgG containing high anti-WNV antibody titres (Omr-IgG-am) has been completed (low enrolment, no demonstrated effect and results unpublished). Two other clinical trials are in progress but no results are available as yet.