MISSION REPORT

West Nile virus infection outbreak in humans in Central Macedonia, Greece

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The ECDC team would like to thank the Greek colleagues, on site in Central Macedonia and in Athens, for facilitating this mission to every possible extent, for their hospitality and for sharing their experiences and knowledge.

A special thanks is due to Professor Saroglou, Assimoula Economopoulou, Adriana Pavli, Linda Pappa, Anna Pappa, Takis Panagiotopoulos and Costas Danis.

Suggested citation: European Centre for Disease Prevention and Control. West Nile virus infection outbreak in humans in Central Macedonia, Greece: July–August 2010. Stockholm: ECDC; 2010.

Stockholm, October 2010

ISBN 978-92-9193-219-1

doi 10.2900/34420

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1 Background

On 7 August 2010, the Hellenic Centre for Disease Prevention and Control (KEELPNO) reported through the Early Warning and Response System (EWRS) 11 cases of probable West Nile virus (WNV) infection in the region of Central Macedonia in northern Greece. Following laboratory testing at the Reference Laboratory for Hemorrhagic Fever Viruses and Arboviruses (RLHFA, Aristotle University of Thessaloniki), two cases were confirmed under the European Union (EU) case definition with presentation of IgM antibodies to WNV in the cerebrospinal fluid. Nine cases fulfilled the probable EU case definition with IgM and IgG positive for WNV in the serum. All cases tested negative for WNV PCR. All cases were hospitalised with symptoms of meningitis and/or encephalitis.

Even though serological studies conducted in the 1980s and in 2007 identified WNV antibodies in about 1% of the population in Central Macedonia [1,2], this was the first report of a recognised human cluster of confirmed WNV infections in Central Macedonia and Greece. The following public health measures were implemented immediately following the recognition of this cluster:

1. Awareness raising amongst clinicians in the region about WNV infection;
2. Issuing of guidelines for recognising the clinical picture of WNV infection, laboratory diagnosis and clinical management;
3. Establishment of enhanced surveillance for WNV infections in humans;
4. Informing prefecture (district) authorities about WNV infection and recommending the strengthening of mosquito control measures where appropriate;
5. Informing the Hellenic Centre for Blood Transfusion to prepare relevant guidelines;
6. Public health education for personal protection from mosquitoes.

This is the second largest outbreak of WNV infection in humans recorded in the EU since the outbreak in Bucharest, Romania, in 1996–1997, when more than 500 cases were reported. Furthermore, other recent events in the EU and in neighbouring countries (probable – now disregarded – WNV human case in Portugal, WNV infection outbreak in horses in Morocco and WNV infection outbreak in humans in Volgograd, Russia) indicate that the ecological parameters for transmission of the virus to humans was favourable during the 2010 mosquito season. In order to better understand the Greek epidemiological picture and use this to better describe and assess the risk for other EU countries, an ECDC expert mission to Greece was proposed in August.

The following report covers the main findings of the ECDC expert mission to Central Macedonia in August 2010.

2 Objectives of visit

2.1 Overall objective

The main objective of the visit was to review the current epidemiological and virological situation of WNV infection in Central Macedonia, including:

- identifying the characteristics of the epidemic (time, place and person in humans) and all information available from veterinary and entomological surveillance, as well as blood safety surveillance; and
- further refining current risk assessment for WNV transmission to humans with relevance to Greece and other EU Member States.

2.2 Specific terms of reference

The ECDC team joined the Greek authorities in defining strategies for:

- human surveillance and further case management;
- veterinary surveillance needs from public health perspective;
- entomological surveillance needs and vector control strategies;
- virology and further laboratory investigations;
- issues around blood and tissue safety; and
- research.

3 Main findings

3.1 Human epidemiological and virological situation

3.1.1 Epidemiological overview

The available epidemiological data is reflected as of 27 August 2010, according to the official report of KEELPNO on their website.

Personal characteristics

Until 27 August, 134 laboratory-confirmed cases of WNV infection were reported at the national level in Greece; of these, 111 (83%) presented with clinical manifestations that were consistent with neuroinvasive disease. Nine deaths were reported in persons over 70 years of age. Of the 134 cases, 37 were hospitalised and eight were in the intensive care unit.

Table 1 shows the age and gender distribution of the notified neuroinvasive cases. The majority of cases (57%) were in persons over 70 years of age. Only 12% of reported neuroinvasive cases were in persons under 50 years of age. Fifty-six percent of these were men.

Table 1: Age and gender distribution in reported neuroinvasive cases of confirmed WNV infection (n=108)

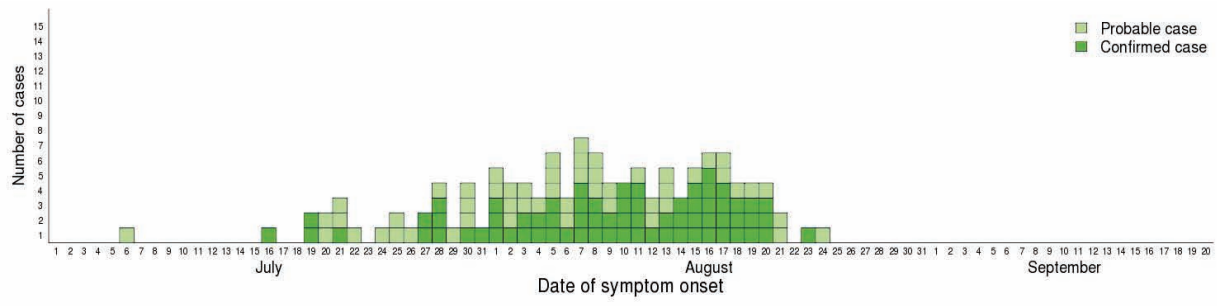
	Number of reported cases
Age group (years)	
<20	3
20–29	3
30–39	5
40–49	2
50–59	14
60–69	19
70–79	44
≥ 80	18
Gender	
Male	61
Female	47
Information unavailable	3

Time

The first cluster of neuroinvasive disease was detected at the Hospital for Infectious Diseases on 4 August 2010, samples were tested on 5 August and the official notification of confirmed human cases of WNV infection was made on 7 August.

According to the most recent epidemic curve by date of onset of disease (Figure 1), the first reported case had disease onset on 6 July and the last on 20 August. The epidemic curve illustrates that between 16 July and 20 August new cases were being reported consistently in the affected areas, which suggest that transmission was ongoing during this period. It is expected that some delays exist following identification of cases in hospital settings, obtaining the relevant samples, laboratory testing and notification. This explains why there are no cases with reported dates of onset after 20 August 2010.

Figure 1: Epidemic curve for reported cases of neuroinvasive WNV infection as of 27 August 2010, Greece (n=108)



Place

Until 27 August 2010, the geographic extension of reported human cases had been restricted to the region of Central Macedonia and the district of Larissa, which is located to the south of Central Macedonia (see Figure 2). Of the 108 neuroinvasive cases for whom the place of residence was known, the majority were from the city of Thessaloniki (31%, n=33) and the districts of Imathia (26%, n=28) and Pella (21%, n=23).

Figure 2: Geographic distribution of neuroinvasive cases by place of residence of laboratory-confirmed WNV infection, Greece (n=108)

3.1.2 Surveillance activities

The current surveillance strategy in Greece for human cases of WNV infection includes passive surveillance, which is implemented nationwide on a routine basis. Enhanced surveillance has been implemented in the region of Central Macedonia and the district of Larissa since the recognition of this outbreak. They are further described below. The healthcare system in Greece is structured in a way that it is likely that most (if not all) cases of severe clinical presentation of WNV infection would be referred to a hospital. Furthermore, it is understood that even mild

clinical presentation of any disease would present to outpatient departments of district and regional hospitals. The use of the primary care system is very limited.

Passive surveillance

All health structures in Greece are obliged to notify cases of WNV infection in humans to the national level according to the EU case definition (see Annex 1). Since the recognition of the human cluster of WNV infections in Central Macedonia in early August, a slightly modified case definition is being used, which excludes the epidemiological criteria, as there is an absence of recent surveillance data on WNV in animals. Laboratory testing for all suspected human cases in Central Macedonia is carried out by the RLHFA in Thessaloniki.

The case definitions currently in use are:

Probable case: any person that presents with encephalitis or meningitis or fever without specific diagnosis and that has a WNV-specific antibody response (IgM or IgG) in their serum sample.

Confirmed case: any person that presents with encephalitis or meningitis or fever without specific diagnosis and at least one of the four laboratory criteria: (i) isolation of WNV from blood or CSF; (ii) detection of WNV nucleic acid in blood or CSF; (iii) WNV-specific antibody response (IgM) in CSF; and (iv) WNV IgM high titre, and detection of WNV IgG, and confirmation by neutralisation.

A database for all nationally notified cases is maintained at the RLHFA and at KEELPNO in Athens. For all notified cases, a case investigation is initiated to collect further demographic information, information on their location of residence and some further details that might indicate their risk for exposure for WNV infection. The majority of these case investigations are conducted by telephone from Athens. For a subset of notified cases, those that are reported from the city of Thessaloniki or from 'new' villages and affected areas, an effort is being made to conduct face-to-face interviews in order to collect more precise information on possible areas of exposure to the infection.

Active surveillance

The case definition used for active surveillance focuses on the clinical presentation of neuroinvasive disease of WNV infection (specifically meningitis and encephalitis). The surveillance staff from KEELPNO in Athens makes daily phone calls to all major hospitals in Central Macedonia and the district of Larissa to follow-up on any new cases of neuroinvasive illness that have presented to the hospital in the previous 24 hours and to encourage the treating physicians to take samples for processing at the RLHFA for diagnostic testing.

Syndromic surveillance

There are some initiatives to implement syndromic surveillance in a selection of hospitals and health centres in five prefectures of Central Macedonia (Imathia, Perea, Kilkis, Pella and Thessaloniki) with the objective to have a better understanding of the milder clinical presentation of WNV infection in humans and to potentially support the strategy behind the current vector control activities.

The syndromic surveillance would look at persons that present to the health facility, since 1 August 2010, with any of the following five clinical syndromes:

- central nervous system (CNS) manifestations (spasms, ataxia, disorientation) + fever (> 38^{o}C);
- rash, excluding the face + fever (> 38^{o}C);
- gastrointestinal illness (nausea, vomiting, diarrhoea, abdominal pain) + fever (>38^{o}C)
- generalised symptoms (e.g. headache, swelling of the lymph nodes) + fever (>38^{o}C)
- fever (>38^{o}C) of unknown origin

For those for whom samples were taken, the RLHFA will run the test for WNV infection. For those for whom no clinical samples are taken, active follow-up is conducted to obtain a serum sample for testing.

The syndromic surveillance data collection will initially be conducted by the outbreak response team of KEELPNO in the affected areas, but will gradually be handed over to the nurses working in the respective healthcare facilities.

This system had not been implemented by the end of the ECDC mission.

Virological and laboratory activities

The RLHFA was the only reference laboratory for arboviruses in Greece at the start of this outbreak. It is equipped with a BSL2+ laboratory with negative pressure. The laboratory participated in the WN External Quality Assurance provided by the European Network for Viral Imported Disease – Collaborative Laboratory Response network (ENIVD–CLRN).

The initial samples from the human cluster of neuroinvasive illness identified at the Hospital for Infectious Diseases in Thessaloniki included blood serum and CSF. Initial laboratory tests conducted included PCR detection for herpes and enteroviruses. Furthermore a series of arboviruses were tested, including phleboviruses (by immunofluorescense assays [IFA]), Crimean-Congo haemorrhagic fever (CCHF), tick-borne encephalitis, dengue and West Nile virus by Elisa.

Diagnostic tests for WNV were conducted using commercial ELISA assays for WNV IgM and IgG antibodies. The results were very specific for WNV infection as there was no cross-reaction observed for tick-borne encephalitis and a weak positive test was obtained for dengue (for the highly positive IgM samples). Further confirmation tests by neutralisation were to be conducted on a batch of approximately representative 20 samples at the V. Sambri laboratory in Bologna, Italy, starting in the week of August 30 2010.

In order to attempt virus isolation from samples from patients who were identified early in their clinical course, viral genome detection by RT-PCR was conducted (technique from Antonio Tenorio, ISCIII, Madrid, Spain). All tested samples were negative for viral RNA detection; to that regard viral isolation using Vero E6 cells was not conducted.

Since the identification of the current outbreak, samples have been received daily from hospitals throughout Central Macedonia and other parts of the country. Results are sent daily to treating clinicians and to the national surveillance database at KEELPNO. Around 20–30 specimens are received on a daily basis. Results are usually available on the same day and are shared with the treating clinician as well as the Ministry of Health.

The RLHFA also conducts laboratory diagnostics on collected mosquitoes pools and samples from horses and birds that are shared with them.

3.2 Entomological situation

3.2.1 Historical

The affected area is situated close to the delta of the rivers Axios and Aliakmonas. These rivers feed the irrigation channels of the rice paddies (about 20 000 hectares of rice fields) that are located 15 km from Thessaloniki. The monitoring activities of larva and adult mosquitoes are mainly done due to the nuisance problem in the rice paddies and in villages and urban settings close to touristic places. In 2002, 21 mosquito species were caught by human landing collections done in human settlements close to the rice fields. *Aedes caspius* was the most dominant species followed by *Ae. detritus* and *Ae. vexans*. *Anopheles* species contributed 6% of the total catch including *An. sacharovi* and *An. pseudopictus.* Other species that were collected were *Coquilettidia richiardii*, *Cx. pipiens* and *Cx. modestus* [3].

3.2.2 Present

As the mosquito monitoring is mainly done because of the mosquito biting nuisance, a good overview of the mosquito species occurrence in Central Macedonia is currently lacking. However, the known vectors of WNV, *Culex modestus* and *Culex pipiens*, are widely present in affected areas. *Culex modestus* is associated with rice paddies and predominates in villages close to the rice fields. *Culex pipiens* is found in villages more distant from the delta and rice field ecosystem and breeds in open sewage system, which provides suitable breeding sites for this mosquito species (especially in urban and peri-urban settings). Even though the affected area is dry (August 2010) *Culex pipiens* finds suitable breeding places in villages. According to data collected locally, human cases of WNV infection have been reported from villages where either *Cx. modestus* or *Cx. pipiens* is present.

A *Culex* mosquito pool was collected in the village of Nea Sanda (north of Thessaloniki), where a human case was reported from, on 6 August 2010. This pool was tested at the RLHFA and was positive for WNV by generic RT-PCR and a sequence of 140 nucleotides showed a close relationship with lineage 2 WN viruses, including those reported from Hungary in 2003. A viral isolation attempt on cell culture failed. The complete sequence of the virus is to be conducted by Norbert Nowotny (the University of Veterinary Medicine, Vienna,) in Austria.

3.2.3 Control measures implemented

For more than 10 years mosquito larval control activities have been implemented throughout Central Macedonia to reduce mosquito biting nuisance by a private company (ECO Development). The control measures are focused predominantly on the flooded rice paddies between June and August each year. Treatment of sewages in villages is implemented to reduce the *Culex pipiens* biting nuisance. Two larvicides are used: diflubenzuron (DU-DIM) in rice paddies and sewage systems, and *Bacilllus thuringiensis israelensis* (Vectobac) in the natural delta.

Since the start of the WNV outbreak, adult mosquito control has been implemented in villages where neuroinvasive human cases have been detected. The mosquito control is done by ULV spraying using pyrethroids (Deltamethrin, Tetramethrin or D-phenothrin). Two treatments are normally done with an interval of four to seven days. CO_2 traps are set before and after the treatment to monitor its impact. The mosquitoes are counted and, because of time constraints, they are only identified up to genus level. A random selection of 50 specimens of *Culex spp* of each collection batch is sent to the lab for virus detection.

ECO Development carries out all the vector control and monitoring under contract with the Ministry of Health through the Development Agency of Thessaloniki S.A. Communication teams from KEELPNO visit villages the day before spraying for social mobilisation and communication. An additional identified challenge has been that local

honey farmers have been resistant to the adulticide activities as they are required to also move their bee populations. Recent considerations are being made by the Ministry of Health to initiate aerial spraying with insecticide in the affected areas.

3.3 Veterinary situation

3.3.1 Historical

From serological studies, there is some evidence of circulation of WNV in horse populations in Greece from the late 1970s and early 1980s as well as in other animals, including goats, cattle and rabbits [4].

3.3.2 Birds

The lowland areas are excellent breeding sites for birds. Additionally, several parts of Central Macedonia currently function as stopover places for migratory birds.

During the ECDC mission there was no indication that there had been an unusual increase in bird mortality from any of the affected areas. It is known that bird mortality in the Old World is not reliable as an indicator of WNV circulation.

There is currently a sentinel surveillance system established to monitor the infection of influenza A/H5N1 in dead birds, which is funded by the European Commission (EC). This system relies heavily on ornithologists and hunters to bring in samples. The Ministry of Agriculture is currently considering activating this system also to start testing bird samples for WNV. However, it might be limited by budget constraints, which would restrict obtaining primers for laboratory testing and incentives for hunters to bring in the samples.

3.3.3 Equines

According to the Ministry of Agriculture, there are 35 000 registered horses in Greece. There is a national system for the surveillance of equine encephalitis in place according to the EU directive for animal health. However there is no other established surveillance for the monitoring of WNV circulation in horses.

Even though the horse population in the affected area is limited, there are horses present in several riding schools in and around Thessaloniki, and there are several privately owned horses throughout the affected areas (see photos in Annex 2). Furthermore, it is known that there is a population of approximately 70 wild horses that are located in an isolated part of the river delta.

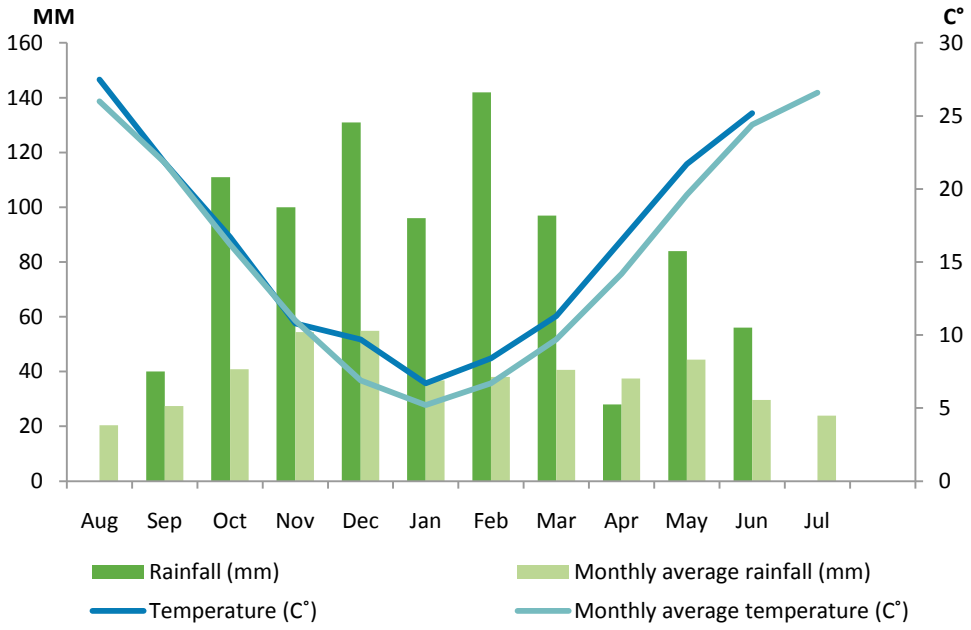
During the current outbreak, initiatives were established between the Veterinary Medicine Department of Aristotle University of Thessaloniki and large riding schools in the city. One of these riding schools identified sick horses during August 2010 and sent samples to the RLHFA. Of these, six tested positive for WNV specific IgM antibodies. Subsequently, five cases of equine encephalitis were reported to the Animal Disease Notification System (ADNS) on 27 August. It is known that disease in at least two of these horses was fatal.

3.4 Meteorological information

The importance of changing meteorological patterns in the emergence and re-emergence of vector-borne disease is a topic of active scientific discussion. For WN there are studies in the United States, Russia and Israel that suggest that heat and above-average temperatures might favour the transmission of WNV and cause outbreaks in humans [5-7]. The effect of heat, and potentially increased humidity would facilitate the reproduction and propagation of mosquito populations (causing increased abundance) and reduce the time needed for the virus development inside the mosquito. The first factor could have increased the man/horse-mosquito contact and the second would result in a higher proportion of mosquitoes able to transmit the WNV.

Meteorological data from 2010 for Central Macedonia suggest that 2010 has been unusually wet and that July temperatures were higher than average, as shown from the comparison of the 2010 rainfall and temperature patterns with a 38-year average covering 1959–1997. However, at the present time, no more detailed data to better understand the potential impact of the weather on the current outbreak are available.

Figure 3: The rainfall and temperature patterns in Thessaloniki, August 2009 to June 2010



**Monthly average rainfall and temperature period: 1959–1997.*
Data from the Hellenic National Meteorological Service (http://www.hnms.gr/hnms/english/climatology/climatology_html)

3.5 Impact on blood transfusions

Following the notification on EWRS of the existence of a human outbreak of WN, on 11 August, the Greek contact point for human tissues and cells distributed an action plan to the EU network of competent authorities for blood and blood components. In this notice, the Coordinating Haemovigilance Centre (SKAE) and the Advisory Scientific Committee for Blood Transfusion of the National Blood Centre proposed the following activities (http://www.keelpno.gr/keelpno/2010/id990/action_plan_en.pdf):

- Distribution of the current situation to all blood transfusion services.
- Reinforcement of the donor clinical evaluation, especially for persons reporting having visited the affected area for one day or more.
- Deferral of potential donors for a period of 28 days after they leave the affected areas following the EC Directive 2004/33/EC, annex II.
- Deferring all blood donor sessions in the districts at high risk for WNV transmission.
- Quarantining and subsequent NAT testing of all blood units collected from the districts with high risk of WNV transmission.
- Requesting donors to report any fever-like illness up to 15 days after their donation.
- Conducting a study on the prevalence of WNV contamination in units from 3 000 donors from Central Macedonia and Thessaly regions.
- Post-transfusion surveillance for WNV infection.
- Special attention to the optimal use of blood components and appropriate management of national blood supplies.

During the ECDC visit to the blood safety laboratory at the University Hospital of Thessaloniki on 27 August, the team was informed that affected villages in the following prefectures had been deferred from blood donations: Kilkis, Kalemi, Pella and Verea. Also, NAT screening capacity had been established in this facility (which tests the majority of blood donations from Central Macedonia) on 15 August. According to the information provided by the manufacturer, the NAT in use in Thessaloniki blood bank can detect lineage 2 viruses (limit: 30 copies). To date a single donation tested positive for WNV with the NAT test, and will be further used to try and isolate and sequence the virus. The donor of this WNV-positive unit of blood was contacted and reported having a mild fever-like illness in the days after her donation. The current impact on the total number of donations appears to be around 25% as the blood bank reportedly received between 70–100 donations less per day.

There was no available information on the measures that have been implemented for organ donor safety.

3.6 Other public health measures

3.6.1 Risk communication and public education

A communication campaign using leaflets and posters informed the general public on the WNV outbreak, transmission, symptoms and the personal protection measures that can be taken to prevent mosquito bites. One leaflet deals with frequently asked questions, such as cause and transmission of WNV, symptoms, treatment and cure of the disease. Personal protection measures address the use of repellents and the measures that can be taken in and around the house to reduce mosquito bites. A special leaflet addresses in more detail the personal protective measures that the general public can apply. Similar information was presented in different posters. Leaflets and posters were distributed in villages where cases were found and through the prefectures.

In addition, KEELPNO maintains a daily and weekly report on their website. Apparently several of the outbreak response team had given interviews to the local and national press. Also, during the ECDC team's stay, the Minister of Health, Mariliza Xenogiannakopolou, visited Thessaloniki, and gave a press conference in which she pledged additional budget from the Ministry of Health to fight the outbreak and also suggested that new control measures would include aerial spraying to tackle the adult mosquito populations.

4 Risk assessment for Europe

4.1 The importance of the current event

This current outbreak in Greece is noteworthy for several reasons:

1 Size of the outbreak:
This is the second largest outbreak of WNV infection in humans reported at EU level. The first was a predominantly urban outbreak in Bucharest in 1996–1997. As the cases reported represent only a small proportion of the total number of infected persons, the incidence of the infection during this summer season in 2010 in affected areas is much higher than can be estimated from neuroinvasive cases alone. Furthermore, with the increasing numbers of cases being reported from the city of Thessaloniki, an urban cycle of transmission cannot be excluded and might lead to an increase in reported human cases from there.

2 Evidence of circulating lineage:
Even though virus has only been isolated from a pool of mosquitoes to date, the evidence of lineage 2 WN viruses in known mosquito vectors of this disease suggest that this lineage of virus is circulating in the affected areas. As viral isolation from human cases is pending, it is not certain that there are not other co-circulating WN viruses also causing human infection. However, lineage 2 virus has only so far been reported from Hungary and Austria, where it was detected in animal populations, and in Volgograd in 2007 from a human fatal case [7]. The current outbreak would increase the number of EU countries where this lineage has now been isolated. The full importance of this finding, in terms of epidemiological outcome, remains to be understood.

3 Pathogenesis of disease / virulence of circulating virus
The demographic picture of reported cases in northern Greece is consistent with what is known about WNV infection, in that the majority of neuroinvasive cases are in the elderly and about a 10% case-fatality rate is present in cases with this severe clinical presentation. It has been suggested that lineage 2 virus is associated with lower pathogenesis of disease as lineage 1 WNV [8]. If confirmed that lineage 2 virus is infecting the human cases in Central Macedonia, it would strengthen results from previous outbreaks of WNV-lineage 2, in Russia in 2007 and in South Africa, that recorded equal pathogenesis of disease in human cases [7, 9].

4 Vector species involved in the outbreak
Both *Culex pipiens* and *Culex modestus* are present in the affected areas and, based on the available evidence, involved in the current outbreak. This is consistent with what is known about competent vectors of WNV. However, Central Macedonia presents a unique environment for the reproduction of mosquito vectors (rice fields and urban sewage as examples) and intense interaction with migratory and resident birds, which probably favour the onwards transmission of virus.

4.2 Risk for sustained transmission in Greece

With the presence of birds (migrating and resident) and bridge vectors, the region of Central Macedonia presents all the ecological components for successful transmission of WNV to humans. A clear epicentre of the outbreak has been identified in the lowland plains between and around the Aliakmonas and Axios rivers, from where the majority of cases were reported. The human cases reported from the city of Thessaloniki, however, suggest that there might be an established urban cycle of transmission. For the remainder of cases residing outside of the outbreak's epicentre, it is possible that several have acquired their infection by spending time in the epicentre of the outbreak. For others, such exposure is less clear following case investigations, and therefore transmission of virus to humans in these areas cannot be excluded.

Publicly available information regarding viral circulation in horse and bird populations in Central Macedonia is limited at the present time. However, earlier serological studies indicate that WNV has been circulating in these animal populations and humans in the region for several years [2, 10]. The identification of lineage 2 virus in a *Culex spp* mosquito pool caught in a village from where a confirmed human case was reported in August 2010, as well as the ongoing reporting of human WNV infections in this region strongly indicate that viral circulation and its transmission to humans is currently well established.

Public health measures to control the outbreak have been implemented at present in the whole region of Central Macedonia as well as in the district of Larissa, in the neighbouring region. Enhanced surveillance for neuroinvasive disease is ongoing (passively nationwide and actively in affected areas). However, the current outbreak may well continue (including newly identified villages) until the mosquito season ends. While unlikely, a further extension of the outbreak outside of the region of Central Macedonia cannot be excluded; close surveillance of the surrounding areas will assure rapid identification of newly affected areas.

4.3 Risk for spreading to the remainder of Europe

Considering the current epidemiological situation of WN in Central Macedonia, as well as the particular climatic factors and their influence on the local mosquito populations, this outbreak is considered to be of limited risk for spreading to other EU Member States (MS).

However, ecological parameters in the European region are currently favourable for viral activity. This is evidenced in recent reports of a large outbreak in humans ongoing in Russia, a single probable human case from Portugal and outbreaks of WN in horses from Morocco. For this reason, it is likely that there is additional active viral transmission between birds and mosquitoes, as well as mosquitoes and humans/horses, throughout the southern part of Europe (including Balkans and Mediterranean countries). This reinforces the need for strengthened surveillance as well as preparedness plans that cover all sectors involved in the transmission of this disease, including human surveillance, entomological surveillance, veterinary surveillance (including birds and horses) and blood and tissue safety.

5 Conclusions

The current outbreak in Greece is the first large outbreak of WNV in humans in Europe since the Romanian outbreak in 1996–1997, even though the presence of West Nile virus is well documented in several European countries. Climatic conditions, temperature and humidity favour the presence and the multiplication of *Culex spp*. from May to October in the affected zones. At the same time, there has been an increase in the number of cases in the EU over the past decade. The possibility that this reflects a changing epidemiology needs to be considered seriously. Also, preparedness in countries where WNV transmission is likely needs to be strengthened by increasing epidemiological capacity to detect clinical cases of WNV infection in humans (neuroinvasive disease) and horses, by increasing laboratory capacity for the diagnosis WNV infections (humans, horses, birds and mosquitoes) and supporting multisectoral collaboration to ensure early warning of potential outbreaks..

6 Next steps and considerations

6.1 For Greece

Human surveillance:

- Harmonising existing active and passive surveillance systems to ensure that they are using the same criteria, that reporting is harmonised at healthcare facility level and information is exchanged between the two systems;
- Strengthening the surveillance in Thessaloniki, specifically, in order to detect any early changes in a potential start of an urban outbreak. By continuing the in-depth case interviews with recent cases from Thessaloniki and newly identified affected areas, it will be easier to understand the history of exposure of these cases;
- Consider the extension of the active surveillance to include all hospitals in the prefecture of Serres as well as the entire region of Thessaly;
- In order to have a comprehensive description of the clinical presentation of the disease in neuroinvasive cases, request a detailed case follow-up by a single neurologist;
- Implementation of some additional operational research to assist in understanding: the clinical manifestation of WNV in Greece and the transmission dynamics of this virus in humans.

Entomology

- Understanding the impact of current vector control strategies in terms of mosquito populations and potentially human epidemiology (though difficult to measure);
- Better understanding species composition in affected and non-affected villages and the transmission in rural versus urban sites;
- Reviewing of entomological situation in other parts of Greece;
- Understading the impact of climate on mosquito abundance – any historical data that can be used?;
- Better understanding the transmission cycle and bird-mosquito-human interface;
- Understanding viral survival during the winter in mosquito populations.

Veterinary aspects

- Strengthening routine and in-depth collaboration and information exchange between the veterinary and public health sector, both at the national and local level;
- Better understanding the situation regarding viral circulation in bird populations, both in Central Macedonia and elsewhere in Greece. Bird mortality in Europe is generally not a good indicator for WNV transmission, but was an important piece of information in Israel from 1997–1998 and in Hungary in 2003 with domestic geese;
- Implement active surveillance of horse populations as a complimentary early warning system for new human cases, by monitoring horses on the borders of the currently areas affected with human cases in order to try and define to where the viral circulation has reached.

Blood safety

- Additional questions to be considered for risk assessment on blood safety include:
  - What is the acceptable risk for WNV contamination of blood supplies in Greece (e.g. in 2009 Israel conducted no screening on any blood supplies for WNV);
  - What is the ability of NAT screening of blood to identify contaminated units from non-excluded areas;
  - Using daily epidemiological updates to determine new areas from where blood donations might be excluded and an immediate quantification on what the possible impact will be for national blood supplies;

  - A quantification of possible risk for contamination in blood donations from currently included prefectures for donation (e.g. Serres, which has cases but no deferral in place);
- Conducting some type of risk assessment on organ donor safety is not yet in place and would be useful.

6.2 For Europe

- Conducting a thorough and active review of existing preparedness and response plans for WNV transmission to humans in countries in the Balkans and Mediterranean region. Such a review can start by addressing the existing surveillance systems for human cases, the laboratory capacity to diagnose probable and confirmed human cases and the existence of other early warning systems in place for WNV;
- Continuing to closely monitor the activity of WNV circulation throughout the region, including the ongoing outbreaks in humans in Volgograd, Russia;
- Reviewing WNV epidemiology and outbreaks in different countries in Europe to better understand the causes of the current WNV circulation;
- Reviewing the impact of current existing vector control strategies to prevent and control WNV outbreaks;
- Identifying experts that can facilitate and support any of the above-mentioned considerations in Greece or other countries in the EU upon request.

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8 Composition of ECDC Team

- Hervé Zeller, virologist;
- Wim Van Bortel, entomologist;
- Annick Lenglet, epidemiologist.

9 Schedule of visit

Day	Activities undertaken
Tuesday, 24 August 2010	• Meeting the outbreak response team from Thessaloniki • Field visit to recent case's village in Posidi, Kassandra
Wednesday, 25 August 2010	• Outbreak team meeting • Visit for mosquito trapping • Visit to Infectious Diseases Hospital in Thessaloniki • Visit to epicentre of outbreak area • Visit to Reference Laboratory for Arboviruses • Follow-up meeting with Takis Panagiotopoulos to address some outstanding questions on surveillance
Thursday, 26 August 2010	• Outbreak team meeting • Visit to villages with positive *Culex* mosquitoes and breeding sites • Drafting TA • Meeting to understand syndromic surveillance system
Friday, 27 August 2010	• Follow-up visit to Reference Laboratory for Arboviruses • Visit to blood centre in Thessaloniki • Meeting with vector control company • Drafting debriefing presentation and start of mission report

10 Persons met

Hellenic Centre for Disease Control (KEELPNO) team

- President KEELPNO: Professor George Saroglou
- Coordinator in the field (KEELPNO): Assimoula Economopoulou
- Coordinator in the field (travel medicine, KEELPNO): Adriana Pavli
- Chief Reference Laboratory for Hemorrhagic Fever and Arboviruses, Aristotle University of Thessaloniki: Anna Pappa
- Scientific Health, Department of surveillance and intervention (KEELPNO): Takis Panagiotopoulos
- Team member (surveillance team, KEELPNO Athens): Costas Danis
- Team member (crisis room, KEELPNO Athens): Linda Pappa
- Team member (syndromic surveillance, KEELPNO): George Ferentinos
- Chief Internal Medicine, Infectious Disease Hospital: Dr Bakas
- Professor of Microbiology (University of Athens): Dr Tsakris

Blood safety

- Director HEPA University blood bank: Dr Balaouni and team

Development Agency of Thessaloniki S.A.

- Vasilis Papavasileiou

ECO Development: private company for vector control

- Manager: Spiros Mourelatos
- Manager: George Iatrou
- Principle field activities coordinator: Sandra Gewehr

Annex 1: EU case definition for human WNV infection

Clinical criteria

Any person with fever OR at least one of the following two:

- encephalitis;
- meningitis.

Laboratory criteria

- Laboratory test for case confirmation (at least one of the following four):
  - isolation of WNV from blood or CSF;
  - detection of WNV nucleic acid in blood or CSF;
  - WNV specific antibody response (IgM) in CSF; or
  - WNV IgM high titre AND detection of WNV IgG, AND confirmation by neutralisation.
- Laboratory test for a probable case:
  - WNV specific antibody response in serum.

Laboratory results need to be interpreted according to flavivirus vaccination status.

Epidemiological criteria

At least one of the following two epidemiological links:

- animal-to-human transmission (residing, having visited or having been exposed to mosquito bites in an area where WNV is endemic in horses or birds); or
- human-to-human transmission (vertical transmission, blood transfusion, transplants).

Case classification

A. Possible case:

N/A

B. Probable case:

Any person meeting the clinical criteria AND with at least one of the following two:

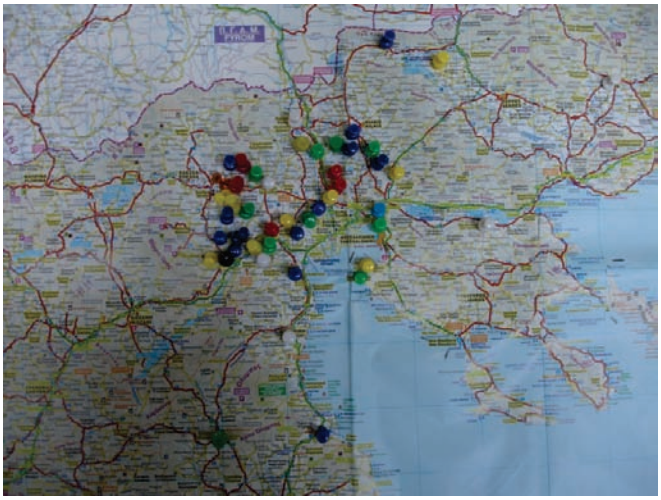
- an epidemiological link; or
- a laboratory test for a probable case.

C. Confirmed case:

Any person meeting the laboratory criteria for case confirmation.

Annex 2: Photos from affected area

Picture 1: Active case finding in hospital registers at Thessaloniki Infectious Disease Hospital



Picture 2: Map used by vector control company to keep track of villages with human cases

Picture 3: Privately owned horses in and around areas reporting human cases

Picture 4: **Identified *Cx. pipiens* breeding site. In background, privately-owned horses and a sewage treatment plant**

Picture 5: **Sandra Gewehr, Wim Van Bortel and Hervé Zeller looking for mosquito larvae**

Picture 6: **CO_2 trapping for mosquitoes**

Picture 7: **Location where positive pool of *Culex* were caught**

Picture 8: **WNV Outbreak Team coordination meeting**