EuroWestNile Publishable Summary

Summary description of project context and objectives

The project context. West Nile virus (WNV) is is an African flavivirus originally maintained in sylvatic cycles mostly between mosquitoes and birds with evidence of circulation outside its original ecological African niches since the 1950's. It is one of the most evident examples of emerging/re-emerging pathogens one can nominate, which is characterized by occasional and often unpredictable virulent epizootic outbreaks. Since its initial introduction in Europe, relatively large outbreaks of West Nile neuroinvasive disease (WNND) have been recorded in humans and/or horses in an increasing number of European and neighbouring countries. The increasing incidence of WNND, the appearance of new foci, and the endemic virus circulation in temperate areas have promoted significant efforts in terms of research and innovation supported by important financial investment of the European Commission under the 7th Framework Programme when this and other projects has been funded.

Being a generalist pathogen par excellence, its eco-epidemiology is extraordinarily complex, involving hundreds of different vectors and hosts, which differ among locations. In addition, as other RNA viruses lacking proofreading replication, its genome is highly variable and consequently of extraordinary plasticity. As a result, many WNV lineages and variants have evolved independently in different parts of the world. As the virus is introduced into new habitat and ecological niches, either by migrating birds or as consequences of human activities (commerce and/or exchanges of goods and livestock, international travels with introduction of infected vectors), different WNV variants (lineages) from different origins can coexist and co-evolve in a particular area. This is the case in Europe, where now with at least seven WNV lineages identified to date. This situation is clearly different from that of North America.

The Eurowestnile project (www.eurowestnile.org) aimed at conducting comprehensive and interdisciplinary studies on WNV in Europe and neighboring countries to fill the gaps in knowledge on many aspect of its virology, ecology, pathogenesis, vector competence, diagnostic and modelling focusing on the European socio-ecological context, since most of the current knowledge on this emerging zoonotic virus derive from research work carried out in USA. Furthermore, the project aimed to develop new animal models and approaches for research on treatment, vaccination and prevention of WNV disease, as well as to produce new diagnostic methods taking into account the variability of the strain circulating in the Euro-Mediterranean region other than other co-circulating flaviviruses.

Main objectives. The strategic aim of the Eurowestnile Project was to develop an integrated European research capacity on WNV in Europe and neighbouring counties including Middle East (Israel) and Africa (Senegal), specially focused on generating new knowledge and innovative products of specific interest to the European citizens, through the cooperation between experts from different countries and scientific background under the "One–Health" perspective. To fulfil this goal, the Consortium brought together highly experienced experts (16 Partner institutions)

from different scientific fields working in Countries where WNV was actively circulating at the start of the project.

Our integrated research efforts, organised in nine Work Packages, was devoted to:

- Obtain a **biobank** of WNV lineages and strains available for each participant country and characterize their **genomes;**
- Develop new **animal models** for research on West Nile **pathogenicity** and **neuroinvasiveness** of the diverse West Nile viruses detected in Europe;
- Develop **infectious clones** with the most and the least pathogenic WNV strain, along with their recombinants, in order to use them for reverse genetic studies and to develop a **vaccine** strategy in the future;
- Characterize the vectors involved in the natural cycle of WNV and in its **transmission** to birds, humans and equines in different geographical areas with different epidemiology of West Nile disease;
- Characterize **vector competence;**
- Characterize the **viral ecology** interactions between WNV and other viruses commonly detected in its most probable vectors;
- Develop innovative prototype kits for WNV **diagnostics and surveillance**, able to detect any of the WNV lineages and differentiate it from other flaviviruses;
- Develop **mathematical models** integrating also data on the virus and its interactions with other viruses and its vectors.

Description of work performed and main results

WP 1. **Project management**. We promoted cooperation and synergies with other FP7 funded projects, in particular Edenext (www.edenext.eu), Wings (www.west-nile-shield-project.eu) and Vectorie (www.vectorie.eu) with the organization of a joint meeting in February 2014 aimed at presenting the most important achievements obtained by the four projects to the European Commission, International Agencies (as ECDC, EFSA, OIE) and the stakeholders' community (report available).

WP 2. **Database and biobank of WNV strains**. We collected a wide variety of WNV strains from all over Europe, establishing a virus biobank and a database. We demonstrated a huge genetic variability of the strains currently circulating in Europe, which has significant implications for both public and animal health. Genetic and phylogenetic analyses conducted during this work package revealed novel WNV strains and even new WNV lineages.

WP 3. Pathogenicity, prevention and treatment in animal models. We developed and standardized new animal models of WNV disease, in particular a mouse model and new birds models, to determine the virulence of WNV isolates and to develop standard assays for efficacy testing of vaccines and treatments against WNV infection. These models are of utility for the

differentiation among highly and low pathogenic WNV strains, assess their neurovirulence and for the rapid typing of emerging WNV strains.

WP 4. **Virulence determinants**. We constructed infectious clones of the most and the least pathogenic WNV strains, along with their recombinants (called chimeras). The virus obtained from chimeras that contained the region encoding the envelop of IS98 and the replicative region of KJMP retained both neuroinvasiveness and neurovirulence, demonstrating that the envelop region is indeed a major viral component involved in pathogenicity.

WP 5. **Vectors, hosts and transmission**. Our aim was to better understand the ecology and feeding preference of mosquitoes in Europe in order to identify and characterise the most competent vector of WNV in different socio-ecological setting in Continental Europe and the Mediterranean Basin. The use of new molecular tools allowed us to identify the most relevant host species selected by mosquitoes for feeding and therefore to identify the candidate key amplifiers for West Nile virus in the Old World.

WP 6. Vector competence, capacity and extrinsic incubation period for the best bridge vector. **T**he results of our experimental infections highlighted variation in the vectorial competence of *Culex pipiens* populations obtained from various regions. Specifically, two *Cx. pipiens* populations derived from WNV outbreak areas (Italy and Russia), showed the highest values of PTR suggesting the possibility of a higher vector competence. *Ae. albopictus* was able to transmit WNV under laboratory condition, thus supporting the hypothesis that this invasive species could represent an additional potential vector of WNV in Europe especially in urban areas.

WP 7. WNV interactions with viruses usually infecting the most probable WNV vectors. We detected many novel viruses belonging to several viral families which are closely related with some new groups of viruses recently described which have the ininability to replicate in vertebrate cells. This characteristic suggests that they are insect viruses with an unlikely vertebrate host. The co-infection experiment using a combination of Nidovirus and Negevirus revealed the potential strong effect of viral co-infections on WNV dynamics.

WP 8. Virus and antibody detection tools. We developed prototypes of WNV diagnostic Nucleic Acid Tests (NAT) for quick diagnostic and for high throughput screening able to detect the different lineages. PCR protocols for detection of all lineages of WN and/or specific lineages were developed and shared. A SPEED-OLIGO® test (quick visual oligo chromatography) for detection and differentiation of WNV Lineages 1-8, WNV L1, WNV L2 and USUV was produced and preliminary validated. Four different MAb-capture antigen detection ELISAs were set up for detection of pan-flaviviruses, WNV, USUV, WNV L1 and WNVL2. Moreover, a LFD test (pen-side test) using MAbs to domain III of E protein was set up.

WP 9. Modeling WNV ecology. We developed an early warning mathematical model for *Culex pipiens* population dynamics and identified environmental conditions favouring West Nile virus outbreaks in Europe. We also studied heterogeneity of mosquito-borne diseases in multi-host

models and identified potential key avian host species for West Nile virus amplification and spreading.

Expected final results and potential impacts

Novel West Nile virus (WNV) strains emerged recently in Europe and have been spreading into new territories. Considering the expected future changes in land use and climate, it is likely that WNV would disperse also in other western and northern European countries since transmission-competent mosquito species are often already occurring. The lack of a specific therapeutic treatment and human vaccines against WNV disease in humans constitutes a significant limiting factor for the public health authorities to mitigate the impact of the disease. Furthermore, elderly people are highly susceptible to WNND, rendering the disease of great societal impact because of the ageing of the European population. Therefore the tracking in real time of the virus spread and ability to predict future possible outbreaks is of utmost importance for the European public health, also in relation to the need to guarantee the safety of blood transfusion and organ donation. We built an impressive biobank of virus strains and an even larger database of the circulating strains. These viruses proved to be of considerable genetic heterogeneity, and we even identify new WNV lineages circulating in Europe. This is of paramount importance because various co-circulating WNV strains may suppress or enhance each other, which may lead to less or more severe clinical outcomes. The variety of WNV strains in Europe has also a significant influence on the diagnosis of the infection due to cross-reactivity between various lineages other than with other co-circulating flaviviruses especially in serological assays. Therefore, we developed new prototypes of WNV diagnostic Nucleic Acid Tests (NAT) for quick diagnostic and for high throughput screening able to detect the different lineages. PCR protocols for detection of all lineages of WN and/or specific lineages were developed as well. A SPEED-OLIGO® test (quick visual oligo chromatography) for detection and differentiation of WNV Lineages 1-8, WNV L1, WNV L2 and USUV was produced and preliminary validated. Four different MAb-capture antigen detection ELISAs were set up for detection of pan-flaviviruses, WNV, USUV, WNV L1 and WNVL2. Moreover, a LFD test (pen-side test) using MAbs to domain III of E protein was set up. To assess WNV pathogenicity and identify new treatment and vaccination protocols, we developed new animal models using WNV strains circulating in Europe, since most of the knowledge on WNV derive from studies carried out in USA on different strains. The avian models standardized in this project includes different European wild bird species, belonging to orders that can respond differently to WNV infection. Results are fundamental in better understanding the epidemiological patterns observed in our continent. We also demonstrate that the design of chimeric WNV may lead to the identification of viral components involved in the severity of WN disease. The virulence determinants we identified (envelop, glycosylation status) can in turn become targets for antiviral drugs, as well as be included in the design of new safer vaccines.

One of the important milestones achieved with EUWN was to build a pan EU-African network and research capacity on WNV and new emerging vector borne infections with the sharing of common methodologies, knowledge and technologies bringing together various laboratories and research

institutions and SMEs belonging to the consortium but also to other sister projects. The successful exchange of innovative ideas and cooperation among teams is proved by the high number of scientific publication so far produced (62 so far but other are under submission). Furthermore, for te first time it was possible to compare ecological properties shared by host and vectors of WNV in Europe, Middle East and Africa. One example is the description of the feeding preferences of the *Culex pipiens* including the introduced species *Aedes albopictus*. We also increased our knowledge on the complex relationships involving viral agents and mosquito vectors, not only in term of variation of vector competence among various *Culex* populations but also in term of understanding the outcome of viral co-infection in mosquitoes and the potential consequences on WNV transmission. This new knowledge will improve the capacity to produce more reliable predictive risk models. However, significant progress have been already made by our modellers in early predict *Culex* dynamics over time and to identify environmental condition linked to WNV risk in space and time.