EuroWestNile

NEWSLETTER

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Editorial

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Dear EWN project followers,

In this issue we are very please to provide an overview of some the most important achievements obtained within the Eurowestnile projects during the last three years. We discussed such advancements during our last Eurowestnile meeting held in Madrid on 17th-18th February 2014

Exciting new insights regarding West Nile virus virology, diversity and pathogenicity in some European birds species have been obtained. A virus biobank of the most relevant WNV lineages and strains circulating in Europe and neighbouring countries was established providing a reference collection for WNV research. A number of new viruses have been identified in mosquitoes and studied to better understand their interaction and the consequences of such co-infection on the vector capacity. New animal models for research on West Nile virus pathogenesis have been developed, enabling for a full characterization of various West Nile virus strains detected in Europe in terms of virulence and transmissibility, revealing important differences between strains which may help to understand the different eco-epidemiology of this pathogen in different areas.

Important advances in our understanding of West Nile virus ecology have been acquired, with the identification of the most important mosquitoes vectors and their preferred vertebrate hosts which are suspected to act as WNV super-spreaders in Europe. These main vectors and hosts represent highly specific targets for virus surveillance and vector control in Europe.

Vector competence studies on various mosquito subpopulations has revealed differences which can partially permit to understand spatial variation in the number of annual human cases reported in various Countries.

New tools and prototype kits for WNV diagnostics and surveillance in animals able to detect the whole range of WNV lineages allowing its differentiation from other related viruses (as Usutu, tick-borne encephalitis, Dengue and Japanese encephalitis viruses) have been developed.

Finally, new mathematical models for understanding the impact of climate on mosquito population dynamics and decision support systems for WNV risk analyses have been developed.

Publication and dissemination of the project results are now included within our next priorities. For this reason we have also organised a joint meeting with other three EU funded projects on West Nile virus (EDENext, WINGS, Vectorie) which had been organised in Madrid on 19th of March 2014. The meeting was quite intense and productive, providing the opportunity to identify knowledge gaps and research priorities on West Nile virus and related mosquito borne viruses for the next future.

Enjoy the reading.

Main achievements of Work Package 8: New products and tool for the diagnosis of West Nile and related viruses.

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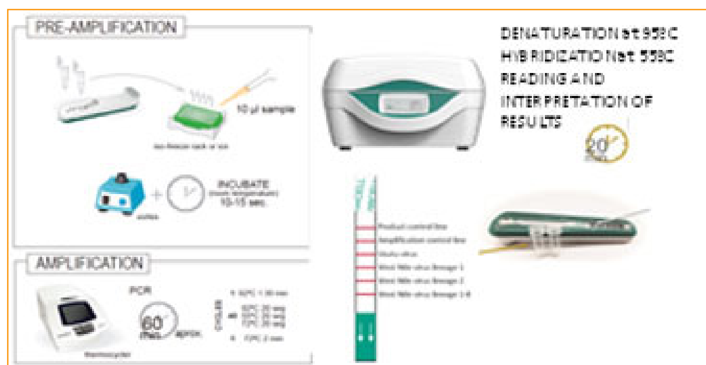
Several progress and new products have been developed in the EuroWestNile project Virological and molecular diagnosis of WNV infections:

1. Quantitative multiplex real time PCR for WNV L1, L2 and USUV. Able to simultaneously detect and differentiate between WNV lineage 1, lineage 2 and USUV.

2. Development of a SPEED-OLIGO® test (qick visual oligo chromatography) for detection and differentiation of WNV Lineages 1-8, WNV L1, WNV L2 and USUV. Adaptation of prototype test strip to SpeedOligo new format-SpeedOligo Cassette, which allows for an automated contamination-free protocol.



3. Development of MAb-capture antigen detection ELISAs that uses two monoclonal antibody (MAb) pairs as catcher and tracer. Four different ELISAs were set up for detection of pan-flaviviruses, WNV, USUV, WNV L1 and WNVL2. For each ELISA, analytical sensitivity and specificity were calculated by testing several dilutions of viruses with known infectious titres and analyzing several flaviviruses (WNV lin 1, 2; BAGAV; YFV; USUV, DEN 1, 2 , 3, 4; JEV) respectively. Analytical sensitivity values were: pan-flav ELISA and WNV ELISA -103,5TCID/50µl; USUV ELISA – 102,5TCID/50µl.

4. Setting up of WNV antigen detection lateral flow device.

Several WNV strains (E101, NY99, Mo'03, Aust08, Malasya, Kunjin, B956, Koutango, Senegal) resulted positive; other flaviviruses (BAGAV and USUV) resulted negative.

Serological diagnosis of WNV infections

1. Production of EDIII and NS1 recombinant proteins of WNV lin.1, 2, USUV and TBEV.

Recombinant antigens produced were E (complete and/or domain III) and NS1 proteins of West Nile virus (Lineage 1 and 2), USUTU virus and TBEV and were expressed in both E.coli and baculovirus.

EIII and NS1 expressed in baculovirus are recognized by MAbs and by infected horse serum. Some MAbs to NS1 are able to differentiate between NY99 isolate (Lineage 1) and B956 isolate (Lineage 2). EIII and NS1 of Usutu virus are recognized by some MAbs. Different constructions of DIII –WNV and NS1 recombinant antigens have been used for two main purposes: a) setting up of ELISAs for antibody detection and b) immunization of mice for production of MAbs. MAbs against DIII and NS1 could be useful for setting up competitive serologic assays and antigen detection assays.

2. Production and characterization of MAbs:

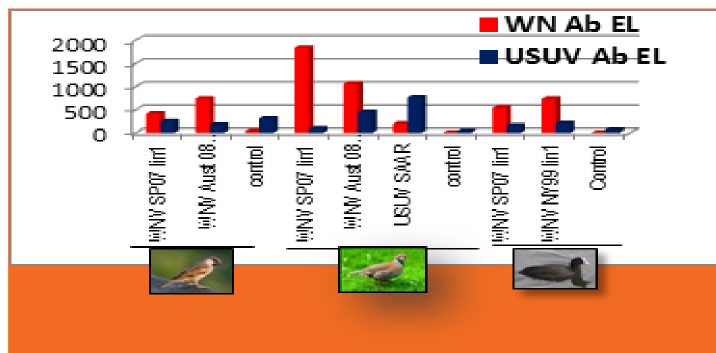
2.1 - MAbs specific to WNV lin.1 and 2, USUV and TBEV. Several MAbs were produced against different strains of WNV, belonging to the lineages 1 and 2, USUV, and TBEV. Table 1 reports a summary of MAbs produced at IZSLER and the characterization of their reactivity.

Virus MAbs obtained Specific MAbs cross reactive MAbs VN activity cloned MAbs Protein reactivity of cloned MAbs

2.2 - Broadly reactive to avian immunoglobulins – pan-avian MAbs. 31 and 74 MAbs were obtained from mice inoculated with a pool of purified immunoglobulins of chicken, quail, jay and pigeon origin and purified chicken immunoglobulins repectively. Selection was done according to the reactivity against sera of different avian (domestic and wild birds) and mammalian species. MAbs reactive against the majority of analyzed avian sera and negative on mammalian sera were further and better characterized.

3. Development of Antibody detection ELISA tests:

3.1 - WNV Ab detection competitive ELISA. A MAb-based ELISA test was developed and validated to detect antibodies against WNV (WN-Ab ELISA) in equine sera using the ROC (Receiver Operating Characteristic) curves approach. This ELISA is a solid phase competitive assay which uses the specific WNV MAb 3B2 previously produced and characterized, which recognizes a conformational epitope located into domain III of the E protein and does not show cross-reactions against other Flaviviruses. For validation, a total of 630 (233 pos and 397 neg) equine sera were tested in parallel using WN-Ab ELISA and Virus-neutralization (SN) assay. Diagnostic performances of this test were very good with Se=98.4% and Sp=98.5%, area under curve=0.996.



3.2 - IgM antibody detection ELISA (Ingezim IgM Wnv). This ELISA used an anti-equine IgM coated to the plate to capture IgM antibodies in sera. After addition of antigen, the final step was a peroxidase conjugated Mab specific to EDIII protein. This assay was shown to be very sensitive (PT organized by ANSES) as well as highly specific. At present, this assay is commercially available and has been registered by Spanish Ministry of agriculture.

Using these two tests we participate to the Proficiency Test for serological diagnosis for West Nile Virus (FR- EILA/ ANSES LSAn/UNITE VIRO2013 / 01) with excellent results: WN-Ab ELISA offered the best performances and proved to be the most specific (TBEV, USUV, JEV infected ponies resulted negative) and sensitive (serum sampled on day 8 PI resulted positive); Ingezim IgM Wnv resulted to be more sensitive than other tests. Infact, sera collected from WNVL1 infected pony on day 35 diluted and from WNVL2 infected pony at several dilutions resulted positive.

3.3 - WNV and USUV antibody detection indirect ELISAs for avian sera.

The protocols selected were: recombinant EDIII proteins (WNV and USUV) coated to the plate; sera; pan-avian MAb HRP. Sera collected from three wild avian species collected 1-3 weeks PI after different experimental infections (WNV lin 1, 2, USUV) were tested in parallel with these two ELISAs. Results of sera tested in parallel with WNV and USUV indirect ELISAs are reported in the graphic. For each group geometric mean was calculated.

The two ELISAs in parallel provided promising results and can discriminate between anti-WNV and anti-USUV antibodies. Furthers trials analyzing a high number of sera should be undertaken in order to better define the discrimination power of these two tests.

Conclusions

Flavivirus infections are a significant public health problem, since several members of the Flaviviridae family are highly pathogenic for humans. Moreover these infections presented often similar clinical features as well as flaviviruses contain cross-reactive epitopes, posing a problem in differential diagnosis, especially in regions where several flaviviruses are present; indeed in several European countries WNV of lineages 1 and 2, USUV, BAGAV, TBEV co-circulate in the same areas. This also implies that specimens should be tested against different flaviviruses to ensure accurate diagnosis. Therefore, there is a strong need in laboratories, for rapid and easy-to-perform assays, with high specificity and sensitivity against the various flaviviruses for routine and epidemiological studies.