MAISONS-ALFORT LABORATORY FOR ANIMAL HEALTH

AND

DOZULÉ LABORATORY FOR EQUINE DISEASES

EU REFERENCE LABORATORY FOR EQUINE DISEASES

2012 Scientific program of the European Union Reference Laboratory for Equine Diseases

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RÉPUBLIQUE FRANÇAISE

This document describes the 2012 scientific program of the European Union reference Laboratory (EU-RL) for equine diseases. The described program is established in accordance with six main activities which are listed as follows:

- Activity 1: Equine Viral Arteritis (EVA) , Equine Infectious Anemia (EIA) and Equine Herpes Viruses (EHV)
- Activity 2: West Nile and other exotic encephalitis and Vesicular stomatitis
- Activity 3: Dourine
- Activity 4: Contagious equine metritis (CEM)
- Activity 5: Glanders
- Activity 6: Coordination and management (this activity is not described in this work-program)

1. Program in Virology

1.1. Equine Viral Arteritis (EVA) , Equine Infectious Anemia (EIA) and Equine Herpes Viruses (EHV)

- Equine Viral Arteritis (EVA):

  - Set up a real-time RT-PCR for EVA diagnosis: it is a collaborative research program (Covetlab organization) with our colleagues from the CVI (Netherlands), VLA (UK), SVA (Sweden) and the DTU (Denmark).
  - Harmonization of the serological test for EVA in Europe by providing materials (virus, cells, sera...) and scientific and technical support to the network and to the Commission.
  - Training sessions for the EU NRLs will be planed.

- Equine Infectious Anemia (EIA):

  - Harmonization of the serology tests for EIA in Europe (regarding 2010 proficiency testing results).
  - Molecular characterization of EIAV strains circulating in Europe. This project is achieved in collaboration with the NRLs from Belgium, Italy, Germany and Romania. The aim of this study is to better understand and describe the different viral population of EIA viruses circulating in Europe. Then, a molecular diagnostic tool (PCR) will be implemented on the basis of this work (project will be on several years).
  - Set up a real-time RT-PCR for EIAV diagnosis: it is a collaborative research program (Covet lab organization) with our colleagues from the CVI (Netherlands), VLA (UK), SVA (Sweden) and the DTU (Denmark).
  - Training sessions for the EU NRLs will be planed.

- Equine Herpes Viruses (EHV):

  - A workshop is planned in 2012 on this topic.
  - Training sessions for the EU NRLs will be planed.

1.2. West Nile and other exotic encephalitis

- West Nile virus (WNV)

  ✓ *EU RL Information*

  - Training sessions for the EU NRLs will be planed.
  - A workshop on viral equine encephalitis (West Nile, exotic encephalitis, Borna, herpes viruses..) will be organised in 2012.

✓ *Research activity*

- Program for the "Identification of molecular determinants of WNV neuropathogenicity

Biological properties of virions produced from the plasmid construct were checked and a full validation of the Infectious clone derived virions has been completed in 2011. The next step will be to insert mutations or specific fragments of less pathogenic European strains, in order to evaluate the molecular determinants of the pathogenicity of European WNV strains. Indeed, conflicting results have been observed between the NY99 strain and European strains. Brault et al. (2007) spotted the role of a T249P point mutation located in NS3 to explain the virulence of NY99 found in American crows, while Sotelo and al. (2009) showed that a 2007 Spanish strain bore the same mutation but was much less virulent in mice than the NY99 strain. We chose to work on this mutation as a start to check its importance in a controlled environment.

Work will also progress on the characterisation of viral infection on NK-N-SH cells (neuroblastoms). The molecular construct will be achieved in 2011. Therefore, the work in 2012 will consist in producing virions, and compare the biological properties on the available models (*in vitro:* Vero cells, *in vivo*: mice and chicken embryos) to know the nature of cell death (apoptosis or necrosis) occurring after West Nile Virus infection. Various WNV strains, belonging to 5 lineages amongst the 8 reported lineages (lineages 1, 2, 3, 4 and 7) that are known to circulate or co-circulate, will be tested on this model in order to see if differences in nature of cell death can be observed. This work is a part of the EuroWestNile program (2011-2013). This program aims at collecting new data about the specific European situation as regards WNV. This model will also be used to characterise the T249P mutated Infectious clone recombinant virus.

✓ *Epidemiological surveillance and epidemiological research*

- Epidemiological investigations will be carried out in several African and Asian countries (CIRAD, Montpellier, France).

- The relevance and importance of non vectorial transmission of WNV in nature, and in particular the epidemiological role of amphibians will be addressed (EDENnext program; 2011-2013). Amphibians are abundant in humid regions favourable to WNV circulation and were found infected in Russia, but their actual role in WNV amplification is poorly known. Experimental infections of *Xenopus* in BSL3 animal facilities will be conducted; viremia levels and oral and cloacal excretion over time will be measured and WNV persistence in specific tissues will be assessed. The occurrence of contact infections, in the absence of mosquito vectors, will be considered.

✓ *Vaccine development*

- Advanced investigations in horses will be required in order to fully validate the recombinant CAV-sE WN

• <u>Exotic encephalitis</u>

✓ *Improvement of exotic ELISA tests based on recombinant proteins*

- Development to minimize the cross reactivity observed in current ELISA between JEV and WNV antibodies will be pursued by working on ectodomain DIII of glycoprotein E (EDIII).

- For alphaviruses (EEEV, WEEV and VEEV), development of a sensitive and specific ELISA test should be continued.

- Production of rabbit polyclonal antibodies sera
  The recombinant proteins (purified recombinant protein E2 for alphaviruses and EDIII (rEDIII) from JEV and WNV) synthesised in insect cells will be used to immunise rabbits and produce polyclonal antibodies against these proteins.
  The immunisation will be repeated until the rabbits show high titres for antibodies against the protein. Then the rabbits will be bled and the serum will be used for the establishment of a competitive ELISA independent of the species.

✓ *Production of reference sera*
Vaccination with inactivated vaccine against JEV will be implementing in France to have a reference serum for this disease. For transport and longer lifespan, the lyophilisation of Alphaviruses and JEV reference sera will be planed.

✓ *Improvement of rt RTPCR exotic diagnostic*

A synthetic calibrator will be performed for the exotic encephalitis to have a standard for rt PCR and to calculate the limit of detection of rt RT PCR protocols.

1.3. Vesicular stomatitis

✓ *EU RL Information*

- A ring trial including EU NRLs will be organized.
- Training sessions for the EU NRLs will be planed regarding proficiency testing results.

✓ *Research activity*

- Development of Ag-capture Elisa will be continued.
- Development of multiplex serological diagnostic test based on luminex liquid array technology will be continued.
- Multiplex (duplex) real time RT-PCR will be developed in order to detect in a single reaction viral genome from New Jersey or Indiana VSV.

2. Program in Parasitology (Dourine)

✓ *EU RL Information*

- Training sessions for the EU NRLs will be planed :the training sessions will include theoretical lectures (taking into account the needs of the participants) and practical work in the laboratory . A serological blind test will be organized and results will be expected in the next 6 months following the training sessions.
- A workshop on dourine will be planed in 2012.
- A ring trial including EU NRLs and some other international laboratories will be organized. The organization of this ring trial will be the opportunity to test the antigens and the positive serum that will be produced during the 2011 second semester.

✓ *Research activity*

- The EU RL will continue the annual participation to the OIE ad hoc group on Non Tsetse Transmitted Animal Trypanosomosis and so contribute to the redaction of the "OIE Terrestrial Manual".

- Genomic characterization of *Trypanosoma equiperdum* and *Trypanosoma evansi* will be continued in association with the Sanger Institute (Glascow) and the Institute for Tropical Medicine (Antwerp). The genome of the five strains sequenced will be annotated.

- Based on the production of new antigens, development of a sensitive and specific ELISA test should be continued. Different fractions of the total antigens will be tested.

- Research project "HippoKAMP" : therapeutic potential of equine antimicrobial peptides against rhodococcosis and other major horse infectious diseases
Knowing that resistance to trypanocidal molecules may occurs, efficiency of some antimicrobial peptides against *T. equiperdum* and *T. evansi* will be determined in association with this European research project "HippoKAMP". A screening of candidates will be performed before in vivo tests.

- Supplying the Trypanosomes strains collection associated to the characterization of these new strains will continue.

✓ *Reagent production*

- *Reference antigens:* based on our work on differentiation between *Trypanosoma equiperdum* and *Trypanosoma evansi*, we will use an "*equiperdum* strain" to produce antigens as described by OIE (terrestrial manual, chap 2.5.3). Two strains will be used, the OVI strain from South Africa, and the recently isolated Ethiopian strain Dodola. The antigens will be characterized and validated for a future distribution to the European NRLs.

- *Reference positive serum for complement fixation test and immunofluorescence :* a positive serum has been produced in Ethiopia in association with the Institute for Tropical Medicine from Antwerp – Belgium. The full technical and statistical data on the evaluation of the candidate reference standards, together with the full data sheet information, will be submitted to the OIE Standards Commission.

✓ The EU RL will also provide full assistance to DG SANCO services in charge of animal health policy;

3. Program in Bacteriology

3.1. Contagious equine metritis (CEM)

- Training sessions for the EU NRLs will be planed;

- Based on the results of inter-laboratory proficiency test for the culture and PCR methods carried out in 2011, the harmonization of methods will be performed if necessary;

- The development of the MLST (multilocus sequence typing) tool to characterize the molecular diversity of *Taylorella* genus will be completed and published. The tool will be transferred to EU NRLs wishing to use it. The EU NRLs will also be able to send their *T. equigenitalis* and *T. asinigenitalis* strains analyzed with the tool;

- Nutritional requirements of *Taylorella* genus will be studied to improve culture media used for the isolation and the characterization of the CEM agent;

- Positive cases of CEM will be confirmed according to EU NRLs requirements.

3.2. Glanders

- The validation and the standardization of standard serum produced by horses' immunization in 2011 will be performed in collaboration with the OIE Reference Laboratory for glanders (Dr Mandy Elschner, FLI-IENA).

- Development of Glanders competitive-ELISA using standard serum will be performed.

- Development and evaluation of real time PCR assays to detect *B. mallei* and *B. pseudomallei* on biological samples artificially infected. The validation of this real time PCR for genome detection of *B. mallei* and *B. pseudomallei* in samples will be performed. The objective is to develop a sensitive and specific real-time PCR that could be used for direct diagnosis of glanders and melioidosis in animal biological samples. The aim of this project is to apply real-time PCR methods which would be easy to perform in routine labs. It is proposed to compare the analytical sensitivity and specificity of both PCR on standard concentrations of *B. mallei* and *B. pseudomallei*. The specificity and the sensitivity will be evaluated in 2012. The diagnostic sensitivity of the method could be validated through a ring-trial organised in the end of 2012 together with field validation by voluntary NRLs.