

**Subject:** Study Visit to Reggio Emilia (IZSLER)

**Place:** Reggio Emilia, Italy

**Date:** 28<sup>th</sup> July to 01<sup>st</sup> August, 2014

**Procurement plan:** Training, line 6

Study visit to Reggio Emilia is intended for the employee of Institute of Public Health of Montenegro Danijela Vujosevic. She would go to visit Istituto Zooprofilattico Sperimentale Reggio Emilia, Italy (IZSLER).

### **Study visit goals and activities WP 2 a**

The theme of the visit is: “Detection of West Nile virus (WNV), Usutu virus (USUV) and other flavivirus in mosquitoes pool by Real Time PCRs”

West Nile virus (WNV), is a member of the Japanese encephalitis (JE) virus serocomplex, genus Flavivirus. The virus is a single stranded positive sense RNA of about 11,000bp. WNV can be divided into lineages 1 and 2 on the bases of envelope protein analysis. Lineage 1 is found in North America, Southern Europe, Africa, Asia, and Australia, while lineage 2 remained in sub-Saharan Africa and Madagascar until 2004, when it was detected in a goshawk in Hungary. Nowadays WNVLin2 seems to be present in the eastern part of the Mediterranean basin from Greece to Northeastern Italy across (the Balkans) former Yugoslavian countries. In particular, in many countries is suspected and for sure in Italy, the circulation of WNV is overlapped by the circulation of Usutu virus (USUV), another flavivirus which arrived in Europe (in Italy) at least in 1996 and was responsible for a mortality in blackbirds occurred in Austria in 2001. USUV is not considered to be a significant human pathogen nevertheless two USUV-positive cases of meningoencephalitis were reported in immunocompromised patients in Italy. Moreover, the entomological surveillance has to consider the circulation of Mosquitoes-Only Flavivirus (MOF) in many European countries, although these viruses are distantly related with other flaviviruses. Cross reactivity of serological tests for flavivirus Ab-detection is well known in literature and the seroneutralization test is frequently requested to confirm the serological findings. While less described and reported is the cross reactivity of molecular tests such as Real Time PCR applied in large vector surveillance system.

During the visit the procedure and protocols applied in IZSLER from 2008 will be shared to Montenegrin Researcher and practical demonstration of all the analytical phases will be provided. Main goals of this study visit is training in application of following procedures and protocols: Preparation of mosquitoes pools for RNA extraction; Random primer Retro-transcription reaction protocol assessment; Real Time PCR protocols and reactions set-up; Real Time PCR protocols and results interpretation and RNA extraction with semi-automatic RNA/DNA extractor.

During this study visit it is planned to have the following meeting:

Monday 28<sup>th</sup> of July: meeting with Director of IZSLER, Reggio Emilia, Dr. Michele Dottori and to the personals of Molecular Biology and Entomological laboratory.

The principal objective of the visit will be train Montenegrin researchers for the detection of flavivirus circulation, in particular WNV and USUV in mosquitoes by Real Time PCRs.