Subject: Study Visit to Reggio Emilia (IZSLER)
Place: Reggio Emilia, Italy
Date: 14th to 18th July, 2014.
Procurement plan: Training, line 6

Study visit goals and activities WP 2 a
The theme of the visit was training for : detection of West Nile virus (WNV), Usutu virus (USUV) and other flavivrus 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 meningoencephalits were reported in immunecompromised 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 procedures and protocols applied in IZSLER from 2008 were shared to Montenegrin Researchers and practical demonstration of all the analytical phases will be provided, for more details on the activity see Agenda.

Briefly, I was trained 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 training we discussed, too, about various types of traps, their usefulness for catching some particular species of mosquitoes and possibility to we use them in Montenegro.

At the end of visit a folder with annexes attached to this report was sent to my mail and to the Coordinator of the project.

In particular during the period between 14th July to 18th July, 2014 in the IZSLER laboratories have been run 353 samples and between 28thJuly -1st August run 342 samples of mosquitoes and birds

for West Nile and Usutu virus and a number of positive samples (WNV and USUV) were detected during visits.

Finally, IZSLER Researchers prepared for Montenegrin Researchers positive cDNAs for WNV lineage 1, WNV lineage 2 and USU.

List Annexes attached:

1) Agenda of 14th July to 18th July visit.

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