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PROJECT: SurveilLance of invasive and native mOsquito VeCtors and pathogENs they transmit in Montenegro - LOVCEN

SUBJECT: Report of study visit of Danijela Vujošević to Institute "Istituto Sperimentale Zooprofilattico Reggio Emilia" (IZSLER), Reggio Emilia, Italy, during the period from 28 July to 01 August.

The theme of the visit was training for detection of West Nile virus (WNV), Usutu virus (USUV) and other flavivrus in mosquitos 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 (L1) is found in North America, Southern Europe, Africa, Asia, and Australia, while lineage 2 (L2) remained in sub-Saharan Africa and Madagascar until 2004, when it was detected in a goshawk in Hungary. Nowadays WNV L2 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 immune compromised 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 with me as well as practical demonstration of all the analytical phases (for more details on the activity see Agendas).

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.

At the end of visit a folder with annexes, attached to this report, was prepared.

In particular during the period between 28thJuly -1st August 2014 in the IZSLER laboratories 342 samples of mosquitoes and birds were tested for West Nile and Usutu virus and a number of positive samples (WNV and USUV) were detected during visits.

Finally, colleagues from IZSLER lab have prepared for us positive controls of WNV lineage 1, WNV lineage 2 and USU virus (cDNAs for WNV lineage 1, WNV lineage 2 and USU virus).

List Annexes attached:

Annex 1: Agenda of 28thJuly -1st August visit
Annex 2: Buffer and water for RNA extraction
Annex 3: Protocol for bio molecular analysis on mosquitos samples
Annex 4: Diagnostic scheme WN-USU with reagents and providers

Consultant

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