STSM scientific report

Title of STSM: Development of Massilia phlebovirus in sandflies and interaction with *Leishmania*

STSM dates: 2014-09-10 to 2014-10-19

Reference code: COST-STSM-ECOST-STSM-TD1303-100914-048136

Location: UMR190 EPV "Emergence des Pathologies Virales" Marseille (France)

Host: Prof. Rémi Charrel, UMR190 EPV "Emergence des Pathologies Virales", Marseille

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Purpose of the STSM:

I am cooperating with Laurance Bichaud, a French post-doc from UMR190 (Marseille, France), who is a fellow in the laboratory of Vector Biology at the Department of Parasitology of Charles University in Prague. I work with her on her post-doc project on the development of Massilia phlebovirus in sandflies. All experimental infections are made in the laboratory of Vector biology in Prague, where a unique world-known collection of sandfly colonies is established. My visit to the UMR190 allowed me to participate in the second step of the project: the processing for bio-molecular and genetic analysis of our samples. I learned several new virology methods well established in this lab. It allowed me to combine virology, parasitology and experimental entomology in a worldwide recognised laboratory with renowned scientists.

Description of the work carried out during the STSM

In the laboratory of Vector biology in Prague we have infected *Phlebotomus perniciosus* sandflies with Massilia virus. For further analysis the samples were taken to the UMR190. After crushing of sandflies the supernatant was divided in two parts. The first part was used for RNA extraction through EZ1 from Qiagen 5075 and Eppendorf epMotion 5075 automat

and then for RT – QPCR assays to detect MASV. Second part was used for infection on Vero cells to attempt virus isolation. Every day, the cells were check to look for cytopathic effect and after 7 days supernatant of cell cultures was used for RNA extraction and tested by RT – QPCR.

Description of main results obtained

COST fellowship allowed me to be trained in basics of general virology. I have been trained for (i) inoculation of Vero cell line using either manual protocols or automated protocol when processing large numbers of samples at the same time, (ii) preparation, propagation and inoculation of Vero cells with entomological material and infect them for detection of presence of live virus, (iii) Q-RT-PCR for the detection of viruses. The knowledge accumulated during period will allow me to test the biological material that has been transferred from Prague into Marseille, using the techniques aforementioned. The rest of my stay (planned until December 15th, 2014) will be funded from other financial sources from Department of Parasitology (CUNI, Prague, Czech Republic). In addition, during this period to come, I will be trained for Next Generation Sequencing (Ion Torrent PGM Sequencer) that will be done from material transferred from Prague. This dataset will be used for for sequence and genotype analyses and full characterization of viral metapopulations within sandflies.

Future collaboration with the host institution

Our cooperation will continue also in future. We would like to study together experimentally also (i) susceptibility of various sandfly species to Massilia virus, (ii) kinetics of virus development in sandflies and its organ distribution over time, and (iii) impact of a coinfection with *Leishmania* parasites. Together we established a unique experimental patogenhost model and the results will be useful in understanding the complex interaction between varios pathogens transmitted by sand flies.

Confirmation by the host institution of the successfull execution of the STSM

I hereby confirm that Magdalena Jancarova has successfully executed her STSM training course

Pr Remi Charrel, MD, PhD

Mgr. Magdaléna Jančářová

