SHORT TERM SCIENTIFIC MISSION (STSM)
SCIENTIFIC REPORT

This report is submitted for approval by the STSM applicant to the STSM coordinator

Action number: CA17108 - Aedes Invasive Moquitoes
STSM title: Molecular and serological techniques for Sindbis virus identification
STSM start and end date: 2019-02-11 - 2019-02-24
Grantee name: Coroian Mircea

PURPOSE OF THE STSM:

The main purpose of this Short-Term Scientific Mission was to get acquainted with the molecular and serological methods used at Umeå University, Department of Clinical Microbiology, Umeå, Sweden, for the diagnosis of arthropod-borne viruses, with a special emphasis on the Plaque Reduction Neutralization Test (PRNT), ELISA and PCR regarding the detection of Sindbis virus. One of the reasons for focusing on these assays is the lack of research on this topic in Romania.

Another objective was to understand and learn the protocols used for West Nile virus detection and surveillance.

What's more, this STSM provided me with the opportunity to work in a Level 2 Biosafety laboratory, learn more about microscopy and improve my dexterity in performing laboratory work. I consider that every skill I aquired during this STSM will help my future development as a researcher.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

The training at Umeå University, Department of Clinical Microbiology, Umeå, Sweden, was divided into two parts: the first step consisted of learning the serological and molecular methods used in the detection of Sindbis virus.

Another important part of my training was represented by learning the cell culture techniques, considering it was the first time I had the opportunity to work on this topic.
I learned how to prepare and filter the DMEM Medium (for Maintenance and Growth), PBS solution, used in cell culture techniques, and also the main requirements of cell lines.
I learned how to grow and work with different cell lines including VERO B4, VERO E6, GMK and also various techniques like cell-splitting by trypsinization, how to freeze, revive and infect the cells.
I also practiced two methods used for the production of the antigen – one using centrifugation, glycerol buffer and sonication, and second one included freezing the cells, sonication and ultracentrifugation. These methods were very helpful because we used the antigen in the Immunofluorescence Assay, and also for the in-house IgG ELISA Assay.
After I acquired the basic principles of cell culturing, we performed Plaque Assay to identify the best dilution of the virus considering the cytopathic effect. Then we performed the Plaque Reduction Neutralization Test, using 24 microplate.

The second step of my training consisted of RNA-extractions, a valuable opportunity for learning, due to the fact that this method is not yet performed at the University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania. I used the QIAGEN kit, but I have also received protocol for “manual” extraction.
We performed RT-PCR, nested and q-PCR followed by electrophoresis and interpretation of results.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

During my training at the Umeå University, Department of Clinical Microbiology, Umeå, Sweden I believe that I have accomplished the main goal of my Short-Term Scientific Mission, which was to be able to detect Sindbis virus in different samples and establish new working protocols for this virus detection at the University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania. The most important results for me consist of being able to grow and manipulate cell cultures, perform serological techniques (Immunofluorescence Assay, in-house IgG ELISA, Plaque Assay, Plaque Reduction Neutralization Test) and molecular techniques (RNA extraction, PCR) at University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania, while also having a better set of practical skills for the laboratory work and a better knowledge about Arboviruses.
I have also received all the protocols which we have worked on, and some advice for my PhD project in Romania, regarding West Nile virus surveillance.

FUTURE COLLABORATIONS (if applicable)

First, I have to note that the professionalism and dedication of Prof. Magnus Evander and Dr. Olivia Lwande surpassed my expectations and by this means I want to thank them all for the help and involvement.
I really hope to establish a collaboration for further research on this topic between our University and the researchers team involved in this STSM from the Umeå University, Department of Clinical Microbiology, Umeå, Sweden.
I also want to thank the COST community for this wonderful chance.

The STSM grantee

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Date: 27/02/2019 Signature: