

Report on the outcomes of a Short-Term Scientific Mission¹

Action number: E-COST-GRANT-CA17108

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Details of the STSM

Title: Training in Vector Competence studies

Start and end date: 10/09/2022 to 07/10/2022

Description of the work carried out during the STSM

Description of the activities carried out during the STSM. Any deviations from the initial working plan shall also be described in this section.

(max. 500 words)

Week 1

Previously selected larval competition experiments were conducted at the Institute of Tropical Medicine in Belgium and adult mosquitoes were transferred to the Bernhard Nocht Institute for Tropical Medicine (BNITM), Germany. All experiments at BNITM were performed under BSL-3 conditions. I received safety instructions and trainings by Dr. Anna Heitmann, Dr. Stephanie Jansen, Prof. Jonas Schmidt-Chanasit and Prof. Esther Schnettler. As described by Heitmann et al., 2018, *Culex pipiens* was infected with West-Nile virus (WNV), *Aedes albopictus* with Chikungunya virus (CHIKV) and *Aedes japonicus* with Japanese Encephalitis virus (JEV). Mosquitoes were sorted, twenty females were placed in a plastic vial and starved for one to two night(s). The infectious bloodmeal was provided and afterwards the fully engorged mosquitoes were counted and placed in a new vial. A cotton pad with

¹ This report is submitted by the grantee to the Action MC for approval and for claiming payment of the awarded grant. The Grant Awarding Coordinator coordinates the evaluation of this report on behalf of the Action MC and instructs the GH for payment of the Grant.

fructose was placed between the vial and plug and replenished every 48/72 hours. The mosquitoes were kept at 24 +/- 5°C (mimicking fluctuating temperatures between day and night), with a relative humidity of 70% and 12:12 light:dark photoperiod. Depending on the incubation time, the mosquitoes were kept under these conditions, for WNV and JEV two weeks; for CHIKV one week.

Week 2-3

No forced salivation assay was performed on this experiment, however the method was demonstrated to me in the BSL-3 for other samples.

The mosquito bodies were placed in a reaction tube and were frozen at -80°C for storage. Afterwards 500 µl of homogenization media was added and each sample was homogenized with a hand motor mixer. Then 200 µl of the homogenate was added into a 96-well-plate for purification and heat-inactivated at 60°C for 60-120 minutes. Samples were taken out of the BSL-3 lab and RNA was purified by automated MagMAX CORE nucleic acid purification. Additionally, a Diagnostic-PCR was performed on ten non-fed *Aedes japonicus* samples in order to detect other infections from these field collected specimens.

Week 3-4

The method for crystal violet cell staining was demonstrated on other samples and we checked for the presence of cytopathic effect.

The samples were analysed by quantifying the viral RNA copies using RT-qPCR, the infection rate was calculated to define the number of virus-positive mosquito bodies per number of fed mosquitoes. In addition, the viral titer in the body was calculated for each mosquito. A total of 182 *Culex pipiens*, 32 *Aedes albopictus* and 47 *Aedes japonicus* were analysed during the STSM.

Networking activities

I was introduced to the Department of Arbovirology and Entomology led by Prof. Jonas Schmidt-Chanasit, I met Dr. Dániel Cadar and Dr. Renke Lühken and their lab groups and I worked within the lab group of Dr. Stephanie Jansen and Dr. Anna Heitmann. I joined the arbovirology seminars where Dr. Felix Sauer presented his research and I was invited to the monthly PhD seminar where I met several PhD students from different research groups such as Leishmania Molecular Genetics, Malaria

Cell Biology and Virus Immunology. I also had the opportunity to meet Dr. Rafael Maciel-de-Freitas from the Department of Entomology of Fiocruz, Brazil.

Heitmann, A., Jansen, S., Luhken, R., Leggewie, M., Schmidt-Chanasit, J., & Tannich, E. (2018). Forced Salivation As a Method to Analyze Vector Competence of Mosquitoes. *J Vis Exp* (138). doi:10.3791/57980

Description of the STSM main achievements and planned follow-up activities

Description and assessment of whether the STSM achieved its planned goals and expected outcomes, including specific contribution to Action objective and deliverables, or publications resulting from the STSM. Agreed plans for future follow-up collaborations shall also be described in this section.

(max. 500 words)

The STSM provided a collaboration between ecology, entomology and virology, leading to a better understanding of the vector competence of these mosquitoes. We expected that interspecific resource competition of mosquito larvae increases the vector competence for arboviruses since stress has a negative influence on the fitness of mosquitoes and therefore will have a negative influence on the immune system of mosquitoes. Via this study we can evaluate if this leads to an increased vector competence, meaning a possible higher risk of arbovirus transmission.

Results

The 371 mosquitoes were fed and a total of 261 mosquitoes have been measured. Infection was successful for *Cx. pipiens* and *Ae. albopictus*, however, no infection was found for *Ae. japonicus*. Differential in infection rate in response to the competition treatment has been detected, but these results are not complete yet. During the next weeks, the remaining samples (85 *Cx. pipiens* and 25 *Ae. albopictus*) will be measured, the diagnostic-PCR for *Ae. japonicus* will be finalised and the RT-qPCR for this species will be repeated by the team at BNITM. Afterwards, statistical analysis of the results will be completed.

Discussion

Aedes albopictus proved to be very susceptible for infection with CHIKV since all measured mosquitoes were infected. On the other hand, *Ae. japonicus* showed no infection with JEV. Some samples still need to be analysed in the coming weeks, providing us with more complete data for this research.

These results will lead to a high quality publication named "Investigation on transmission risk from

different synecological patterns of three important mosquito vectors (*Aedes albopictus*, *Aedes japonicus* and *Culex pipiens*)” on which we will further collaborate.

The technique for vector competence analysis, developed by BNITM, was trained to me resulting in a transfer of knowledge from BNITM to ITM and harmonizing the vector competence study conducted in both institutes. This knowledge contributed to Working Group 1 as a training for arboviral surveillance. This experience can be applied at ITM in the context of testing innovative vector control tools targeting the transmission efficiency of arboviruses in the three mosquito species.

I also had the opportunity to build a scientific network with the different lab groups and research groups at the BNITM. The STSM nurtured me as a young medical entomologist with inter-disciplinary expertise in ecology, entomology and virology on *Aedes* invasive mosquitoes related activities and international collaborative perspectives. I am very thankful for this opportunity granted by AIM-COST.