SHORT TERM SCIENTIFIC MISSION (STSM) SCIENTIFIC REPORT

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

Action number: CA17108
STSM title: Predicting mosquito age on Aedes albopictus wild populations based on genes that display age-dependent expression
STSM start and end date: 01/06/2021 - 30/06/2021
Grantee name: ECOST-STSM-Request-CA17108-47020

PURPOSE OF THE STSM:

The purpose of the STSM was to corroborate the methodology proposed by **Hugo et al. (2010)** in order to evaluate differences in the expression of age-related genes targeting a population of *Aedes aegypti* mosquitoes from Madeira, Portugal. Also, the innovative perspective of this research mission was to set, develop and evaluate this technique with orthologous genes in *Aedes albopictus* populations from mainland Portugal, since the age transcriptional profile of this specie has never been investigated. The final aim of this project was to develop a calibration method which would be used as a tool to predict the age of wild mosquito populations.

Hugo LE, Cook PE, Johnson PH, Rapley LP, Kay BH, et al. (2010) Field Validation of a Transcriptional Assay for the Prediction of Age of Uncaged Aedes aegypti Mosquitoes in Northern Australia. PLoS Negl Trop Dis 4(2): e608. doi:10.1371/journal.pntd.0000608

Funded by the Horizon 2020 Framework Programme of the European Union

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

-Mosquito rearing: Eggs from *Aedes aegypti* and *Aedes albopictus* species were hatched in plastic trays with 1L of dechlorinated tap water and fed with fish food pellets (TETRAMin) until pupa appearance. Pupa were placed in small cups inside insect rearing cages until adult emergence and adults were fed with sucrose solution. Every step in the insect rearing process was done in the insectarium facilities at 28ºC of temperature and 80% of relative humidity.

-Selection of age categories: Six age categories were stablished for females of *Aedes aegypti* and *Aedes albopictus* mosquitoes: a) 0-1 days; b) 2-4 days; c) 7-9 days; d) 14-16 days; e)18-24 days; and f) 25-31 days. Only three age categories (2-4 days; 7-9 days; and 14-16 days) were considered for males since they are not involved in arbovirus transmission and they have shorter life expectancy in natural conditions. Mosquitoes were mechanically aspirated from insect rearing cages, anesthetized with carbon dioxide and separated by specie, sex and age in individual Eppendorfs and conserved at –80 ºC for RNA preservation.

-RNA extraction: Total RNA was extracted from individual mosquitoes (4 mosquitoes for each category of specie, sex and age) and a pool of 5 mosquitoes for each category. Extractions were done with Invitrogen commercial kit by following the manufacturer's protocols and then samples were preserved at -80ºC for later analysis.

-qRT-PCR: One age related gene (which encode a calcium binding protein) and one housekeeping gene (which encode the 40s ribosomal protein S17) were selected for each specie based on previous results published in **Hugo et al. (2010, 2014)**. Alignment and primers and probes' design were provided by GenScript. Two different reporter fluorochromes, named FAM and VIC, were selected to be respectively attached to the age-related probe and to the housekeeping probe for multiplex analysis. Due to time limitations, qRT-PCR were only performed on *Aedes aegypti* RNA extractions. One step reagents from Invitrogen and Qiagen commercial kits were used for qRT-PCR, allowing at the same time the reverse transcription (RT) and the qPCR to be done in one single step. Thermal cycling and transcript quantification was performed using LightCycler 2.0 (Roche) with a temperature program adapted from the mentioned bibliography (50ºC for 20min for RT; 95ºC for 5min for denaturation; 45 cycles of 95ºC for 10 s, 60ºC for 15 s and 72ºC for 20 s for amplification). Results from qRT-PCR from Aedes aegypti were visualized with LightCycler software.

Hugo LE, Cook PE, Johnson PH, Rapley LP, Kay BH, et al. (2010) Field Validation of a Transcriptional Assay for the Prediction of Age of Uncaged Aedes aegypti Mosquitoes in Northern Australia. PLoS Negl Trop Dis 4(2): e608. doi:10.1371/journal.pntd.0000608

Hugo LE, Jeffery JAL, Trewin BJ, Wockner LF, Thi Yen N, et al. (2014) Adult Survivorship of the Dengue Mosquito Aedes aegypti Varies Seasonally in Central Vietnam. PLoS Negl Trop Dis 8(2): e2669. doi:10.1371/journal.pntd.0002669

DESCRIPTION OF THE MAIN RESULTS OBTAINED

A total of 90 RNA extractions were done from *Aedes aegypti* and *Aedes albopictus* species: 48 individual females (24 for each specie); 24 individual males (12 for each specie); 12 pools of 5 female mosquitoes (6 pools for each specie); and 6 pools of 5 male mosquitoes (3 pools for each specie). A multiplex qRT-PCR was performed for several samples of *Aedes aegypti* with the housekeeping gene and the age-related gene with two commercial kits (Invitrogen and Qiagen).

First results showed that the Invitrogen kit did not work for this purpose and there was no amplification. Qiagen kit worked properly and the age-related gene showed differences in their expression pattern across age categories. Results from pools were more noticeable than from individual mosquitoes. The housekeeping gene did not produce any fluoresce at all (we think it may be related with a problem with VIC fluorochrome, the multiplex reaction or the quantity of probe). We repeated the qRT-PCR in order to detect the expression of the housekeeping gene by increasing the proportion of probe in the mix and readjusting the cycling conditions. After several attempts to readjust the reaction, no housekeeping gene expression was found at all which make us think that could be any problem with the fluorochrome of with the probe or primers' design. Since we were not able to evaluate this reference gene, age-related gene expression was analyzed for all samples of *Aedes aegypti* anyway (*Aedes albopictus* was no tested due to time limitations). As main result of this research, we proved that the age-related gene that we selected (based on previous bibliography) showed a differential pattern of expression that varies with age. Older mosquitoes for both sexes showed less gene concentration (higher ct number) that younger ones, indicating that the calcium binding protein gene could be a good predictor of *Aedes aegypti* mosquito age for both males and females.

FUTURE COLLABORATIONS

Future collaborations between host and home research institutions are planned to be done along the end of this year in order to complete the work plan initially planned for this STMS. The idea of this future collaboration is to order new primers and probe for the 40s ribosomal protein s17 for *Aedes aegypti* and to test the age-related gene and the housekeeping gene for *Aedes albopictus*. Once we have proved the reliability of the selected genes in predicting the age of both species, we would design a calibration model in order to predict the age of wild mosquitoes based on the expression of these age-related genes.

SIGNATURE:

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APROVED BY HOST INSTITUTION:

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Data: 2021-07-21 às 10:16:58