Report on the outcomes of a Short-Term Scientific Mission[1]

Action number: CA17108 Aedes Invasive Mosquitoes

Grantee name: ELTON ROGOZI

Details of the STSM

Title: "*Molecular detection of arboviruses in Aedes albopictus Skuse, 1895 (Diptera: Culicidae) in coastal and urban areas of Albania"*

Start and end date: 23/10/2022 to 31/10/2022

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.

We intend to investigate the potential of *Aedes albopictus* as a main vector of different viruses like WNV etc. *Aedes albopictus* has been established in Albania since more than 40 years, and has well adapted populations throughout the country. Studies from the Institute of Public Health in the recent 15 years, gave a full insight in the distribution, presence and abundance of this species in the country. As the bioecology, distribution, presence and vertical and horizontal presence studies of *Aedes albopictus* are in-depth studied; the studies of the viral presence load are lacking. This gave an interesting idea to collect adults of the species throughout the coastal area and some urban and rural ones and transport and conserve them directly from live collection with BG-Lure-CO2 traps; as an important link for the virus preserving with dried ice CO2 for a better conservation of the potential virus present in the adult mosquito. We intend to make a first initiative on the collaboration between the two institutions; The Institute of Public Health of Albania and the Friedrich Loeffler Institute, Greifswald, Germany, under the great support of the COST CA17108: "Aedes Invasive Mosquitoes". As *Aedes albopictus* is one of the main vectors of West Nile Virus in humans, while *Culex pipiens* and *Aedes caspius* play an important role in the vectoring in sylvatic cycle of the virus transmission from migratory birds to human and other animals; the adults of *Aedes albopictus* and other species collected in the forests, close to the areas where the presence of migratory birds' abundance is high; gave us the reliability that the possibility to find *Aedes albopictus* infected with WNV, would be higher rather than the urban ones. Adults were captured using two types of adult's traps, BG-Sentinel + Lure-7 + CO2

[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.

traps and IMT – light – CO2 traps. The pools that were formed of the collected adult mosquitoes were separated not only with the species captured, but also with the collection dates separated clearly and sometimes where the number of adults of *Aedes albopictus* captured were high enough the males and females were separated. Fully blood fed female or fully gravid female were kept separated in tubes. These we mostly captured with BG traps or Human Landing Catch technique – HLC. We aimed to study the: 1. Detection of presence of arboviruses (West Nile Virus, other Flavivirus possible present), in *Aedes albopictus* mosquito pools; Potential of *Aedes albopictus* of Albanian urban and coastal areas, on the transmission of West Nile Virus in the sylvatic and urban cycle; Potential arboviral load detection, to strengthen the vector role of *Aedes albopictus* and its competency and capacity as the transmission role. Bioecology and distribution of (*Aedes albopictus*) in Albania during a specified period of time 2019-2021 in the frame of mosquito surveillance throughout the country and its distribution.

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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.

Mosquito samples of the adult's male and females of Aedes albopictus, were stored in -80º and transported with dry ice conditions in the Friedrich Loeffler Institute. Subsequential replace of the dry ice was made during the transport which took 5 days. Samples were arranged, further separation of male and females in different tubes with 1-25 adults for each pool. We prepared sterilized 2ml tubes with rounded end, where two spherical sterilized beads were put in each tube. Tubes were doubled coded with the first code of the collection site and new FLI code, then 1-25 individuals of adult's mosquitoes were put together. We prepared the media for each sample/pool. For the preparation of the medium, we dissolved the RNA Carrier and the proteinase K in the respective supplied buffer. The buffer was prepared adding to the medium (serum free), where penicillin/streptomycin, was added to an amount of cf=100UmL-1 (stock solution: 10000UmL-1 1:100), gentamicin; cf=0.01mgmL-1 (stock solution: 5mgmL-1 1:500) and amphotericin; cf=0.25µgmL-1 (stock solution: 125µgmL-1 1:500). We added 500µL of the medium at the samples with one individual and 750µL of it in the pool with 2-25 adult's mosquito pools. After adding the medium the samples run grinding of the tissue via Tissue-Lyser for three minutes. The samples were then centrifuged and 180µL of the sample was added to the extraction plate, then they were shaken for 15 minutes and then the beads solution prepared for the sample/lysis plate, where we mixed: 20µL B-beads and 575µL VEB per sample (for 100 samples: 2mL + 57,5mL). then we added 595µL B-beads-VEB mix to lysis/sample plate was added followed by another shaking of 5 minutes before running the extraction process. Before the extraction, washing solutions were prepared. We prepared the following components of the washing phase for 96 deep-well plate (for 100 samples): 20µL Proteinase K (2mL), 180µL VL1 (18mL), 4µL Carrier RNA (400µL), 5µL each IC2DNA/RNA (500µL). after this, we prepared the washing solution for the extraction: we filled 600µL of VEW1, 600µL of VEW2 and 600µL of 80% EtOH in a 96-deep well plate and seal with aluminium foil, then we filled 100µL VEL in a 96-well plate and seal with aluminium foil. The six plate were then put in the extraction

machine for 20 minutes. After this we got our DNA/RNA samples and stored it before running the PCR for the detection of Flavivirus and West Nile Virus. We screen 1712 female and males of *Aedes albopictus*, after extraction and PCR running processes. The PCR process was three hours long. At the end of the process the results came out and we found that there was not any West Nile virus positive pool in the tested samples of Aedes albopictus from Albania. Anyway, two poos of the had higher titres for the presence of other Flaviviruses. Next steps would be followed to make the sequencing of the higher pools to find out I this is another Flavivirus rather than West Nile virus, or may be other DNA/RNA sources. The sequencing process would find out the presence for any probable Flavivirus in those pools. As a conclusion we say that the Aedes albopictus are not positive for West Nile virus, but may be very good option as the vector of other unidentified Flavivirus. The two positive pools, would be sequenced to find out if there is any other Flavivirus circulating in the Albanian *Aedes albopictus*. Despite, the negative results we found for West Nile virus presence in Albanian Aedes albopictus, this species still remain an important vector to be studied and screened again to find out the presence of the West Nile virus on them, especially to the areas where there are migratory birds circulating. Our study on the presence of West Nile and other probable Flaviviruses in *Aedes albopictus*, strengthen the idea that these studies should be carried out and followed to continue the research on this frame.

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