

MNE-HERIC-81180-LOVCEN

Surveillance of invasive and native mosquito  
vectors and pathogens they transmit in Montenegro

## Short report - 1<sup>st</sup> year of life time of Project

### WP1 Collaborative research on native/invasive mosquitoes and pathogens they transmit in Montenegro and development of non-chemical control measures

#### WP1a Surveillance of invasive and native mosquito

Trapping by mosquitoes traps was conducted from 5<sup>th</sup> June to 30<sup>th</sup> September at 11 sampling sites, in total 23 sampling nights. Human bite sampling was performed from August 8<sup>th</sup> to 12<sup>th</sup> and September 14<sup>th</sup> to 19<sup>th</sup>. By all sampling methods 2012 male and female mosquito specimens belonging to 9 species were sampled:

1. *Anopheles maculipennis* s.l. (vector of malaria)
2. *Aedimorphus* [*Aedes*] *vexans* (vector of Rift Valley Virus and Celovo Virus)
3. *Dahlia* [*Ochlerotatus*] *echinus*
4. *Ochlerotatus caspius* (vector of Rift Valley Virus and nematodes *Dirofilaria immitis* and *D. repens*)
5. *Ochlerotatus sticticus*
6. *Stegomyia* [*Aedes*] *albopicta* (vector of Chicungunia Virus, Dengue Virus and nematodes *Dirofilaria immitis* and *D. repens*)
7. *Culex modestus* (vector of West Nile Virus and nematodes *Dirofilaria immitis* and *D. repens*)
8. *Culex pipiens* (vector of West Nile Virus, Sindbis virus, Rift Valley Virus and nematodes *Dirofilaria immitis* and *D. repens*)
9. *Culicoides* sp. (vector of Blue Thong Virus)

One new patent procedure is ongoing for Mosquitoes resting trap Zbun TiN MNE.

#### WP1b Mobile phone application for surveillance of invasive and indigenous species (KOMARAC)

First version of the MSRS software is finished and installed. User side application is developed for Android mobile devices in *Eclipse android development work space* while server side application is developed as a *PHP script*. User side application uses Android Location Manager to extract fine (GPS) or coarse (based on position of mobile telephony base stations) geographical location at the moment of a mosquito picture is recorded. Name of the recorded picture is created on the base of geographical position and Email address of the user. After the picture is recorded it is sent to server by wireless or 3G internet connection. Server side *PHP script* sends report to the administrator about new record, the report includes jpeg picture, geographical position of place where picture is recorded and Email address of user.

#### WP1c Detection of pathogens in mosquitoes and humans

17 molecular research samples of mosquitoes proceeded at IZSLER on Pan-flavivirus, Pan-bunvavirus, Pan-alphavirus, West Nile and USUTU viruses. 48 samples of the human sera, found negative, were tested on anti WN, IgM and IgG antibodies in IPH.

#### WP1d Social Impact and Policy Recommendation

The choice of methodology outputted the direct interview with the selected respondents supported by a questionnaire. The selected categories of relevant stakeholders having in view the

potential impact of the implementation of the project were: the citizens, the municipalities and the institutional partners identified by the public institutions in the field of Public Health Services.

The core conception of the questionnaires was centered on the following relevant directions: 1. knowledge and perception about the current regulatory framework; 2. past and present actions in mosquitos control and their impact in terms of quality assessment, efficiency and efficacy; 3. the impact over the tourism sector, the economy and the public health of the mosquitos and the mosquito borne diseases; 4. current expenditure and the readiness to contribute by supporting a national monitoring and control system and 5. the perception about the supporting pillars of the above mentioned system and their share of contribution. The research is on 60%.

### WP1e SIT and other non-chemical control methods

Research in non-chemical control measures, as a part of this Project following research has been done: the first step is a choice of possible product that could be used in controlling mosquito vectors in Montenegro - MMF Aquatain AMF, Liquid Mosquito Film. Preliminary, laboratory experiments have taken place at BTF, larvae of *Stegomyia albopicta* have been collected in the field and brought to the laboratory. The samples from the field have been divided in several groups according to the developmental stage (L<sub>1,2</sub>, L<sub>3</sub>, L<sub>3,4</sub> and Pupae). Each group was exposed to the treatment in several repetitions included control. Evaluation has been carried out in 24 hours intervals. Mortality rate is registered according to the dead individuals, accordingly, the efficacy rate has been calculated. Following first preliminary results, as next research steps it is recommended to continue the experiment using the same method with enlarged number of juvenile stages exposed, higher number of repetitions and in the containers of a bigger volume and larger surface.

Sterile male mosquito fitness (mating frequency and dispersal capacity) is crucial for successful implementation of Sterile Insect Technique (SIT). In 2014, Mark Release Recapture (MRR) experiment was conducted during the period August 8<sup>th</sup> to 12<sup>th</sup> and September 14<sup>th</sup> to 19<sup>th</sup> in the village Radovici on Luštica peninsula, Montenegro, in order to evaluate effects of shipment of mosquitoes (Crevalcore, Italy – Radovići, Montenegro) on flight capacity/dispersal of males. Planned application of SIT\* in invasive mosquito control in Montenegro is environment safe, high quality innovative research tool that represents cutting edge of RTD in Europe. Until now only practiced in Italy, it will be applied to populations of *St. albopicta* at Luštica peninsula. Relatively small settlements and narrow connection to continental part of the coast make Luštica ideal place for implementation of SIT.

\* release of sterile male insects is ranked by FAO as “Biological input that can help manage pest populations” together with release of predators, parasites or pathogens; biological control through fish, ducks, geese, goats; bio-pesticides and biological preparations.

Human bite sampling was performed two, four and seven day after release at 35 sampling points distributed within 50m, 100m, 150m, 200m and 250m from the point of release, by the battery powered aspirator. Sampled mosquitoes were identified in the field laboratory according to morphological characters by the dichotomous key Becker et al. (2014). Suspect coloured mosquitoes are rechecked under the UV lamp. During MRR experiment 529 male and female mosquito specimens belonging to 5 species were sampled: *Aedimorphus vexans*, *Dahlia* [*Ochlerotatus*] *echinus*, *Ochlerotatus caspius*, *Stegomyia albopicta* and *Culicoides* sp.

### WP1f Climate change impact on MV and MBD, adaptation and mitigation

Research was focused on analysis of past climate in Montenegro and selection of appropriate data series. In order to provide as complete as possible data series of past climate it has been decided to use 1981-2010 as a referent period for Montenegro for future CC impact analysis. Second step analysis was focused on scenario and climate model simulations. Data series of future climate for 2001-2100 are separated in three climatological periods: 2001-2030, 2031-2060 and 2071-2100 in order to make comparison between past and future climatic conditions for Montenegro. Additionally: overwintering of *Ae. albopictus* based on average temperature in January and yearly precipitation, as well as Kobayashi GIS model and MCDA model (ECDC) that presents possibility for domestication of species are provided.

**WP2 Twinning through exchange of know-how and experience and dissemination activities**

WP2a Training visits of MCM's young researchers to international partner institutions

Four visits of six researchers:

IHM-IZSLER two visits of two researchers - detection of West Nile virus (WNV), Usutu virus (USUV) and other flavivirus in mosquitoes pool by Real Time PCRs

BTF-FoA one visit of two researchers - Key morphological features of female mosquitoes; Identification of well preserved adult females; Identification of damaged adult females; Identification of adult mosquitoes sampled by participants and

IHMS-FoA one visit of two researchers - Climate change impact on MV and MBD, adaptation and mitigation

WP2b Know-how visits of MCM's researchers to international partner institutions

No activities in 1<sup>st</sup> research year.

WP2c Hosting foreign institutions representatives for *in situ* training

3 *in situ* trainings – hosting 3 persons:

CAA-BTF Practical training: Implementation of mosquito surveillance and non-chemical control measures in Montenegro.

FoA-IHMS Training in climate simulation and climate change assessment

FoA-IHMS Training trained in climate change (CC) phenomenon

WP2d Active participation of the applicant's research staff at international conferences

Multi Country - Workshop on the implementation of mosquito vectors surveillance in the EU and enlargement countries; INT MARKT 56605, Brussels 04 - 05 December 2014, 2 persons attended conference.

Preventing Vector Borne Diseases around the Mediterranean and Black Sea regions by creating new networks, MediLabSecure first “Head of Laboratory” meeting Institut Pasteur, Paris, 14 - 15 January 2015., 3 persons attended meeting.

WP2e Introduction of teaching on MV and MBD

Important improvements have been introduced in modules "Nematology" and "Phytopharmacy" on BTF and

on Faculty of Medicine (IPH) is ongoing process of change it is proposed to allocate more items from individual modules to one separate module "Special epidemiology".

WP2f Designing LOVCEN web site

<http://project-lovcen.me/index.php>

WP2g Internal dissemination meetings

25 meetings was held on: BTF, IHMS, IPH, NHM, TSU, HERIC, Municipality Podgorica.

WP2h Preparing web pages and brochures

No activities in 1<sup>st</sup> research year.

WP2i "Laboratory open days"

On 25. February 2015. has been organized the visit of the students of the high vocational school “Spasoje Raspopović” from Podgorica, to the IHMS. The students were introduced with the work of the laboratory, with the accent on the LOVCEN project and involvement of IHMS in the project. The total number of students was 44, from I to IV grade, from different field of orientation: chemical technician, food technician, environmental protection technician, agricultural technician.

WP2j Public (radio, TV, newspapers) appearances

During first year of lifetime of LOVCEN Project there were one interview and two public appearances. 20<sup>th</sup> May 2014. Portal analitika give news about HERIC Grants. 3<sup>rd</sup> June 2014., Romeo Bellini, Dusan Petric and Igor Pajovic were guests on RTCG 1 – Good morning Montenegro and 19. October 2014. Pobjeda published article "12 species of mosquitoes are found during survey in Montenegro".

WP2k Dissemination and feedback on stakeholder's opinions

LOVCEN Project make contact, or become a partner of couple on-going international Projects: - *European Network for Neglected Vectors and Vector-Borne Infections* – **EurNegVec**, COST Action TD1303.; - *Preventing Vector Borne Diseases Around the Mediterranean and Black Sea Regions by Creating New Networks* – **MediLabSecure** European project (2014-2017) aiming to provide collective responses of viral diseases (respiratory virus and arboviruses) in the Mediterranean and Black Sea regions; - *A European network for sharing data on the geographic distribution of arthropod vectors, transmitting human and animal disease agents* – **VectorNet**. The European network for sharing data on the geographic distribution of arthropod vectors, transmitting human and animal pathogens, funded both by the European Centre for Disease Prevention and Control (Stockholm, Sweden) and the European Food and Safety Authority (Parma, Italy). As well as the project in BTF – TEMPUS LifeADA.

We disseminate the LOVCEN to: Citizens of Montenegro, Municipalities, Health care institutions, University/Academic community and European scientific community.

We have support for activities from: Ministry of health; Ministry of sustainable development and tourism; Luštica Bay Montenegro – SME on Lustica; NGO Regional Environmental Center (REC), Montenegro country office and Union of Municipalities of Montenegro.

The main achievement of dissemination of activities is that *next 20<sup>th</sup> Meeting e-SOVE will be in - Budva, Montenegro – 2016*

WP2l Community participation in mosquito surveillance and control

In correlation with WP1b Mobile phone application for surveillance of invasive and indigenous species (KOMARAC)

**WP3 Acquisition of research equipment and innovation capacity building**

WP3a Acquisition of research equipment

We procure two STEMI 305 binocular microscopes. And in process of procurement are: Deep freezer -86°C; mosquito traps; EUROIMMUN ELISA Analyzer I-2P and QuantStudio™ 7 Flex Real-Time PCR System.

WP3b Innovation capacity building

Capacity building – one person: Professor Steve Quarrie held at BTF on Wednesday, 21<sup>st</sup> - Thursday, 22<sup>nd</sup> January training courses - Good quality research and Scientific writing.

**WP4 Management**

2<sup>nd</sup>-4<sup>th</sup> June 2014. - Kick off meeting, official beginning of Project – hosting 4 persons,  
16<sup>th</sup>-17<sup>th</sup> June 2014. - Facilitating effective implementation of the CoE and Grant projects financed by HERIC

13<sup>th</sup>-15<sup>th</sup> May 2015. - Annual meeting, official overview of the 1<sup>st</sup> year of the Project and planning work in 2<sup>nd</sup> year – hosting 6 persons.

Dr Igor Pajovic, Project leader,  
University of Montenegro – Biotechnical faculty  
Monday, 11<sup>th</sup> May, 2015., Podgorica

**signature**

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