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UNIVERZITET CRNE GORE

BIOTEHNIČKI FAKULTET

n/r dr sci Igora Pajovića

Podgorica

Poštovani,

Dostavljam Vam u prilogu godišnji izvještaj o projektu **“Surveillance of invasive and native mosquito vectors and pathogens they transmit in Montenegro – LOVCEN”** koji se odnosi na sprovedene aktivnosti iz ovog projekta, a vezano za ulogu Instituta za javno zdravlje u njemu.

S poštovanjem,

DIREKTOR

Doc. dr Boban Mugoša



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

Activity report

Period: year 2014

During reporting period several activities according to the Activity plan have been done by IPH researchers included in this Project.

At Kick-off meeting held on 02nd-03rd June 2014 at Biotechnical Faculty in Podgorica Zoran Vratnica presented IPH and its contribution to Project realization. At this meeting Zoran Vratnica was selected at PMT as a member from IPH.

At that period Paolo Bonilauri from IZSLER visited IPH and with Zoran Vratnica and Danijela Vujošević assessed capacities of IPH laboratory for molecular diagnostics of flaviviruses and prepared a plan for training visits of IPH researchers to IZSLER where defined that Zoran Vratnica will visit IZSLER from 14-18th July and Danijela Vujošević should do the same activity at the period from 28th July to 1st August 2014.

IPH agreed with Igor Pajović that IPH will supply with "dry ice" researchers from BTF, needed for NS traps and accepted that all mosquitoes captured during the field work will be pooled in 2ml falcons and placed in deep freezer at -80^oC, at Center for Medical Microbiology - CMM until they will be send to IZSLER.

As previous defined, Zoran Vratnica visited IZSLER from 14-18th July and brought mosquitoes samples for analysis. Danijela Vujošević visited IZSLER at the period from 28th July to 1st August 2014.

The theme of the visits was training for: detection of West Nile virus (WNV), Usutu virus (USUV) and other flavivirus in mosquitoes pool by Real Time PCRs.

During the visit the procedures and protocols applied in IZSLER from 2008 were shared to Montenegrin researchers and practical demonstration of all the analytical phases was provided. Montenegrin researchers were trained in application of following procedures and protocols: Preparation of mosquitoes pools for RNA extraction; Random primer Retro-transcription reaction protocol assessment; Real Time PCR protocols and reactions set-up; Real Time PCR protocols and results interpretation and RNA extraction with semi-automatic RNA/DNA extractor.

During this training Zoran Vratnica discussed with colleagues from IZSLER about various types of traps, their usefulness for catching some particular species of mosquitoes and possibility to some of them can be used in Montenegro.

At the end of visit a folder with annexes attached was sent to our mails and to the Coordinator of the project.

Finally, IZSLER researchers prepared for Montenegrin researchers positive cDNAs for WNV lineage 1, WNV lineage 2 and USUV.

Samples with mosquitoes from Montenegro were analyzed with West Nile and USUTU viruses specific real time PCR and with 4 pan-arbovirus PCR (pan-flavi, bunya, phlebo and alpha). All of them resulted negative in all the researches.

Several meetings about the problem with dry ice have been held with Igor Pajović and director of IPH during this reporting period.

In October the procedure for purchasing of new equipment was initiated. At 16th October Igor Pajović and Zoran Vratnica visited HERIC office and discussed with HERIC team about procurement plan and procedures. At that meeting timeline for procurement was defined. End of October as a deadline for submission of detailed technical specification of equipment and materials was determined. Problem was appeared when new generation of Real time PCR instruments was introduced to the market, because amount of money determined for this purchase was insufficient for this type of instruments. IPH has been tried to find reasonable solution. One of offered solutions was that IPH purchase a -80^oC deep freezer specified in this Project and spared money could be transferred for purchase of Real time PCR instrument. This was a reason why technical specification of equipment and materials for IPH wasn't submitted until the end of 2014.

During whole reporting period samples of the human sera taken from citizens from different cities in Montenegro were tested for anti WN IgM and IgG antibodies in Department for Virology and Serology CMM. ELISA technique was used for this purpose. All of 48 tested samples were found negative.

Additionally, during 2014 at the Center for Medical Microbiology samples for other vector borne diseases pathogens were tested:

- *Borrelia burgdorferi* – 840 IgG ELISA tests (59 positive)
- *Borrelia burgdorferi* – 838 IgM ELISA tests (79 positive)
- *Borrelia burgdorferi* – 76 Real-Time PCR tests (all negative)

- *Leishmania* spp. – 22 microscopy (all negative)
- *Leishmania donovani* – 143 IgG ELISA tests (10 positive)

- *Plasmodium* spp. – 14 microscopy (all negative)

Also, during 2014, the Center for Control and Prevention Diseases at the Institute of Public Health of Montenegro were recorded more mandatory reporting diseases from the group of vector-borne diseases, which are continuously monitored. Based on the electronic database following results were registered:

- Lyme boreliosis – six reports of disease

- Leishmaniasis – three reports of disease
- Infectio West Nile – no registered cases
- Malaria – no registered cases

Among the other activities (meetings, workshops and field work), Dr. Sanja Medenica and Dr. Nebojsa Sekulic attended a workshop, **TAIEX Multi Country Workshop on implementation of EU surveillance for invasive mosquitos for enlargement countries**, held in Brussels, Belgium, in the period from 04th to 05th December 2014. Workshop was organized in cooperation with the ECDC. At the workshop, in addition to representatives of the ECDC and WHO, were present representatives from the following countries: Austria, Belgium, Bulgaria, Croatian, Czech Republic, Denmark, Finland, France, Greece, Hungary, Italy, Portugal, Romania, Sweden, Great Britain, Albania, Bosnia and Herzegovina, Macedonia, Kosovo, Serbia and Turkey. During the workshop, new version of **Guidelines for the surveillance of native mosquitoes in Europe** was presented. Several presentations from representatives from EU and non-EU countries concerning current topics were presented. We presented the project "LOVCEN" and disseminated the paper form of that. In conclusions of discussions which were held during the workshop, LOVCEN was mentioned as a good example.

Report submitted by

Assoc. Prof. Boban Mugosa, researcher 

Zoran Vratnica, D.Sc. M.Sc. MD, key researcher, responsible person for partner organization

Dr. Danijela Vujosevic, researcher 

Dr. Sanja Medenica, researcher 

Dr. Nebojsa Sekulic, researcher 

