SHORT TERM SCIENTIFIC MISSION (STSM) – SCIENTIFIC REPORT

The STSM applicant submits this report for approval to the STSM coordinator

Action number: FA1408
STSM title: Molecular and serological techniques for *Toxoplasma gondii* identification ELISA, PCR, bioassay, genotyping and magnetic capture
STSM start and end date: 04/03/2018 to 17/03/2018
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PURPOSE OF THE STSM

The main purpose of this STSM was to get acquainted with the molecular and serological methods used at the National Reference Laboratory (NRL) for Toxoplasmosis at the Institute for Medical Research, University of Belgrade. NRL Toxo performs *Toxoplasma gondii* infection diagnosis using several techniques such as bioassay, MAT, ELISA and PCR (for detection and genotyping). This was a unique opportunity to develop my personal knowledge regarding the diagnostic methods for *Toxoplasma gondii* infection.

Firstly, it was my aim to improve the bioassay protocols performed in USAMV Cluj-Napoca for both *Toxoplasma gondii* diagnostics and for maintaining the strains that we work with. The bioassay protocol used by NRL Toxo is much more efficient, thus representing an opportunity to improve the future research carried out at our University.

Secondly, another purpose of this STSM, as stated above, was to become familiar with the serological methods, such as MAT and ELISA, but I also had a chance to participate in the whole diagnostic procedure since the arrival of a sample in the lab through all diagnostic steps to PCR genotyping of the *T. gondii* strain.

Finally, this STSM provided the opportunity for me to acquire a set of practical skills for laboratory work and improve my dexterity. I consider gaining additional experience in this field very helpful as a PhD student researcher that is currently utilizing similar techniques. In addition, I had an opportunity to really understand the theoretical aspects underlying each step of the protocols I was interested in, through helpful discussions with the NRL Toxo team.

My purpose was to learn and understand these methods so I can use them in my PhD and to extend my knowledge as much as I can. By the end of my STSM I think that I reached my purpose in learning and understanding these techniques and I think I can do them in the lab of my faculty back home.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSM

During this STSM, I performed the following methods: bioassay, conversion of bradyzoites to tachyzoites, ELISA, MAT, PCR for detection and genotyping. For each method I received theoretical and practical training. The theoretical information consisted of documents where I could find the necessary information about each technique, an explanation of the advantages and limitations of each method, , the necessary materials, and the protocol. Everyone from the lab helped me understand each step and they answered my questions. The practical part started with the presentation of the lab, they showed me all the instruments, the necessary materials and machines. They have a different room for each method, and there you have all you needed from basic instruments to kits, and machines. For each method I worked with a person who knows the method very well. First I assisted and got information about each step and after that, I did the method myself. I was helped if I needed it. All members of the host labwere very helpful and understanding. The methods themselves were performed according to protocols, which were also made available to me.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

One weekly activity that I have participated in was regular passage of the Rh strain, which is done on Tuesday and Friday. For that purpose, Rh tachyzoites are extracted from peritoneal cavity of infected mice, counted and inoculated i.p. into uninfected mice. In addition, I microscopically examined brain homogenates from mice used for regular passage of one of the type III strains maintained in the Serbian NRL Toxo. Briefly, the mice from which the cysts were harvested have been infected per os 3 months prior to sacrifice. I was able to see tissue cysts of various sizes and measure them. Those cysts were used for in vivo conversion into tachyzoites after i.p. inoculation into an uninfected mouse. Five days after inoculation, tachyzoites were collected from the peritoneal cavity and used to infect VERO cells.

Next, I used the MAT method to examine 50 serum samples from experimentally infected chickens from Romania, which are part of my experimental work. Of those, 25 serum samples were collected on day 7 post-infection, 25 on day 28 post infection. The results were negative for the samples collected on day 7 while 7/25 were positive on day 28.

Of the molecular methods used for *Toxoplasma gondii* dection in the lab, q-PCR for the amplification of the 529bp repetitive element was performed on 15 samples with 5 positive results.

The genotypes of the two strains which I used for the experimental infection of chickens are unknown. One strain was isolated from a wild cat (*Felis silvestris*) and one from a domestic cat (*Felis catus*), both

diagnosed at the Department of Parasitology and Parasitic Diseases in Cluj-Napoca, where the strains are being maintained. From the wild cat, we obtained tissue cysts, while from the domestic cat, we were able to collect oocysts. During the STSM, we genotyped both strains using the multiplex nested PCR-RFLP approach. We amplified the following markers: GRA6, altSAG2, Btub, Apico, C22, C29-2, PK1 and CS3. For the domestic cat strain, we were able to amplify all markers except C29-2, while we were only able to amplify altSAG2 and GRA6 for the wild cat strain. Based on the results of the RFLP analysis, the domestic cat strain is a type II on all markers we were able to test, while the wild cat strain is likely to be a recombinant strain, as the alleles for altSAG2 and GRA6 were identified as type I.

FUTURE COLLABORATIONS (if applicable)

The Romanian data about the seroprevalence of *Toxoplasma gondii* in domestic birds is rather vague or scarce, also there is limited research regarding the molecular epidemiology and genetic diversity of *T.gondii* genotypes which infect domestic birds, therefore leaving space for further investigations in this field. During this STSM, I received some advice for my PhD project in Romania. I think that future collaborations are possible because *Toxoplasma gondii* is our common research interest. Collaborations between the two labs occurred in the past through EU projects and this STSM was an opportunity to re-activate this collaboration and long standing partnership.