

SHORT TERM SCIENTIFIC MISSION (STSM) SCIENTIFIC REPORT

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

Action number: FA1408

STSM title: Seroprevalence of *Toxoplasma gondii* in backyard birds from Romania (MAT – Modified Agglutination Test)

STSM start and end date: 07/01/2019 to 01/02/2019

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PURPOSE OF THE STSM:

The main purpose of this STSM was to get acquainted with the serological method MAT (Modified Agglutination Test) that is being used in ANSES - French Agency for Food, Environmental and Occupational Health and Safety, Maisons-Alfort, France for the diagnosis of *Toxoplasma gondii* infection. This was a unique opportunity to develop my personal knowledge regarding the serological methods used for the diagnosis of *Toxoplasma gondii* infection.

This STSM provided me the opportunity to acquire a set of practical skills for laboratory work and improve my dexterity. As a PhD student researcher, I consider very helpful gaining additional experience in this field, since I am currently utilizing similar techniques.

My purpose was to learn and understand these methods, so I can utilise them in my PhD research work and to extend my knowledge as much as I can. By the end of my STSM I believe I have reached my purpose in learning and understanding these techniques and I consider I am able to perform and implement the serological identification protocols for *Toxoplasma gondii*, in birds, at the University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, while also increasing the quality of my scientific work.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSM

The training at the French Agency for Food, Environmental and Occupational Health and Safety, Maisons-Alfort, France (ANSES) was divided into two parts: a theoretical and a practical one, which were included on an everyday basis; additionally after the completion of the scheduled tasks for the day, time was allowed for background research or documentation on the tackled topics.

For a good understanding of the method I received theoretical and practical training. The theoretical training consisted of documents and articles where I could find the necessary information about the technique, an explanation of the advantages and limitations of the method, the necessary materials, and the stages of the protocol. Everyone from the lab helped me understand each step and answered my questions. The practical part started with the presentation of the lab, the equipment and the necessary materials and devices. The Institute has individual rooms for each method, and there you can find everything you need from basic instruments to kits, and devices. First, I assisted and learned about each step and after that, I performed the method myself. I received help whenever I was in need. The method was carried out according to the protocol, which was also made available for me.

During this STSM I performed the serological method MAT (Modified Agglutination Test). The modified agglutination test (MAT) is one of the most commonly used tests for the detection of *T. gondii* antibodies in animal and human serum. Among many serological tests available for the detection of *T. gondii* antibodies in human or animal sera, the modified agglutination test (MAT) is simple, easy to perform, does not require special equipment or species-specific reagents, and can be used for all species, including humans. Antigen for the MAT technique is stable for months, and reagents are commercially available. The procedure that I performed consisted in the following :

- Fill 25 µl of diluted serum (1/3 prepared previously) in the first well of the plate
- Distribute 25 µl of DTT (1/50 prepared previously) across the line of the plate
- Make a ½ serial dilution of the sera to be tested, across the line
- Distribute 25 µl of diluted antigen (1/17 prepared previously) across the line
- Homogenize the plate
- Cover with adhesive film
- Incubated overnight at room temperature
- Reading the next day: a positive result is considered when the agglutination is more than 50% of the well
- Result will be expressed in dilutions : first well = 6: second wells = 12
- If you want to express the titer, must be T= dil. / 2,4,8 depending on the batch of antigen

DESCRIPTION OF THE MAIN RESULTS OBTAINED

During my STSM I used the serological method MAT to assess 1016 serum samples from domestic birds raised in backyard houses from Romania. The serum from the birds was first screened for *T. gondii* antibodies, using four screening dilutions (1:6, 1:12, 1:24, 1:48) for the MAT. Antibodies to *T. gondii* discovered by the modified agglutination test (MAT) were found in 308 of 1016 (30,3%) chickens with titers of 1:6 in 263 of 2016 (25,8%) with titers 1:12, 218 of 2016 (21,4%) with titers of 1:24, 176 of 2016 (17,3%) with titers 1:48.

We also tested 60 birds serum from a farm, but all the sample tested negative for the presence of *T. gondii* antibodies.

I also used the MAT method to examine 150 serum samples from experimentally infected chickens from Romania, which are part of my experimental work. The samples were collected in day 0, day 7 pi, day 28 pi and after reinfection. Positive samples were detected on day 28 in 7 (25%) out of 28 experimentally infected chickens and after reinfection 5 (25%) out of 20 experimentally infected chickens.

FUTURE COLLABORATIONS (if applicable)

Information on the seroprevalence of *Toxoplasma gondii* in domestic birds from Romania is rather vague or scarce, also there is limited research regarding the molecular epidemiology and genetic diversity of *T. gondii* genotypes that infect domestic birds, therefore leaving space for further investigations in this field. I believe 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 longstanding partnership.