SHORT TERM SCIENTIFIC MISSION (STSM) – SCIENTIFIC REPORT

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

Action number: Action FA1408 - 40783
STSM title: Detecting *Toxoplasma gondii* oocysts in water samples
STSM start and end date: March 11, 2018 – March 17, 2018
Grantee name: Huifang Deng, jenny.deng@rivm.nl
Host: Moredun Research Institute
Home: The Dutch National Institute for Public Health and Environment

PURPOSE OF THE STSM/

Toxoplasmosis is caused by the obligate intracellular parasite *Toxoplasma gondii* (*T. gondii*). It is one of the most common parasitic infections in human and other warm-blooded animals. Humans become get infected mainly through consuming undercooked/raw meat containing viable tissue cysts, or by ingesting food/water contaminated with oocysts. *T. gondii* oocysts shed by the primary infected definite hosts, felines, are spread in the environment mainly through wind, water and manure[1]. They can remain infective under unfavourable conditions. Environmental matrices (e.g. fruits, vegetables, soil and water) contaminated with sporulated oocysts are important sources of human infections. A few acute toxoplasmosis outbreaks in humans linked to oocysts have been reported worldwide. However, oocyst isolation in the suspected environmental sources was usually not conducted from those epidemiological studies. This reflects the lack of practical and sensitive methods to recover *T. gondii* oocysts in the environmental samples (such as water/fresh produce).

So far, commercial kits for *T. gondii* oocysts detection in water samples are not available and there is no agreed optimised method for *T. gondii* oocysts detection from water[2]. Thus, the actual percentage of water-borne infection in humans and the relative importance of this transmission route remained unclear.

Moredun research Institute, conducts worlds class scientific research on the infectious disease of livestock, caused by important virus, bacteria and parasites. Currently, the research group of Dr. Frank Katzer is working on detecting and quantifying *T. gondii* oocysts in drinking water from various water supplies throughout Scotland. Therefore, the aim of this STSM Euro-FBP mission was to learn the techniques for processing and detecting *T. gondii* oocysts and *T. gondii* DNA in water samples. More specifically, this STSM was aimed to get practical skills on:

1) How to process and concentrate water samples for *T. gondii* oocysts visualization or DNA extraction
2) How to perform sucrose flotation for *T. gondii* oocysts
3) How to prepare slides for *T. gondii* oocysts visualization and how to remove oocysts from slides
4) How to extract DNA from *T. gondii* oocysts from raw/final water samples and slides

This mission is in line with my PhD project, and the techniques can be directly applied after returning to RIVM.

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DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

Day 1, 12 March 2018

- Introduction of the institute and laboratories
- Introduction of the risk management for entering the laboratories
- Sucrose floatation
  - Prepare sucrose solution
  - Spike a known number of *T. gondii* oocysts into water samples
  - Centrifuge water samples and remove the supernatant
  - Add H_2SO_4 and put the samples at room temperature overnight
- Visualise *T. gondii* oocysts (from pre-prepared slides)
  - New and old oocysts and toxoplasma-like bodies were checked under microscope
- Department scientific meeting

Day 2, 13 March 2018

- Remove oocysts from positive slides (from pre-prepared slides)
  - Remove the cover slip with nail polish remover
  - Add lysis buffer onto the well of the slide
  - Scrape the surface of the well with a sterile plastic bacterial inoculation loop
  - Collect the liquid from the slide well
  - Repeat the scraping of the slide well with the same inoculation loop and collect the liquid
- *T. gondii* oocysts DNA extraction
  - Perform the freeze-thawing procedure in liquid nitrogen and water bath to extract DNA
  - Centrifuge the samples and add proteinase K
  - Incubate the samples at 56 °C water bath overnight
- Protozoology group meeting

Day 3, 14 March 2018

- *T. gondii* oocysts DNA extraction
  - Get the samples out from the 56 °C water bath and continue DNA extraction procedure by following the modified method
  - Obtain *T. gondii* oocysts DNA and store them at -20 °C
- Sucrose floatation
  - Continue working on the water samples with spiked *T. gondii* oocysts
  - Collect oocysts from the samples

Day 4, 15 March 2018

- Making slides
  - Make the slides with the oocysts obtained from Day 3

Day 5, 16 March 2018

- Visualize and count *T. gondii* oocysts under microscope with the prepared slides from Day 4
- Summary and questions on *T. gondii* oocysts detection method
- Close this STSM

DESCRIPTION OF THE MAIN RESULTS OBTAINED

During the STSM visit at Moredun Research Institute all aims described above were achieved. The mission was fully successful in training and getting practical skills.

(1) Hands-on experiences on how to collect and process water samples from Dr. Alison Burrells was obtained.

(2) After the STSM training I am able to perform sucrose floatation, visualization, and DNA extraction for *T. gondii* oocysts. These methods can also be applied on other environmental samples, such as soil and fresh produce.

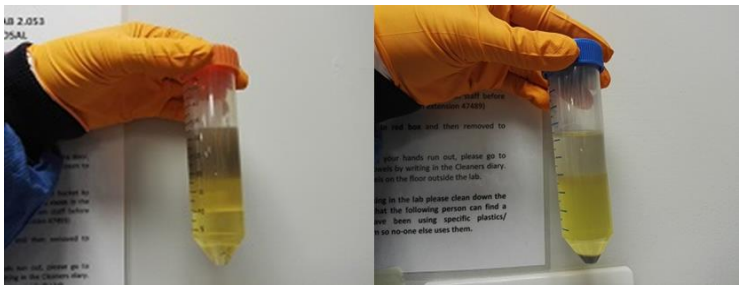

Figure 1. *T. gondii* oocysts sucrose floatation, before (left) and after (right) centrifuge

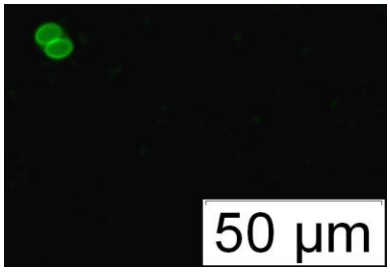

Figure 2. Sporulated *T. gondii* oocysts under microscope.

FUTURE COLLABORATIONS (if applicable)

Moredun Research Institute and the Dutch National Institute for Public Health and Environment already have a good working relationship. This STSM has tightened the relationship and a continued sharing of knowledge between the institutions is foreseen. My supervisor Dr. Joke van der Giessen (the Dutch National Institute for Public Health and Environment) will give a presentation at a panel discussion on 4 April 2018 at Edinburgh. Possibilities for collaboration will be discussed.

OTHER COMMENTS

I would like to express my gratitude to my host and home institutes for arranging and organising this visit. Their purport and willingness to share experiences were invaluable to the success of the STSM. A special thanks to Dr. Alison Burrells for training and supervising me during the visit. The skills I learned in this STSM have certainly enriched my laboratory experience and will be very useful for my PhD study.

REFERENCE

1 Jones, J. L. & Dubey, J. P. Waterborne toxoplasmosis - Recent developments. *Exp Parasitol* **124**, 10-25 (2010).

2 Klotz, C., Soba, B., Skvarc, M., Gabriel, S. & Robertson, L. J. A European network for food-borne parasites (Euro-FBP): meeting report on 'Analytical methods for food-borne parasites in human and veterinary diagnostics and in food matrices'. *Parasit Vectors* **10**, 559, doi:10.1186/s13071-017-2506-9 (2017).