SHORT TERM SCIENTIFIC MISSION (STSM)
SCIENTIFIC REPORT

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

Action number: FA1408 – A European Network for Foodborne Parasites (Euro-FBP)
STSM title: Training of magnetic capture and qPCR for direct detection of *Toxoplasma gondii* in meat and environmental samples
STSM start and end date: 17/02/2019 to 23/02/2019
Grantee name: Nadja Bier
Host Institution: The Dutch National Institute for Public Health and Environment (RIVM)
Home Institution: German Federal Institute for Risk Assessment (BfR)

PURPOSE OF THE STSM:

Toxoplasmosis is one of the most common parasitic zoonosis worldwide with approximately 30 % of the human population infected with the parasite. Toxoplasmosis can be acquired horizontally by ingestion of different infectious parasite stages of *Toxoplasma gondii* through consumption of contaminated food (raw or undercooked meat, vegetables, fruits, and water) or by accidental ingestion of oocysts after gardening or direct contact to cat feces.

Due to the uneven distribution and often small concentration of tissue cysts, molecular detection of *T. gondii* in meat can be challenging and the probability to detect *T. gondii* rises with increasing size of the analyzed sample. At the same time, inhibiting substances in meat and environmental samples may lead to decreased PCR sensitivity or false-negative results.

At the The Dutch National Institute for Public Health and Environment, Marieke Opsteegh developed one of the most sensitive methods for molecular detection of *T. gondii* DNA in meat that addresses these challenges and allows the detection of approx. one tissue cyst per 100 g meat (Opsteegh, 2010). In this method, specific *T. gondii* DNA is isolated and concentrated from a large sample size of up to 100 g meat using magnetic capture and is detected by qPCR. Currently, the method is also being adapted for detection of *T. gondii* oocysts in environmental samples by Jenny Deng.

The aim of this STSM was to gain expertise in the methodology of magnetic capture and qPCR based detection of *T. gondii* in meat and environmental samples. More specifically, the training aimed to get experience in:

1) Preparation of heart muscle samples spiked with tachyzoites and DNA extraction using magnetic capture
2) Preparation of water samples spiked with oocysts and DNA extraction using magnetic capture
3) qPCR for *T. gondii* 529bp repeat element

After the training, the method should be implemented at the BfR laboratory to enable quantitative and sensitive detection of *T. gondii* DNA in meat from different animals as well as in environmental samples.

An additional aim was to discuss strategies for data analysis used at the RIVM in former and ongoing research projects and to gain knowledge in the performance of systematic reviews as well as in the analysis of serological data to estimate the seroprevalence of *T. gondii*.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

Day 1, 18 February 2019
- Introduction of the institute and laboratories
- Safety instructions
- Preparation of water samples
    - Collection of surface pond water samples
    - Spiking of 16 water samples with dilution series of oocysts
    - Centrifugation and removal of supernatant
    - 3x Freeze-/thaw-cycles of pelleted oocysts

Day 2, 19 February 2019
- Workmeeting
- Preparation of meat samples
    - Discussion on sample preparation to eliminate fat, vessel wall and connective tissue
    - Preparation of 10 heart muscle samples from seropositive foxes
    - Spiking of Toxo-negative meat samples with defined number of tachyzoites (positive and negative controls)
- Lysis of water and meat samples overnight
    - Preparation and addition of lysis buffer to samples
    - Homogenization of meat samples on a stomacher
    - Incubation overnight in a 55°C water bath
- Presentation: Magnetic capture and qPCR based detection of *T. gondii* in meat by Marieke Opsteegh

Day 3, 20 February 2019
- Meat samples:
    - Homogenization of sample on a stomacher
    - Removal of 50 ml lysate and centrifugation
    - Removal of 12 ml supernatant and transfer to clean tube
- Water samples:
    - Centrifugation of the samples
    - Removal of supernatant and transfer to clean tube
- Removal of Biotin using Streptavidin-Sepharose
    - Incubation of all samples for 10 min at 100°C to inactivate proteinase K
    - Cooling down
    - Washing of Streptavidin-Sepharose 3 times with PBS
    - Addition of Streptavidin-Sepharose to samples
    - Incubation at room temperature on a rotator
    - Centrifugation
    - Removal of supernatant and transfer to clean tube
- Presentation: Systematic reviews and meta-analysis by Jenny Deng

Day 4, 21 February 2019
- Hybridisation with biotin-labelled capture oligos
    - Addition of 10 pmol capture oligos to the samples
    - Incubation of samples at 95°C for denaturation of DNA
    - Cooling down and incubation on a rotator for hybridization of *T. gondii* DNA with capture oligos
- Magnetic capture
    - Washing (1x) and resuspension of dynabeads in B &W buffer.
    - Addition of 5M NaCl and washed Dynabeads to the samples.
    - Incubation on a rotator to enable binding of Streptavidin-coated magnetic beads to the *T. gondii* DNA-biotinylated capture oligo-complex
    - Magentic separation of beads
    - Resuspension of dynabeads in 1X B &W buffer and transfer in clean tube
    - Washing of dynabeads with1X B &W buffer
- Removal of dynabeads
    - Incubation of samples for 10 min at 100 °C to release *T. gondii* DNA
    - Magnetic separation and transfer of DNA sample to clean tube
- Skype Meeting with European partners to discuss future collaborations in an EJP-project

Day 5, 22 February 2019
- qPCR on 529bp RE of *T. gondii*
- Analysis and discussion of qPCR results
- Presentation: Analysis of serological data using binormal mixture models to assess seroprevalence, cut-off value and characteristics of serological tests by Marieke Opsteegh
- Workmeeting: Final discussion of the results obtained in the STSM and future collaborations

DESCRIPTION OF THE MAIN RESULTS OBTAINED

During the STSM at The Dutch National Institute for Public Health and Environment all of the above mentioned aims have been successfully achieved. I was trained in the methodology of magnetic capture and qPCR for detection of *T. gondii* in meat as well as in water samples and will be able do establish the method in our laboratory.
All ten heart samples of seropositive foxes examined during this STSM tested positive for *T. gondii* DNA with Cq values between 28.4-32.8. In the next months, the method will be implemented at the BfR laboratory to examine heart muscle samples of wild boar, roe deer, and red deer collected during the hunting season 2018/2019 in Brandenburg, Germany.
Additionally, the application of magnetic capture for detection of *T. gondii* in water samples has been evaluated. The preliminary protocol allowed the detection of 10 oocysts in 50 ml water. Due to PCR inhibiton in some of the samples, a more thoroughly washing of the magnetic beads has been discussed to eliminate PCR inhibitors.

FUTURE COLLABORATIONS (if applicable)

This STSM has tightened the relationship between RIVM and BfR. Future collaborations to assess the seroprevalence of *T. gondii* in different game species in Germany have been discussed. Moreover, a close cooperation within a potential EJP project is sought.

OTHER COMMENTS

I would like to highly express my gratitude to my host institute for arranging and organising this visit on such a short notice. I want to especially thank Dr. Joke van der Giessen for her great hospitality and the opportunity to visit her lab as well as Dr. Marieke Opsteegh and Jenny Deng for their willingness to share their knowledge and experience with me. The training and discussions were very valuable for me and my future work.

References:

Opsteegh, M., Langelaar, M., Sprong, H., Den Hartog, L., De Craeye, S., Bokken, G., et al. 2010. Direct detection and genotyping of Toxoplasma gondii in meat samples using magnetic capture and PCR. Int. J. Food Microbiol., 139, 193-201.