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: Training of PCR and sequencing methods for the identification of not-*Trichinella* isolates of Nematodes
STSM start and end date: 26/11/2018 to 30/11/2018
Grantee name: KAESTNER Carolyn

PURPOSE OF THE STSM:

The short term scientific mission "Training of PCR and sequencing methods for the identification of not-*Trichinella* isolates of nematodes" took place in the National Reference Laboratory (NRL) *Trichinella* at the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) in Paris and was held between 19/02/2018 and 26/02/2018. The training was conducted by Dr. Grégory Karadjian under the supervision of Dr. Isabelle Vallée.

Trichinella spp. is detected in meat by artificial digestion followed by a molecular typing method (multiplex PCR). During this process, Nematode larvae other than *Trichinella* spp. are often found and cannot surely be identified by morphological examination.
The aim of this project therefore is to identify these "other Nematodes" using molecular methods.
The NRL *Trichinella* in France has developed a PCR based method for the identification of these parasites.
The purpose of this Short Term Scientific Mission was to get trained in the identification and differentiation of nematodes other than *Trichinella* spp. by using the above mentioned molecular detection methods and morphological orientation.
Furthermore, molecular data should be gained by identification of nematodes other than *Trichinella* spp. which were isolated at the BfR and sent to ANSES for molecular detection.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSM

The STSM took place according to the following work plan provided by the host institution:

Day 1
DNA extraction of not *Trichinella* isolates available at ANSES
amplification of 18S locus, preparation for sequencing

Day 2
Sequence analysis based on former results
comparison with other nematode sequences in GenBank (BLAST, alignments in BioEdit, …)
Introduction to WormbaseParasite database
Day 3

Microscopy on BfR isolates to look for morphological characters
DNA extraction, amplification of fragment of 18S locus of BfR isolates without discriminant morphological caracters

Day 4

Sequence analysis of first round of 18S locus amplifications, followed by either ITS2 or 28S PCR

Day 5

Further excercises on sequence analysis
DNA extraction, amplification of 18S locus of BfR isolates

In the beginning, we looked for morphological criteria of the BfR isolates by microscopy. On the basis of this morphological orientation (if possible/if no 18S PCR was performed), we decided which specific PCR is senseful to be done.

Protocol:

The DNA extraction is realized according to the *Trichinella* extraction method prior to multiplex PCR (from EURL).

a. 18 S PCR

The sequence of a 600 bp fragment of the 18S locus is enough for orientation to a taxonomic group (Family, genus, … depending of the group).
All primers are 0.3 µM final concentrations.

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- Mix /reaction: Water: 30.5 µl
Buffer HF 5X: 10 µl
Primer 18S_965S: 1.5 µl
Primer 18S_1573R: 1.5 µl
dNTP: 1 µl
Taq: 0.5 µl

+ 5 µl of extracted DNA

- Cycle:
(Heat Lid to 112°)
Denaturation / Taq activation: 98°, 2 min
40 cycles: - Denaturation: 98°, 10 sec
- Hybridation: 60°, 1 min
- Elongation: 72°, 1 min
Final elongation: 72°, 10 min
(Store forever: 10°)

b. ITS2

These PCRs are used to discriminate species within the different families by morphology or the 18s PCR, i. e. Toxacaridae/Ascaridae.
All primers are 0.5 µM final concentrations.

-Mix /reaction: Water: 28.5 µl
Buffer HF 5X: 10 µl
Primer ITS2F: 2.5 µl

Primer ITS2R: 2.5 µl
dNTP: 1 µl
Taq: 0.5 µl

+ 5 µl of extracted DNA

- Cycle:
(Heat Lid to 112°)
Denaturation / Taq activation: 98°, 2 min
40 cycles: - Denaturation: 98°, 10 sec
- Hybridation: 60°, 1 min
- Elongation: 72°, 1 min
Final elongation: 72°, 10 min
(Store forever: 10°)

c. 28S (ref : Tkach et al., 2013)

This PCR is used to discriminate species within the Strongylida/Rhabditida given by morphology or the 18s PCR.
Primers are 0.5 µM final concentrations.
- Mix /reaction: Water: 28.5 µl
Buffer HF 5X: 10 µl
Primer ritf: 2.5 µl
Primer 1500R: 2.5 µl
dNTP: 1 µl
Taq: 0.5 µl

+ 5 µl of extracted DNA

- Cycle:
(Heat Lid to 112°)
Denaturation / Taq activation: 98°, 2 min
40 cycles: - Denaturation: 98°, 10 sec
- Hybridation: 53°, 30 sec
- Elongation: 72°, 2 min
Final elongation: 72°, 10 min
(Store forever: 10°)

Very often, more than one band is obtained and the interesting band (~3200-3400 bp depending on the species) has to be purified prior to sequencing. For sequencing, we send PCR products and 4 more primers than the ones used for the PCR (ITS5, ITS4, 300R and ECD2).

Reagents references

PCR reagents	
Phusion Hot Start II DNA Polymerase (2 U/µL)	Thermo Scientific F-549S
dNTP Mix (10 mM each)	Thermo ScientificR0192
Primers synthesis (100µM)	Eurofins-Genomics Custom DNA Oligos

Positive controls used for each PCR	
18 S	*Trichinella spiralis* maintained in our lab
ITS2 Toxocaridae/Ascaridae	*T. cati* or *T. canis* from vet school
28 S	*A. vasorum*

Primer sequences	
18S_1573R	TACAAAGGGCAGGGACGTAAT
18S_965S	GGCGATCAGATACCGCCCTAGTT
ITS2F	TGGATCGATGAAGAACGC
ITS2R	TTAGTTTCTTTTCCTCCGCT
Ritf	GCGGCTTAATTTGACTCAACACGG
1500R	GCTATCCTGAGGGAAACTTCG
ITS5	GAAGTAAAAGTCGTAACAAGG
ITS4	TCCTCCGCTTATTGATATGC
300R	CAACTTTCCCTCACGGTACTTG
ECD2	CTTGGTCCGTGTTTCAAGACGGG

DESCRIPTION OF THE MAIN RESULTS OBTAINED

In total, 20 isolates of nematodes other than *Trichinella* spp. were sent to ANSES by the BfR. 8 of them came with photos of the larvae and were analysed by morphological criteria first. 12 were sent separately without any pictures but with preexamination by 18S PCR.

Results for the 8 samples (with photos):

Based on the morphological orientation, 5 out of 8 samples looked like *Toxocara spp*. and were directly examined by ITS 2 PCR. 3 samples did not give any orientation, so we did the 18S PCR for further analysis.

The ITS 2 PCR confirmed the suspected *Toxocara* spp. for all 5 isolates, so the bands were sent for sequencing to differ between *Toxocara canis* and *Toxocara cati*. The 18S PCR also showed bands for the 3 other isolates which were sent for sequencing as well.

Results for the 12 samples (without any photos but with 18S PCR already done):

Based on the results of the 18S PCR, ITS 2 PCR was done for 6 samples suspected *Toxocara/Ascarida*. Further, 28S PCR was done for the other 6 samples suspected *Strongylida/Rhabditida*.

The ITS 2 PCR showed bands for all 6 samples which were sent for sequencing. The 28S PCR showed positive results for 4 out of 6 samples, 2 of them were negative. The bands of the 4 positive samples were sent for sequencing as well. The 2 negative will be tried to be examined by COX 1 PCR, but there are no results available yet.

Results of sequencing for the 8 samples (with photos):

According to the sequencing, the 5 samples which were examined by ITS 2 PCR are *Toxocara cati*. The 18S sequence results for the 3 other samples were good and orientated well.

Results of sequencing for the 12 samples (without any photos):

According to the sequencing, the 6 samples which were examined by ITS 2 PCR are *Ascaris suum /Ascaris lumbricoides.* Up-to-date it is not clear if they are the same species or not in the literature.

The results for the 28S sequence are not that good. One of them orientate to *Metastrongylus* and one to *Crenosoma*. The 2 others gave no results and will be tried to be analysed by another 28S PCR using more amount of extracted DNA, as well as with COX 1 PCR. There are no results available yet.

FUTURE COLLABORATIONS (if applicable)

In conclusion, the STSM has been successful concerning the described aims. The NRL *Trichinella* at the BfR will be able to identify most of the Nematode larvae other than *Trichinella* spp. by using the PCR protocol developed by the NRL *Trichinella* at ANSES.

Furthermore, it would be lovely to continue the collaboration between ANSES and the BfR by exchanging the results and eventually publishing them.