Deliverable D-JRP-TOXOSOURCES-WP5.3

Report on the inter-laboratory comparison and the pilot studies of WP5

Workpackage 5 of JRP22-FBZ4.1-TOXOSOURCES

Responsible Partners:
FLI, UCM, ISS, SSI

GENERAL INFORMATION

European Joint Programme full title	**Promoting One Health in Europe through joint actions on foodborne zoonoses, antimicrobial resistance and emerging microbiological hazards**
European Joint Programme acronym	**One Health EJP**
Funding	**This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 773830.**
Grant Agreement	**Grant agreement n° 773830**
Start Date	**01/01/2018**
Duration	**60 Months**

DOCUMENT MANAGEMENT

JIP/JRP deliverable	D-JRP-TOXOSOURCES-WP5.3
Project Acronym	JRP22-FBZ4.1-TOXOSOURCES
Authors	Maike Joeres (FLI), Rafael Calero Bernal (UCM), Pavlo Maksimov (FLI), Simone Caccio (ISS), Luis Ortega-Mora (UCM), Pikka Jokelainen (SSI), Gereon Schares (FLI)
Other contributors	TOXOSOURCES consortium
Due month of the report	M59
Actual submission month	M59
Type *R: Document, report* *DEC: Websites, patent filings, videos, etc.; OTHER*	R **Save date**: November 30, 2022
Dissemination level *PU: Public (default)* *CO: confidential, only for members of the consortium (including the Commission Services)*	PU
Dissemination *Author's suggestion to inform the following possible interested parties.*	OHEJP WP 1 ☐ OHEJP WP 2 ☐ OHEJP WP 3 ☒ OHEJP WP 4 ☐ OHEJP WP 5 ☒ OHEJP WP 6 ☐ OHEJP WP 7 ☐ Project Management Team ☐ Communication Team ☐ Scientific Steering Board ☐ National Stakeholders/Program Owners Committee ☐ EFSA ☒ ECDC ☐ Other international stakeholder(s): …… Social Media: ..... **Other recipient(s):**.....

This activity is part of the European Joint Programme One Health EJP.
This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 773830.

D-JRP-TOXOSOURCES-WP5.3
REPORT ON INTER-LABORATORY COMPARISON AND NEXT GENERATION SEQUENCING-MULTILOCUS SEQUENCE TYPING PILOT STUDIES

BACKGROUND

This is a public deliverable of One Health EJP Joint Research Project:
JRP22-FBZ4.1-TOXOSOURCES – *Toxoplasma gondii* sources quantified
(https://onehealthejp.eu/jrp-toxosources/);

Work Package:
JRP-TOXOSOURCES-WP5 Novel *Toxoplasma* gondii typing method to detect within-genotype variation;
Task:
JRP-TOXOSOURCES-WP-T3 Inter-laboratory comparison and Next Generation Sequencing-Multilocus Sequence Typing pilot studies.

Project Leader: Pikka Jokelainen, SSI; Deputy Project Leader: Joke van der Giessen, RIVM.
WP Leader: Gereon Schares, FLI; Deputy WP Leader: Pikka Jokelainen, SSI.
Task Leader: Luis Ortega-Mora, UCM; Deputy Task Leader: Pikka Jokelainen, SSI.

Contacts: Pikka Jokelainen, PIJO@ssi.dk ; Gereon Schares, gereon.schares@fli.de

TOXOSOURCES addresses the research question – **What are the relative contributions of the different sources of *T. gondii* infection?** – by using several multidisciplinary approaches and novel and improved methods to yield robust estimates that can inform risk management and policy makers.

Objectives of TOXOSOURCES WP5:

- ✓ To develop a novel *T. gondii* typing method with the discriminatory power required in the epidemiological European scenario
- ✓ To apply the novel method in pilot studies

TOXOSOURCES WP5 was successful in its aim to identify highly polymorphic regions in genomes of very closely related *T. gondii* strains across Europe. Preliminary NGS data on European type II *T. gondii* revealed substantial variation between isolates and relative to reference strains. Using a panel of representative strains, regions in the genome with a higher SNP density were identified and used to establish a novel high-throughput

targeted Next Generation Sequencing-Multilocus Sequence Typing (NGS-MLST) method. This Deliverable reports on the inter-laboratory comparison of typing and pilot studies of TOXOSOURCES WP5.

INTERLABORATORY COMPARISON

Three sample sets were generated for interlaboratory comparison of typing methods.

One TOXOSOURCES consortium partner had the possibility to be part of an interlaboratory comparison using the new typing method, and five laboratories (including three TOXOSOURCES and two external partners) participated in an interlaboratory comparison using the microsatellite typing method.

1. Interlaboratory comparison on the novel NGS-MLST method

A standard operating procedure (SOP) was established. A list of reagents was prepared which are necessary to perform the new typing method. Some of the reagents (e. g. primer pools, barcode adapter) were offered to be shared with interested consortium partners.

2. Interlaboratory comparison on microsatellite typing

The current state-of-the-art *T. gondii* typing standard technique is based on 15 microsatellite (MS) regions (Ajzenberg et al. 2010). It is able to type/discriminate lineages but also to detect intra-lineage genetic variability.

Five European laboratories agreed to be part of a ring trial on microsatellite typing. In this ring trial a number of key factors were identified causing differences in typing results and a guideline to reach an optimal level of agreement between laboratories' results was established. This was the first time a ring trial on the method was organised.

3. Samples used for the validation of the novel NGS-MLST method and the comparison with the MS typing technique

Different DNA concentrations of the *T. gondii* reference strains RH (type I), Me49 (type II) and NED (type III) were used for the ring trial of the MS technique and also for the validation of the novel NGS-MLST method, including tests to assess the analytical sensitivity of the new method.

Furthermore, a large number of European field samples were collected, including fresh tissue, formalin-fixed paraffin-embedded (FFPE) tissue and faecal samples. DNAs extracted from selected samples were used for the validation of the new typing method. In addition, negative tissue specimens from different host species and also negative faecal samples were spiked with different amounts of DNA.

PILOT STUDIES USING NOVEL NGS-MLST METHOD

Field samples were collected from different parts of Europe. Each sample was characterized by real time PCR to assess the different levels of *T. gondii* DNA. Samples with Cq values ≤ 30 were typed by a currently available microsatellite (MS) technique, using 15 markers. In total, 215 samples were completely typable and thus potentially suitable for pilot studies using the novel typing method.

Altogether five pilot studies were conducted:

1. Multicentre study across Europe, different host species and different matrices

- Research question: Are there regional differences in molecular epidemiology of *T. gondii* across Europe?
- Samples received via TOXOSOURCES partners but also from external partners and collaborators
- Sampled host species: 24 different host species
- Sample types: 13 different matrices (fresh tissue, FFPE tissue, oocyst samples)

2. Local study in oocyst shedding cats and intermediate hosts in Germany and neighbouring countries (Czech Republic, Austria, Switzerland, …)

- Research question: To which extent differ typing results in various regions of single country and neighbouring countries?
- Samples provided by: Bretislav Koudela (University of Veterinary Sciences, Brno), Walter Basso (University of Bern), Majda Globokar (IDEXX, Kornwestheim), Martin Peters (CVUA Arnsberg), Christoph Schulze (LL-BB, Frankfurt/O.), Nelly Scuda (LGL, Erlangen)
- Sampled species: Cat, various intermediate host species
- Sample type: Oocysts, tissues (fresh, paraffin-embedded)
- Sampling period: Ongoing
- Number of samples, typable by 15 MS markers: n>30 (type II)

3. Local study in cats from Denmark

- Research question: Overview of locally circulating strains.
- Samples provided by: Pikka Jokelainen (SSI), collaboration with University of Copenhagen
- Sampled species: Cat
- Sample type: Tongues of euthanized feral cats
- Sampling period: 2020
- Number of samples, typable by 15 MS markers: n=30 (type II)

4. Local study in sheep and pigs in Spain

- Research question: Are there regional differences and differences between livestock production types? Are there differences between samples collected from different cases in the same outbreak? Differences between samples collected from adult sheep (slaughterhouses) vs. samples collected from abortion cases?
- Samples provided by: Luis Ortega-Mora (UCM)
- Sampled species: sheep, pig
- Sample type: fresh tissue (Spanish sheep flocks: abortion outbreaks, endemic abortions, foetuses, placenta, adult sheep; Iberian pigs)
- Sampling period: 2017-2019
- Number of samples, typable by 15 MS markers: n=7 (type II)

5. Local study in squirrels with toxoplasmosis from the Netherlands

- Research question: Overview of locally circulating strains. Are there geographical differences?
- Samples provided by: Margriet Montizaan, DWHC
- Sample type: Tissue (lung, liver, heart)
- Sampling period: 2014-2020
- Number of samples, typable by 15 MS markers: n=39 (type II)

DISCUSSION AND PERSPECTIVES

The work was performed successfully as an international collaboration of scientists, which included in addition to the TOXOSOURCES partners also external partners. The results on the new typing method, the results of the first microsatellite typing ring-trial, and results of the pilot studies are of interest and importance to the scientific community and will be disseminated as scientific publications in open access peer-reviewed international journals and presentations at scientific conferences and gatherings. The pilot studies confirmed the usability of typing to a wide variety of samples, and the results of the pilot studies add to the understanding of circulation of *T. gondii* across Europe.

ACKNOWLEDGEMENTS

We thank all colleagues in TOXOSOURCES and beyond for providing isolates and DNAs and for their contributions to the work. This work would not have been possible without an international collaborative effort.

References

Ajzenberg, D., Collinet, F., Mercier, A., Vignoles, P., & Dardé, M. L. (2010). Genotyping of *Toxoplasma gondii* isolates with 15 microsatellite markers in a single multiplex PCR assay. Journal of clinical microbiology, 48(12), 4641–4645. https://doi.org/10.1128/JCM.01152-10