COST Action FA1408

European Network for Foodborne Parasites in Europe (EURO-FBP)

WG2 Minutes:

Guideline development with emphasis on validation, standardisation, and QC

Meeting date: 5th-6th July 2018

Attendees:
Rachel Chalmers (Rachel.Chalmers@wales.nhs.uk); Suzanne Jordan (Suzanne.Jordan@campdenbri.co.uk); Frank Katzer (frank.katzer@moredun.ac.uk); Christian Klotz (KlotzC@rki.de); Stefanie LaCarbona (s.lacarbona@actalia.eu); Marco Lalle (marco.lalle@iss.it); Anne Mayer-Scholl (anne.mayer-scholl@bfr.bund.de); Ivona Mlanideo (mladineo@izor.hr); Lucy Robertson (lucy.robertson@nmbu.no); Gereon Schares (gereon.schares@fli.de); Chiara Trevisian (CTREVISAN@ITG.BE).

Apologies: Mirek Rozycki (mkarozycki@gmail.com)

Minutes prepared by: Rachel Chalmers and Christian Klotz based on personal notes and assessment summary by RC

Aim for COST Action: to address parasite-specific issues in the development and validation of detection methods that are not addressed in currently published micro guidance

Output for COST Action: a commentary including/citing the relevant guidelines stating parasite-specific issues.

Programme outcomes

TOP 1: Welcome and introductions by Lucy Robertson and Christian Klotz. Housekeeping issues by Chiara Trevisian. Round robin of the attendees.

TOP 2: Synthesis of the information gathered on detection methods – (1 presentation per parasite group – 15 min allocated per parasite for presentation and questions)

For each parasite group one presenter covered the following topics. The outcomes are briefly summarized in the following table:

- Parasite and foods that have been reviewed
- Outline the general approach, search terms, and databases used for the literature review
- A brief, critical review of the detection methods and a summary of your findings (are they any good) and key issues identified
- Whether further method development or validation is needed for this parasite/food
- What are the deficiencies in current guidance for method development, if applied to this parasite?

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Parasite	Pres	Approach	Any good, key issues	Further method dev. needed?	Validation needed?	Current deficiencies
Echinococcus multilocularis Echinococcus granulosus	LR	Based on PubMed search	Limited number of studies (only 2) describing "crazy" methods	Yes	Yes	Issue of highly pathogenic organism really needs surrogate for validation and QC but getting appropriate surrogate is an issue that none of the papers address.
Toxoplasma gondii	GS	Used published reviews and PubMed for most recent papers	Highlighting many issues including: -Ethical issues (bioassays) -Facilities (cats) needed to produce oocysts -Bioassays for viability (PCR det or viability) -DNA conc. issues -Purification issues - In vitro culture may be hard for meat -Different life cycle stages – and need to select appropriate sample -Role of Serology / antibody assays -Is it a tissue test or a whole animal test?	Yes	LOD of molecular assays If bioassay is the gold standard, is there an alternative that the labs can use as benchmark	Variety of tests and conditions used In some animals serology good comp PCR (sheep) others not (cattle)
Trichinella spiralis and* other *Trichinella spp.	AMS	PRISMA systematic review	Digestion Mostly pork	YES PCR needs further work and is not in Standard Method	Some descriptions and there are EURL ring trials Digestion and aggultination Standard methods	EU leg methods only validated for pork, wild boar; validation for meat products lacking but come from countries outside EU

					There are guidelines for critical points - could look at the guidelines for a model? Proficiency testing data available but have not been sumarized	
Cryptosporidium spp.	SL	Systematic review		Yes N18s cross reacts with food matrices qPCR – all spp?	Was limited for ISO method –Did not cover the range of leaf types which may influence performance (as demonstrated during a STSM in this Action) Transfer of validation of 18744 to fruit juice? But performance would need validation.	ISO method for fruit and veg but no proficiency scheme
Anisakis	IM	Defined search terms	Raw fish – no non-destructive methods	New tools need to be integrated Larvae migration during test period/interval	Artificial digestion vs UV press in ring trial incl. single larvae spike Size of fish is an issue	EU reg method artificial digestion Fish get eviscerated, visual inspection won't detect; freezing kills larvae but doesn't prevent allergic reaction therefore need to DNA detection methods…or spectrometry to detect protein (harder than PCR)
Taenia saginata	CT	Scientific report to EFSA and	No ring trials, lack of sensitivity	Relies on visual inspection (motivation etc)	EFSA gave no clear answers; should our	PM meat inspection method sensitivity is low and specificity is questionable

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		Pubmed search	Serology (Ag Elisa) has been developed but not used – sensitivity lacking	Meat inspectors are trained but there are no proficiency schemes	group do ths or refer to Cystinet output? Is there one relevant?	
Giardia duodenalis	ML	Systematic principles	Storage temp is important (keep it cool)	qPCR DNA extraction – alkaline lysis?	Recovery data mostly from papers using ISO; other methods no data. Limited food stuffs and types (same as Crypto)	No proficiency scheme
Commentator	Suzanne Jordan	Provided interpretation of the presentations from an industry testing point of view (see below)				

TOP 3: Overview and discussions of where we are at by identifying gaps and establish need for FBP guidance (led by RC). Questions covered included: 1. which parasites/foods need better methods, 2. parasite-specific issues that are not addressed in the published guidance

- During the discussions it was agreed that the only available standard methods are : *Trichinella* (legal EU reg), C&G fruit and veg (ISO standard), *Anisakis* (legal EU reg) and *Taenia saginata* (legal EU reg)
- Which have proficiency schemes?: *Trichinella*, *Anisakis* - and *Toxoplasma* in animals, but not meat
- A general question that arose is: ”is legislation / testing effective?”

Themes/Topics from the presentations that were discussed or found to be important for further consideration for the development of the commentary (guidelines):

- Suzanne Jordan pointed out that whole ISO 17468 / 16140 process is lacking and need to be included in publications. This statement should also be included in the final commentary (guideline) document.
- Surrogates for spiking studies: Choosing relevant surrogate is important (e.g. proglottids may not be representative of taeniid eggs). Surrogates might be OK for microscopy but for PCR?
- Methods may provide an over estimation of human health risk: non-human pathogen detection; non-viable/infective

- What numbers should be used for spiking studies? (not really addressed in current validation guidance based on bacteria).
- Data about LOD, LOQ and Recovery is needed (but currently mainly lacking)
- PCR related issues were discussed: 1) pre- concentration issues, DNA purification issues are generic, selection of PCR primers and conditions. Would need to move to "black box" tests especially for field (boat, for Anisakis) work; 2) PCR specificity issues (especially if using mRNA assays – closely related parasites e.g. Toxoplasma and Hammondia; 3) qPCR won't pick up empty cells.
- The question arose whether focus on presence/absence might be a better start for risk assessment than quantification.
- Suzanne Jordan pointed out that from industry point of view – don't like tests that they don't understand the implications of, or know how to solve. Testing without a solution is scary. Context of the use of the method – e.g. knowing if HACCP controls are working or for product release?
- Methods suitable for faeces etc do not seem to translate well to foods (could draw on Kristoffer's STSM for example)
- Suzanne pointed out that for the food industry – bioassays are not acceptable. But for development level evaluation/validation toxo cat bioassay could be used, but not for ring trial or interlab. Would need to identify a suitable benchmark.
- Beta testing with industry partners - this would be a really good thing to include in our commentary (guidance)
- Cost of methods – don't dwell too much on this, not for us to decide, but for users
- Time to results may be more important
- Also Port Health Authorities are stakeholders too (importation)
- Definition: Are animals at slaughter meat? Yes, if dead. But herd testing may be wanted by meat companies (e.g. Toxoplasma)
- Trichinella gives a good example of what happens if bring legislation testing in – difficult to stop!
- Availability of certified reference material (ISO has a standard for this too); other ref material standards
- FDA guidelines provide useful suggestions
- Suzanne Jordan pointed out that "Representative sampling" might not always be what's needed – depends what the question is. Where would testing be best directed (from industry point of view also make sure controls are in place):

TOP 4: Discussion of how to present the work done so far – papers/publications (led by CK)

- It was agreed that a review paper will be produced based on the literature reviews done by the parasite groups. After this review has been finished a second document will be produced as a 'commentary' of the current guidelines and standards based on examples such as Toxoplasma and Trichinella
- Possible target journals were discussed (Food Control, Comprehensive Reviews in Food Science and Safety or Trends in Food Science and Technology). It was decided to start with the Trends journal as Lucy Robertson pointed out that WG3 has a paper under review and that the editorial support was good. CK will contact the journal.

- Trade journal – Suzanne Jordan will lead (E.g. "New Food"): after the first paper

- Further outcome of discussions and a draft structure of the proposed manuscript are provided in **Annex 1** (Synthesis)

TOP 5: Miscellanous

- Lucy Robertson requested some input/ideas for the final meeting. It was discussed that selected STSM projects could be presented. The parasite group leaders could provide their vision «What's next». Ideas for speakers to invite: H. Wesseling? (Toxoplasma in pigs), Armin Weiser (FoodChain-Lab, BFR), Santiago Pascual (Anisakis).