ApicoWplexa virtual Meeting, June 24, 2021:

Apicomplexan parasites and One Health
Abstracts

Keynote 1:

En route to PARADISE

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The PARADISE project primarily focuses on the zoonotic pathogens *Cryptosporidium parvum* and *Giardia duodenalis*, which can cause diarrhoeal disease in humans and animals, worldwide, and have been associated with food- and water-borne outbreaks in Europe and elsewhere. The main aims include the development of new tools for the genetic characterization of isolates and new strategies for enrichment of these pathogens from complex matrices.
The project is organized into three research-oriented work packages. WP2 activities are focusing on NGS-based genomics and metagenomics, WP3 focuses on development and validation of new molecular typing schemes, and WP4 explores the use of nanobodies, aptamers and hybridization probes for new enrichment strategies.
The generation of many new *Cryptosporidium parvum* and *Giardia duodenalis* whole genomes has allowed a rationale design of novel typing schemes with improved resolution. Detection of foodborne parasites in complex matrices is being explored using amplicon-based and shotgun metagenomics, as well as with protocols to capture parasite-specific DNA sequences. Nanobodies and aptamer technologies are being optimized to design novel enrichment protocols.
This project will place Europe at the forefront in the fields of comparative genomics and metagenomics and will have a large impact on the molecular epidemiology and the detection of

parasites/parasitic DNA in complex matrices in a one health setting (human, animal, environment, food).
The PARADISE project is supported by funding from the European Union's Horizon 2020 Research and Innovation Programme, under grant agreement No 773830: One Health European Joint Programme.

Keynote 2:

Characterization of *Sarcocystis* spp.

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Apicomplexan protozoan parasites from the genus *Sarcocystis* have heteroxenous life cycles, generally using herbivores as intermediate hosts and carnivores/omnivores as definitive hosts. Most *Sarcocystis* spp. are related to a predator-prey relationship. Sarcocystosis in intermediate hosts (IH) is generally asymptomatic and chronic, producing muscle cysts or sarcocysts containing banana-shaped bradyzoites. The definitive hosts (DH) become infected by ingestion of sarcocysts and usually show mild or no clinical signs. The gametogony and sporogony stages take place in small intestinal cells and the thin sporulated oocyst wall breakes, releasing the sporocysts, each containing 4 sporozoites and a residual body. More than 200 *Sarcocystis* spp. have been named and described, partially based on cyst morphology and life cycles. Humans are DH for *S. hominis* and *S. suihominis* with bovines and swine as IH, respectively. Humans can also act as IH, as muscular sarcocystosis has been reported in people traveling in Malaysia, presumably caused by *S. nesbitti* (snakes as DH). In addition, a digestive symptomatology has been reported as "food poisoning" by ingestion of horse and South American camelids meat containing *Sarcocystis* spp. cysts. During the last years, morphological characterization established on ultrastructure of cyst walls and molecular identification of several new *Sarcocystis* species were performed, allowing improved descriptions and re-descriptions. The use of molecular tools, specially PCR and sequencing, is being increasingly used for *Sarcocystis* spp. diagnosis, which has attenuated the need for bioassays. The principal DNA targets are the rRNA genes (*18S*, *28S*, *5.8S* and ITS), mitochondrial gene *Cox1*, and some genes coding for surface antigens (SAGs), however, several species have no sequences deposited in public databases. This presentation is intended to summarize the last advances on *Sarcocystis* spp. characterization and diagnosis. Emphasis is

given on zoonotic *Sarcocystis* spp. and those using cattle, swine and cervids as IH, and opossums, canids and snakes as DH.

Keynote 3:

Do we really appreciate the *Toxoplasma gondii* sources for human infection?

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Toxoplasma gondii is the most successful zoonotic parasite in the world and it is estimated that about 30% of the human population is infected with the parasite. Food-borne transmission, through the ingestion of tissue cysts in undercooked or raw meat, has been suggested as the main route of transmission for humans. However, *Toxoplasma* infections can also occur following the ingestion of oocysts, shed in the faeces of infected felids, contaminating either water or fruit/vegetables/leafy-greens. *Toxoplasma* may also be transmitted transplacentally from mother to her unborn baby, with potentially fatal consequences. This keynote presentation will focus on some of the food-related transmission risks that have been highlighted in recent years, which may have been underappreciated in the past. This presentation will also raise the question "do we really comprehend the true extent of the genetic diversity of *Toxoplasma* within Europe/North America?" Previously, pork and lamb/mutton were considered to be potentially high-risk sources of *Toxoplasma* infection and beef was regarded as low-risk due to the low detection rates of the parasite within beef samples. However, to fully assess the relative risk posed by different kinds of meat products, eating habits have to be taken into account. For example raw meat is consumed in different countries: "filet americain" in the Netherlands, Mettwurst/Zwiebelwurst in Germany and steak tartare in France. Consumption of these meat products leads to a much higher risk of infection, even if the parasite burden is low, because these meat products are not cooked and therefore the parasite is not killed. In recent years the focus of potential *Toxoplasma* infection sources has been widened and studies have investigated the presence of *Toxoplasma* in wildlife species (boar, deer, kangaroo, game birds, fish and oysters) and results show that meat from wildlife can be sources of *Toxoplasma* infection for humans. In addition, hunting some wildlife species has been associated with *Toxoplasma* infections, which may reflect poor hygiene or may be a consequence of eating undercooked meat from wildlife species. This presentation will address the role of "wild" meat in foodborne toxoplasmosis and whether wildlife may harbor different parasite genotypes, which could lead to atypical infections in humans.

Keynote 4:

TOXOSOURCES: What are the relative contributions of the different sources of *Toxoplasma gondii* infection?

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Toxoplasma gondii infection can be acquired by ingesting oocysts (environmental pathway) or tissue cysts (meatborne pathway). TOXOSOURCES is a Joint Research Project of the One Health European Joint Programme that investigates the relative contributions of the transmission routes and sources of *T. gondii* using multidisciplinary approaches. TOXOSOURCES consortium comprises more than 20 partner institutes, and the project runs 2020-2022 with a budget of 3 million EUR.
The consortium has collected data for a multicenter quantitative microbiological risk assessment for *T. gondii*. A literature review supported the selection of a method to detect *T. gondii* oocysts in fresh produce for a multicentre study. The project also explores serology for detecting *T. gondii* infections caused by oocysts, and an unprecedented effort of Whole Genome Sequencing of *T. gondii* isolates was used to identify polymorphic marker regions for the establishment of a new typing method to detect within-genotype variation.
The outcomes of TOXOSOURCES will include quantitative estimates of the contribution of the main sources and transmission routes to *T. gondii* infections. Moreover, the consortium itself is a major outcome - cross-sectoral, international One Health collaboration is needed to address this zoonotic parasite.
TOXOSOURCES has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 773830.

Short presentations:

Short presentation 1:

The IMPACT project: standardising molecular detection methods to improve risk assessment capacity for foodborne protozoan parasites, using *Cryptosporidium* in ready-to-eat salad as a model

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Protozoan parasites are important causes of human and animal disease, some of which can also manifest as outbreaks. The range of human disease includes, for example, diarrhoea (*Cryptosporidium spp.*, *Giardia duodenalis*), and congenital or acquired infection that can be fatal (*Toxoplasma gondii*). Between them, these three parasites were estimated in 2010 to cause about 47 million cases of illness (incidence 185 cases per 100,000 population) in Europe. Outbreaks are increasingly recognised and attributed to consumption of food; fresh produce and leafy greens in particular seem especially vulnerable to contamination by cysts or oocysts, including from animal sources. This can happen through direct faecal contamination or via contaminated water used in growing and processing. There is a lack of readily-applicable standardised methods for testing food and feedstuffs, which could provide additional evidence in risk assessments and validating control points and interventions.
The aim of the IMPACT project is to increase the European-level capacity for risk assessment of foodborne protozoa using *Cryptosporidium parvum* and ready-to-eat salad leaves as a model example, delivered by strengthening laboratory networks, and enabling knowledge exchange and transfer. A review of procedures for the detection of *Cryptosporidium* in fresh produce has led to the production of guidance for undertaking artificial contamination studies to verify and validate detection methods as well as to the selection of a real-time PCR to help improve detection and workflow. These were achieved through literature review, a market survey of oocyst producers and suppliers, questionnaires, e-meetings and a workshop of experts. An SOP for molecular detection of *Cryptosporidium* oocysts in leafy greens by real-time PCR was developed and evaluated in two laboratories and rolled out to four other recipient laboratories for optimisation. Video tutorials were compiled to facilitate SOP implementation. The next step is validation through a ring trial involving five partners from the consortium and five further laboratories is planned for September this year. In addition to statistical analysis, the attributes of the SOP will be assessed through a questionnaire administered to the laboratories focusing on technical application and practicability of the method (including equipment used, any variation to the SOP,

hands-on and turnaround times, problems encountered, etc.). To facilitate a harmonised approach for data collection in future risk assessments, the findings of the project and final SOP will be disseminated primarily using the network of the National Reference Laboratories in Europe and the EFSA focal points, an extended network previously built during the COST Action Euro-FBP, as well as the OHEJP project network.
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Short presentation 2:

Environmental contamination with *Toxoplasma gondii* oocysts: a systematic review

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Toxoplasma gondii is a major foodborne pathogen capable of infecting all warm-blooded animals, including humans. Therefore, it represents a paradigm of the One Health initiative. Although t oocyst-associated toxoplasmosis outbreaks have been documented in the past few years, the relevance of the environmental transmission route remains poorly investigated. The objective of this study was to provide a comprehensive review of the existing literature on contamination of environmental matrices with *T. gondii* oocysts.
A systematic literature review on the occurrence of *T. gondii* oocysts in soil, water, fresh produce (vegetables and fruits) and bivalve mollusks was carried out following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines. Worldwide studies, published until December 2020, were searched in public databases (PubMed, Web of Science and Scopus). The bibliographies of the selected articles were also screened to identify additional studies.
A total of 3,200 articles were obtained during the search process and 102 articles were selected which met the eligibility criteria. Among them, 13 articles focused on the analysis of two or more matrices, and specifically, 34 articles reported on soil, 40 on water, 22 on vegetables and 22 on

bivalves. *Toxoplasma gondii* oocysts were detected in all types of matrices worldwide. In fresh produce, different rates of *T. gondii* detection and oocyst loads could be attributed to distinct variables such as geographical location, or sampling and detection methods. Lack of standardisation in the procedures (including oocyst recovery) applied was evident, and risk factors for the contamination have not been well defined in the context of the whole food chain including agricultural production and processing. Therefore, sampling guidelines and standardisation of procedures could help to obtain more accurate and comparable results in further studies.

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Short presentation 3:

rTgOWP1-f, a specific biomarker for *Toxoplasma gondii* oocysts

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Detection of *Toxoplasma* oocysts in water is complex, and no standardized methods are available. *Toxoplasma gondii* oocyst wall protein 1 (TgOWP1) integrates a family of seven proteins, consensually assumed as specific antigens of *Toxoplasma gondii* oocyst stage, located in the outer layer of the oocyst wall. Based on that, TgOWP1 was indicated as a possible marker for environmental oocysts. A recombinant antigen, rTgOWP1-f was expressed derived from a fragment selected on basis of its structural homology with *Plasmodium* MSP1-19. This peptide is involved in the interaction of *Plasmodium* merozoites with red cells membranes, and it is highly immunogenic in malarial infections. Rabbit polyclonal antibodies anti-rTgOWP1-f evidence ability for specific identification of environmental *T. gondii* oocysts. We assume, rTgOWP1-f, as a possible biomarker of oocysts. In addition, we present findings supporting this vision, including the development of an immunodetection methods for *T. gondii* oocysts identification.