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Research brief

Why we need a European focus on foodborne parasites

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ABSTRACT

Foodborne parasites (FBP) are recognized as being a neglected pathogen group, often associated with marginalized or disadvantaged populations, especially those living in regions where water supply or sanitation are inadequate. Nevertheless, we are also increasingly recognising that FBP are not just restricted to such places, and even those that do have a circumscribed endemic area may also travel further in our globalised world; FBP are relevant everywhere, including Europe.

Against this background, COST Action Euro-FBP (FA1408) was established and ran for a period of 4 years, addressing a number of different questions related to FBP, particularly in the European setting. In this special issue (SI), some of the issues and outputs associated with Euro-FBP are considered in greater depth, as an output also of the final Euro-FBP meeting. As well as more general issues regarding, for example, globalization and climate change, use of economic models, and the value of risk-based surveillance that puts the topic in perspective, individual articles are included that address specific parasites. These include protozoan parasites, such as *Cryptosporidium, Giardia*, and *Toxoplasma*, as contaminants of water, shellfish, and fresh produce, fishborne parasites such as Anisakid nematodes, and meatborne parasites, such as *Trichinella*. Some of the works provide specific data on occurrence or outbreaks, whilst others are concerned with techniques. In addition, implementation of some of the educational and collaborative tools that are unique to COST Actions are described. COST Actions are not generally intended to deliver a scientific endpoint, and Euro-FBP does not do so. However, the articles in this SI, along with other articles published elsewhere during and subsequent to the course of the Action, as direct outputs of the Euro-FBP activities, indicate that FBP are indeed a relevant topic for European scientists.

1. Introduction

Foodborne parasites (FBP) are recognized as a neglected pathogen group, due to a variety of reasons (Robertson, 2018). These include that many, but not all, infections with FBP do not manifest as acute diseases, but rather have a chronic, more insidious, impact on their hosts. Another important reason that FBP are often neglected is that there is a perception that they are mostly associated with poverty. However, although populations living in areas where basic infrastructure elements, such as water supply, sanitation, housing, and transport, may be lacking are often more exposed to some FBP, and are thus at greater risk of infection, this not always the case. Furthermore, in an increasingly globalised world, with considerable movement of people and animals both between and within countries, as well as an internationalisation of commerce and globalised food supply (Robertson et al., 2014), it is clear that foodborne pathogens are relevant to Europeans too.

On the basis of this, as well as variations within Europe regarding the prevalences and relevance of different FBP, along with a wide range of skillsets available in different European countries, COST Action “A European Network for Foodborne Parasites” (Euro-FBP; FA1408; see: https://www.euro-fbp.org/ and https://www.cost.eu/actions/FA1408) was initiated and ran for a period of 4 years (2014–2018). This Action, which included over 100 institutions from 38 countries, had specific aims and related activities, although its long-term and overriding objective was to “decrease the impact on human health from FBP …” and to “use an interdisciplinary, One Health perspective to assimilate information, coordinate research and harmonize diagnostics, surveillance, analytical methods, potential interventions and mapping of global trends regarding FBP.” One outcome of the final meeting of Euro-FBP (held in Oeiras, Portugal in 2018) was to harness the Europe-wide

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expertise on FBPs that was assembled there to gather opinions on the main drivers for FBP in Europe and to identify research priorities for FBPs in Europe. The results of this expert knowledge elicitation indicated that eating habits, lack of control in the food chain, a paucity of awareness of the problem among relevant agencies, continued globalization, and deteriorating water quality were major drivers for FBP in Europe, while research should focus on methodological gaps, as well as surveillance concerns, impact-assessment issues, and the role of microbiota (Trevisan et al., 2019). Interestingly, some of the issues listed had actually been included in the activities of Euro-FBP, and, furthermore, some activities had already resulted concrete outcomes (such as scientific or popular articles) that had been published during the course of Euro-FBP. Nevertheless, an additional outcome of the final meeting of the Action was a decision to encourage submission of relevant articles to this Special Issue (SI) that could be considered as direct outcomes, or directly related to the outcomes, of the Action. These articles are combined in this SI and include general papers of relevance to the topic of Euro-FBP, individual articles on specific parasites or techniques, along with descriptions of how some of the COST Action tools, such as Short-Term Scientific Missions (STSM) and Training Schools (TS), within Euro-FBP have improved not only our knowledge, but also our skillset, within Europe. These activities should assist us in being better prepared to address the challenges posed by FBP in the future.

In the following sections, we provide an insight into the background of some of the articles that form the basis of this SI.

2. General issues of concern regarding FBP

Setting FBP in the context of our present situation and our changing world is important for understanding their implications and impacts. This SI starts by taking two different approaches on this issue, in both of which the authors emphasise the importance of interdisciplinary collaboration and a One Health perspective; it is no coincidence that one of the articles is co-authored by the vice Chair of a COST Action with focus on One Health; NEOH – Network for Evaluation of One Health. The first article sets the context by considering how we can use "systems thinking" to understand the costs in a broader socio-economic perspective (Aragrande and Canali, 2020). In this article, the authors demonstrate how integration of epidemiological and economic models can be used to identify costs associated with foodborne disease, and they use *Echinococcus granulosus*, an important zoonotic FBP ranked as being of 4th highest importance in Europe in terms of prioritization, and highest priority in southwestern and southeastern Europe (Bouwknegt et al., 2018), as a model to illustrate this approach. Despite FBP being recognized as emerging pathogens, in general FBP continue to be relatively neglected (Robertson, 2018). Thus, this approach demonstrates that even when symptoms are not acute, the associated costs may be extensive, and recognising these costs involves communication between disciplinary silos. Such collaboration is a keystone of COST Actions. In addition, the authors demonstrate that by using such approaches, those areas where data are lacking are highlighted, and thus informs policy makers and researchers alike where focus should be placed to fill these data gaps (Aragrande and Canali, 2020). The second approach for considering the implications and impacts of FBP, takes a more broad-based approach and considers how two major issues of our time, globalization and climate change, may affect the epidemiology and impact of FBP (Pozio, 2020). Although, as the author is careful to point out, the complexity of interactions means that it is impossible for us to predict the consequences of global trends, use of a One Health approach should provide a basis by which the problem should be approached. With a firm grounding in of the author's long experience (over 40 years) in the parasitology field, this article uses examples from across the whole spectrum of FBP, to give an evidence-based insight into where we are now, and which trends and factors may affect our future trajectory (Pozio, 2020).

3. FBP transmitted by meat and fish

Foods of animal origin are consumed daily by most Europeans. The safety of these foods may be compromised by agents of physical, chemical, and biological nature; indeed, foods of animal origin have been identified as the major vehicle for foodborne disease in the EU, both in terms of illness and fatalities (Da Silva Felicio et al., 2015). Amongst the biological agents, parasites constitute an often-underestimated group of pathogens. However, a global risk-ranking conducted by FAO/WHO in 2012 (FAO/WHO, 2014) ranked *Trichinella*, *Toxoplasma,* and *Taenia solium* as being of greatest importance in meat, and nematodes (in particular *Anisakis*) and trematodes as being of greatest importance in marine and freshwater fish. A similar risk-ranking, but restricted only to Europe (Bouwknegt et al., 2018), ranked *Toxoplasma* and *Trichinella spiralis* in 2nd and 3rd positions, respectively (among all parasites), with *Taenia solium* (position 10) as being of lesser importance compared with the FAO/WHO global ranking. In fish, *Anisakis* was ranked as being of highest importance, and, being prioritised in position 8, was considered as a higher prioirty than *T. solium*, with the Opisthorchiidae in position 12. Of particular interest regarding these meat- and fish-borne parasites, is that in western Europe, *Toxoplasma* ranked as of greatest importance among all FBP, and that in all 5 European regions either *T. spiralis* or other species of *Trichinella* were ranked in position 3. Prioritization ranking of the Anisakidae varied between regions, ranging from position 4 in southwestern Europe to position 10 in western Europe. The Opisthorchiidae, in contrast, were below position 10 in all regions of Europe, apart from southwestern Europe and northern Europe (both position 10).

The impacts of these hazards on public health depend on various factors, with the core parameter being the introduction of the specific parasite into primary production. In some settings, this can be effectively controlled, e.g., in the case of *Trichinella* and pig production, where the required biosecurity prerequisites have been laid down in legislation (European Commission, 2015). In contrast, low biosecurity (e.g., free-ranging pigs), presents a higher risk of exposure to the pathogen (Pozio, 2014). Given the virtual impossibility of establishing high standards of biosecurity in some areas of primary production (e.g., wild-caught fish or hunting wild boar), additional lines of defence must be implemented; basically, these are inspection and processing. With respect to inspection, testing of pig carcasses for *Trichinella* is a typical example. Similarly, traditional meat inspection examines carcasses for tapeworm cysts (European Commission, 2019), but with moderate sensitivity, and there is no legal provision for testing of meat for *Toxoplasma*. Likewise, some requirements are in place for checking fish for nematode infections.

Biosecurity aims at prevention of parasites entering the food chain, and inspection aims at removal of parasites or infested tissues from the food chain. In addition, processing and preparation of food have the potential to kill or remove the parasites. The increasing popularity of consuming raw fish and raw or rare meat impairs the latter set of measures. Knowledge of the survival of parasites along the food chain and effective inactivation methodologies are cornerstones for implementing food-safety concepts, such as HACCP. However, an extensive literature survey (Franssen et al., 2019a) generated within the framework of Euro-FBP revealed that the quantity, and sometimes quality, of such data is limited. These knowledge gaps relate particularly to quantification of inactivation and to the possible impact of species differences as well. In line with the aims of Euro-FBP, the implications of these shortcomings have been communicated to relevant industries (Franssen et al., 2019b; Paulsen et al., 2019).

In essence, the control of meat- and fishborne parasites is a shared food-safety responsibility of producers, processors, authorities, and, finally, requires educated consumers. This is augmented by the public health requirements for adequate diagnosis, tracing, and treatment of foodborne disease. All measures in this multi-stage approach have to be effective and proportionate. The contributions in the area of meat- and fishborne parasites follow this rationale. In this Euro-FBP SI, Alban et al. (2020) introduce the concepts of risk analysis and risk-based surveillance, and present a surveillance tool (SURVTOOLS: https://survtools.org/). Particular emphasis is put on how hazard prioritization can be achieved and on effective sampling strategies. The authors discuss how risk-based surveillance is implemented for three meatborne parasites with different coverage in meat inspection legislation, i.e., *Trichinella*, *Taenia saginata,* and *Toxoplasma*.

Although the lifecycle of *Trichinella* was elucidated around 150 years ago, and control measures have been implemented since that time, this parasite continues to be an issue in the meat chain. *Trichinella* species other than *T. spiralis* have been reported from wildlife in virtually all European countries; this, along with biosecurity issues (backyard farming, hunting of wild boar), lack of testing of meat for *Trichinella* in some situations, and meat preparation habits that do not inactivate the parasite, is why human cases or outbreaks still occur in Europe. Some countries are more associated with cases than others, and underlying socio-economic and traditional reasons contribute to the regional persistence of this biological agent in the food chain. In this Euro-FBP SI, the *Trichinella* issue has been considered from two perspectives.

Firstly, precise characterization of parasites is required in order to conduct meaningful tracking and tracing of outbreaks, and to study any regional differences. Karadjian et al. (2020) applied matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) for identification of *Trichinella* and provided a new Main Spectra (MSP) library from samples of *Trichinella* spp. from 3 different countries (France, Germany, and Poland). The authors describe the suitability of the MADI-TOF-based protocol as an alternative to more commonly used PCR-based methods.

Secondly, the realization that human trichinellosis still occurs in Europe in certain settings, and that this is likely to continue, indicates the need for preparation for diagnosis and treatment. The work of Vutova et al. (2020) reports on clinical and epidemiological features of 72 cases of trichinellosis associated with five outbreaks in Bulgaria between 2009 and 2011. Initial treatment was often not effective due to diagnostic issues, with various generalized symptoms being exhibited by patients at hospital admission. However, elevated serum creatine phosphokinase levels and marked eosinophilia in over 80% of cases indicated parasitic infestation, and this was confirmed anti-*Trichinella* antibody-positive results by ELISA and indirect haemagglutination; albendazole treatment proved successful. In some of the outbreaks, the *Trichinella* species could be determined (two *T. spiralis* and one *T. britovi*); associations with backyard farming and circulating *Trichinella* species in wildlife were also noted.

Parasites in fish, their food safety implications and control measures are addressed in this SI in an article from Portugal (Ramos, 2020), with a summary of 10 years of laboratory testing of fish and fish products. The article not only describes implementation of workshops on parasites in fishery products for professionals within the fish supply chain, which improved the diagnostics capabilities of the attendees and enabled implementation of certain biosecurity measures in fish farms, but also training activities for consumers, including children. The most frequently recovered parasites were *Anisakis* larvae, but viable larvae were recovered only from fresh fish and no viable larvae were recovered from farmed fish and Japanese-style raw fish foods. Plerocercoids of *Gymnorhynchus gigas*, *Hepatoxylon trichiuri* and the myxosporean *Kudoa* spp. were detected less frequently.

All four of these contributions that focus on meat- or fishborne parasites address the major goals of the Euro-FBP COST Action, which, in essence, are based on the Risk Analysis concept. They provide information on identification of hazards, assess the impact of the hazards on food safety and public health, and suggest approaches on how such hazards can be managed such that they do not pose an unacceptable risk for consumers. The authors demonstrate that ensuring food safety is a multi-disciplinary approach and a collaborative effort.

4. FBP transmitted as contaminants of water, shellfish or fresh produce

A main vehicle for transmission of different protozoan FBP, including *Cryptosporidium* spp., *Giardia duodenalis,* and *Toxoplasma gondii,* is represented by water contamination with parasites' resistant stages (i.e., (oo)cysts) released in the faeces of the definitive hosts. *Cryptosporidium* spp. and *G. duodenalis* are the most commonly reported aetiological agents in waterborne outbreaks (Efstratiou et al., 2017), whereas waterborne outbreaks associated with *T. gondii* oocysts have been reported less frequently, probably because the often non-acute nature of infection with *Toxoplasma* means that it is seldom identified soon after transmission (Jones and Dubey, 2010). Humans and animal hosts can be infected not only by direct ingestion of (oo)cysts in contaminated water, but also through the consumption of raw fresh produce (fruits and vegetables) that has been irrigated or washed with contaminated water (as well as directly contaminated by faeces during growth, processing, or preparations, via animals, food handlers, surfaces, or equipment) (Shapiro et al., 2019; Ryan et al., 2018, 2019). Molluscan bivalves, lightly cooked or consumed raw, can also be a transmission vehicle as, by filter feeding, they can concentrate contaminants in their organs, including protozoan (oo)cysts (Robertson, 2007). However, identification of FBP in these matrices is particularly challenging due to the generally low level of contamination, and thus requires efficient concentration strategies combined with highly sensitive and specific detection methods (such as molecular assays) (Chalmers et al., 2020). Two papers in this SI address detection of FBP in mussels; one study (Durand et al., 2020), reports the preliminary results of a Euro-FBP Short Term Scientific Mission (STSM) in which spiking experiments were used to evaluate the performance of a molecular assay relying on loop-mediated isothermal amplification (LAMP) for assessing contamination of blue mussels with *T. gondii* oocysts. The authors' main findings suggest that LAMP could provide a promising alternative to qPCR, being able to detect down to five *Toxoplasma* oocysts in 1 g of mussel tissue or 1 ml of haemolymph. The other study on FBP in mussels (Ligda et al., 2020), which arose from collaboration between Euro-FBP participants, aimed at correlating the presence of *Cryptosporidium* and *Giardia* (oo)cysts in wastewaters from sewage treatment plants in the north of Greece and the risk of potential accumulation of (oo)cysts in Mediterranean mussels cultivated in the proximity of the treatment plants. With only low amounts of *Giardia* cysts and *Cryptosporidium* oocysts detected in wastewater, mainly by immunofluorescence microscopy, no significant contamination of bivalves was observed. These data suggest that, in this specific situation, the risk associated with consumption of raw mussels for humans is negligible.

Another paper arising from a COST Action-promoted collaboration was a cross-sectional study on the presence of intestinal human parasitic infections (including *Cryptosporidium* and *Giardia*) in stools of healthy and diarrhoeic individuals from different areas of Greece, to provide a current status overview for surveillance and control activities at the national level (Kostopoulou et al., 2020). The low prevalence of *G. duodenalis* (1.3%) and *Cryptosporidium* spp. (0.6%) and, generally, of intestinal parasites (4%), is similar to that in many other European countries. Molecular detection was achievable for a few samples of *G. duodenalis* and only assemblage AII was found. Thus, the authors proposed that anthroponotic transmission was probably the route of *Giardia* infection in their study, although whether direct (human to human) or indirect (foodborne or waterborne) transmission predominated could not be determined.

Implementation of water testing for detection of *Giardia* and *Cryptosporidium* (oo)cysts in Serbia was the subject of a Euro-FBP STSM described by Ćirković et al. (2020). This resulted in the first report on the occurrence of *G. duodenalis* and *Cryptosporidium* spp. in surface waters in Serbia, and showed that > 50% of river samples are contaminated (0.2–3.3 cysts/L for *Giardia* and 0.2–1.2 oocysts/L for

Cryptosporidium). Possible explanations for differences in contamination levels of different rivers were discussed, along with the possibility of point or diffuse contamination sources. Molecular analyses of positive samples were relatively limited, but indicated the presence of both *Giardia* Assemblages A and B, indicating a potential concern for public health.

These four contributions in the SI clearly mirror the main objective of the Euro-FBP COST Action: dissemination and sharing of expertise and promotion of collaborative activities among the participants to improve parasite detection skills in food and water, and to provide new information on the risks associated with parasite contamination of food and water for human infection in European countries.

5. Tools used in the Euro-FBP COST Action

COST Actions are designed for creating research networks, not for funding research *per se*, and therefore many researchers, especially those whose employment depends on obtaining funding via grant proposals, approach them with some degree of distrust. However, specific tools that are common to all COST Actions do promote research, and, in this SI, the use of two of these tools are described in the context of fostering and supporting research on FBP across Europe. One article is grounded in the Training School (TS) and addresses the question of “Why do we need training” (Deksne et al., 2020). Here, the authors discuss how the advent of molecular techniques within the last two or three decades has resulted in the proliferation of results in the field of FBP that are based on poorly evaluated molecular techniques (in terms of sensitivity and specificity), and that these endanger the position of FBP on the public health agenda (due to erroneous reports) and may result in inappropriate targeting of research funds. The popularity of the TS run during Euro-FBP is demonstrated by the fact that, due to over-subscription, only just over 50% of applicants were able to attend, coming from 23 countries in total. The TS involved both theoretical and practical (hands-on) sessions, and, although the long-term outcomes are impossible to evaluate at this time, the short-term results, demonstrating which techniques covered in the training were implemented in home laboratories, indicate successful knowledge transfer (Deksne et al., 2020).

Another important tool of COST Actions is the STSM, where researchers have a short-term visit to a laboratory in another COST country to learn a particular technique or collaborate in a particular project. Some of the articles in this SI are direct outputs from STSMs (see above). An overview article of the STSMs completed in Euro-FBP (Sotiraki et al., 2020) indicates that a range of different FBP and techniques were the subject of different STSMs; the authors suggest that the focus on specific parasites may reflect the priority ranking exercise for FBP in Europe conducted as part of the Euro-FBP COST Action (Bouwknegt et al., 2018). The emphasis on obtaining knowledge on different molecular detection techniques during STSMs also reflects the prioritised research agenda that was developed as part of Euro-FBP (Trevisan et al., 2019). Some subjects on this research agenda – such as parasite inactivation, estimating health and economic burdens, and investigating aspects of prevention of transmission from the host perspective – were not, however, STSM topics, perhaps indicating a lack of expertise in Europe (Sotiraki et al., 2020). The use of Euro-FBP budget on STSMs (over 30 were funded) indicates their importance as a tool in COST Actions, and their usefulness to researchers, particularly those who may not have access to particular knowledge or experience in their own laboratories. That some of the outputs from the STSMs have already resulted in research publications and research collaborations is also encouraging.

6. Conclusion

FBP are still neglected, and the Eurocentric perspective often considers that they are an issue of other countries and cultures. Various outputs from the COST Action, Euro-FBP, along with the articles combined in this SI, clearly demonstrate that FBP are also relevant for Europe. The Euro-FBP COST Action was completed in 2019, but outputs continue to be generated and collaborative research projects, that are at least partly associated with the Euro-FBP network, are now in progress. An opinion article from the European Food Safety Agency, published in 2018 (EFSA, 2018), was at least partially grounded in the Euro-FBP network and also demonstrates that FBP remain an issue of importance for policy makers in Europe. The collection of articles in this SI provides further support to the opinion that there is no doubt that FBP should remain in focus in Europe, both now and for the foreseeable future.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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