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Trends in Parasitology

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Opinion

Foodborne Parasites in Europe: Present Status and Future Trends

Chiara Trevisan,[1] Paul R. Torgerson,[2] and Lucy J. Robertson[3,*]

Although foodborne parasites (FBPs) are becoming recognized as important foodborne pathogens, they remain neglected compared with bacterial and viral foodborne pathogens. As drivers for infection with FBPs are variable, it is often unclear for funding bodies where research should be prioritized. Through a COST Action (Euro-FBP; FA1408), we harnessed Europe-wide expertise to address these questions, using an Expert Knowledge Elicitation approach. Eating habits, lack of food-chain control, lack of awareness from relevant agencies, globalization, and water quality were identified as major drivers for FBP infection. Prioritized research needs to be largely focused on methodological gaps, but also on surveillance concerns, impact-assessment issues, and the role of microbiota. Despite the European focus, these responses should be relevant to those concerned with FBPs globally.

Highlights

FBPs remain neglected despite increasing recognition of their importance as foodborne pathogens.

For funding bodies, it remains unclear where research on FBPs should be prioritized.

An expert knowledge elicitation exercise identified globalization, water quality, changing culinary habits, gaps in surveillance and control, and lack of awareness from agencies as being among the drivers for infections with FBPs.

Among the research needs, filling methodological gaps and optimizing viability-assessment technologies were identified as priorities, together with improving surveillance, evidence-based impact assessment, and exploring interactions with microbiota.

Foodborne Parasites in Europe: What Are the Issues?

In 2014, the Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) global risk ranking of foodborne parasites (FBPs) was published [1], followed in 2015 by the global burden of foodborne parasitic diseases [2]. These reports indicated the increasing global recognition of the importance of FBPs [3]. Although much of the burden from FBPs lies in low-income and middle-income countries, the large disease burden of toxoplasmosis in The Netherlands has long been recognized [4], and has been increasingly recognized from other countries, both within and outside Europe [5]. Furthermore, a European risk ranking of FBPs [6] provided the impetus for the **European Food Safety Authority** (**EFSA**) (see Glossary) to develop a document describing public health risks associated with some of the highly ranked FBPs [7].

Despite this growing interest, the wide diversity of FBPs and varying regional importance mean that some fundamental issues remain unresolved. For example, it is difficult to decide on research priorities for FBPs in Europe, as the main drivers for infection with FBPs in Europe are unclear. In order to control FBPs, these ambiguities require evidence-based clarification. Answers from Europe could provide a basis for addressing similar questions elsewhere, as many issues presented are also of concern for other global regions, both developed and developing.

As part of the **COST Action** 'A European network for foodborne parasites' (Euro-FBP; FA1408), we harnessed Europe-wide expertise on FBPs to address these questions, initially using an **expert knowledge elicitation (EKE)** approach to obtain information. An online free-text questionnaire asking participants to identify main drivers for FBP infection in Europe and current and future research priorities was distributed to 224 Action participants (covering 30 European countries) in early 2019. In total, 77 responses were obtained (34% response rate). Summarized responses were then distributed to Action participants, and they were asked to choose from these the top five drivers of FBP infections in Europe, and the top ten and

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15 research priorities, both now and in 10 years' time. Responses were received from 59 participants. Here we present the main results, using published literature as a basis to discuss the responses in depth.

Emerging Drivers of FBPs in Europe: What Can We Expect for the Future?

Despite advances in research on FBPs, they remain an important concern for public health [8], and continue to contribute to substantial socioeconomic impacts [9]. The Euro-FBP COST Action network identified 19 drivers (see full list in Box S1 in the supplemental information online) of foodborne parasitic diseases. Below, we list and discuss the five selected as being of greatest importance (Figure 1).

Globalization of Food Supply

Globalization of the food supply means that foods that were once 'exotic' are now commonplace, and food product ingredients might originate from almost anywhere in the world. This broad distribution of foods has largely been enabled by changes in the food industry. Improved transportation, particularly the cold-chain, is an important reason for globalization of the food supply [10]. Consequently, foodborne disease outbreaks today are less likely to be limited geographically, and international foodborne disease outbreaks have become more common [11,12]. One example is recurrent outbreaks of cyclosporosis in the USA associated with imported fresh produce [13]. Traditional preservation methods (e.g., smoking, fermenting, drying, freezing) may have different effects on survival of parasite infectious stages in meat and fish, whereas other more novel methods (such as high-pressure processing or irradiation processes) have variable efficiencies at inactivating parasite transmission stages, while preserving the food for

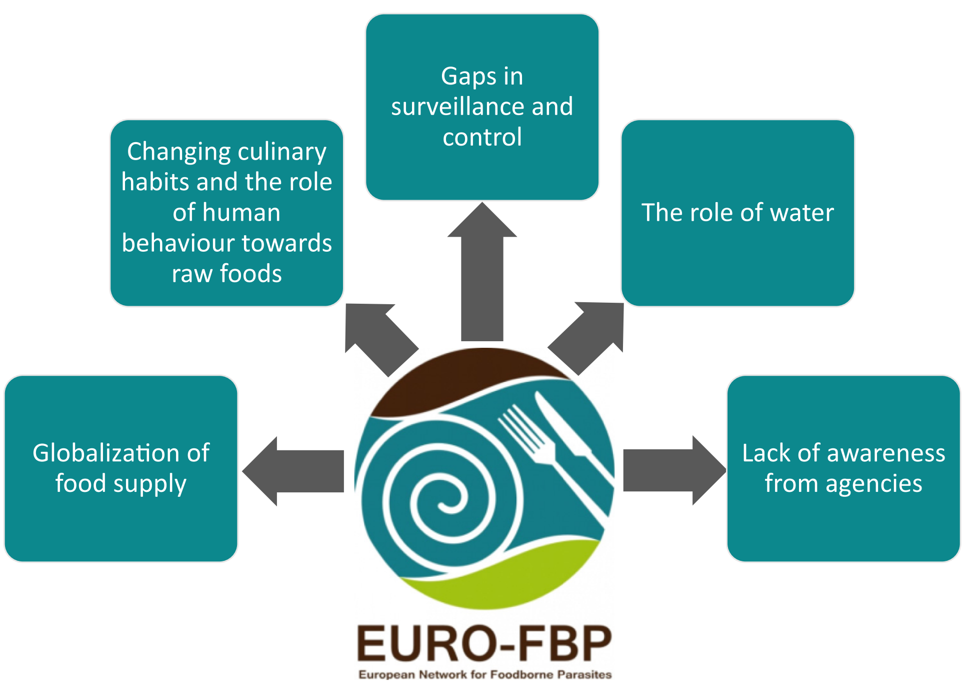

Figure 1. Top Five Drivers of Foodborne Parasitic Diseases in Europe. The COST Action 'A European Network for Foodborne Parasites (Euro-FBP)' identified 19 drivers of FBPs in Europe (listed in Box S1 in the supplemental information online), among which five are selected as being of greatest importance and discussed here.

Glossary

COST Action: a COST Action is a network dedicated mostly to scientific collaboration. It is based on a bottom-up approach, and utilizes a range of networking activities, such as workshops, short-term scientific missions, training schools, meetings, conferences, publications, and other dissemination activities, to advance science, stimulate knowledge sharing, and pool resources. COST Actions are EU-funded, but participation is not restricted to EU countries. See https://www.cost.eu/ for further details.

European Food Safety Authority (EFSA): an EU agency with the remit to provide independent scientific advice on risks associated with the food chain. Most of the work is in direct response to requests for scientific advice on specific issues from the European Commission (EC), the European Parliament, and EU member states. As EFSA's work program is organized according to priorities agreed with the EC and others, and is limited by available resources, FBPs do not necessarily reach high on the agenda. Nevertheless, partially, at least, as a response to outcomes of the COST Action described in this article, EFSA supported one activity on FBPs during 2018, and from 2019 will support a Partnering Grant (aimed at promoting EU-level capacity building) for knowledge and expertise transfer regarding molecular method development and validation for analysis of fresh produce for *Cryptosporidium* (as a model for other parasites).

Expert knowledge elicitation (EKE): consists of a number of methods that attempt to synthesize the knowledge of experts within a certain domain. EKE is a scientific consensus methodology and happens typically through some form of direct interaction with the expert. As unaided expert judgment, particularly the uncertainty, is often biased, methods have been developed to ensure that the knowledge elicitation occurs in an unbiased way.

Matrix-assisted laser desorption/ionization–time of flight mass spectrometry (MALDI-TOF MS): a versatile analytical technique to detect and characterize mixtures of organic molecules. It can be used as a cost-effective, fast, and accurate method to identify a series of microorganisms such as viruses, fungi, and parasites. MALDI-TOF generates characteristic transport [14,15]. A further effect of globalization of the food supply is the introduction of new pathogens to naïve populations, and to regions where health professionals may be unfamiliar with the FBP, resulting in underdiagnosis. One example is increasing cases of *Anisakis pegreffii* infection in Italy associated with consumption of undercooked sardines [16]. Even more 'exotic' foodborne parasitoses that may be associated with food supply globalization – and not just with returning travelers or immigrants, and may provide serious challenges to diagnosticians – include gnathostomosis [17], sparganosis [18], and fishborne trematodoses [19].

Changing Culinary Habits and the Role of Human Behavior towards Raw Foods

Consumption of raw and undercooked foods is increasing, and with it are the opportunities for exposure to FBPs and unfamiliar risks [7,10,20]. Increasing travel brings new culinary habits. Cultural preferences and behavioral changes in eating habits have led to an increase in incidence of zoonotic infections; consumption of undercooked, pickled, or smoked fish, meat, and crustaceans has resulted in transmission of a number of nematodes (e.g., *Anisakis simplex*), cestodes (*Taenia* spp., *Diphyllobothrium* sp.), trematodes (*Paragonimus* spp., *Clonorchis* sp., *Opisthorchis* spp.), and protozoa (*Toxoplasma*). For example, eating raw fish, prepared as sushi and sashimi, has become popular worldwide [21,22], resulting in greater recognition that some Anisakids may be responsible for gastroallergic disorders in consumers, even causing occupational asthma in workers processing fish [23]. In Europe, Spain probably has the highest incidence of anisakiosis, predominantly through consumption of anchovies marinated in vinegar [24]. A quantitative risk assessment model estimated that previous European incidence reports of 500 anisakiosis cases annually were considerable underestimates [25]. Rapid growth of the aquaculture industry means that other fishborne parasites, such as *Diphyllobothrium,* are being introduced into regions where these parasites never previously occurred [21,22].

The rising demand for protein of animal origin, coupled with increasing concern about animal welfare, is prompting changes in farm-management practices. In several European countries, this is reflected in trends towards organic foods or preferences for animals raised outdoors, and, with this, the greater possibility of infection with *Toxoplasma gondii* or reintroduction of FBPs such as *Trichinella*, or even *Taenia* spp. [26–28]. Other drivers of changes in culinary habits include a shift in national economic standards, changes in gender-biased employment (such that, in many European countries, women are less likely to be solely responsible for meal preparation), and meal preparation being generally allocated less time, resulting in fast food, including **ready-to-eat** (**RTE**) salads, becoming more widely eaten [22].

Gaps in Surveillance and Control

Surveillance of foodborne disease is a fundamental component of food-safety systems [7]. A prerequisite for control is public health surveillance, and outbreaks can signal breakdown within the system [29]. For some FBPs, such as *Trichinella* and *Taenia* spp., there are EU-level regulations. For other FBPs, surveillance systems are absent and substantial gaps exist. One example is *Toxoplasma*, for which no country or regulatory authority demands meat inspection, mainly due to the ubiquity of this infection and technical challenges, such as the lack of simple detection methods at the slaughterhouse. Thus, the extent of occurrence of *T. gondii* in meat products is largely unknown [10]. Although several seroprevalence surveys for *T. gondii* among meat animals have been conducted in Europe, such studies do not necessarily provide information regarding the occurrence of infectious bradyzoites in the tissue (e.g., concordance between seroprevalence results and other detection methodologies is lacking in cattle [30]). Furthermore, meat inspection might not provide the optimal control strategy; additional control strategies, including risk-based sampling, should be considered and implemented.

mass spectral fingerprints which are unique signatures for each microorganism. Bioinformatics pattern profiling is used to accurately identify the pathogen at the genus and species levels.

Next-generation sequencing (NGS): a term that is used to describe a series of modern sequencing techniques which allow for sequencing of DNA and RNA much more quickly and cheaply than previous sequencing tools such as, for example, Sanger sequencing; NGS revolutionized the study of genomics and molecular biology, and it permits highly adaptable, high-throughput whole-genome-scale assays.

Ready-to-eat (RTE): food products that are prepared in advance and can be eaten, as sold, without any further cooking or preparation. In some contexts, it is understood that the food product does not need further preparation to achieve a designated level of food safety.

Whole-genome sequencing (WGS): a technique used to determine an organism`s complete DNA sequence. WGS provides a comprehensive picture of both the coding and noncoding regions of chromosomal and mitochondrial DNA. WGS can help to solve foodborne outbreaks sooner and improve the efficiency of surveillance.

The Role of Water

Water uses in food production include, among others, irrigation, washing of fresh produce, and processing. In the last century, the food industry saw substantial changes regarding food production, processing, and preservation [31,32]. Extensive population growth and the concomitant increase in food requirements resulted in the development of new areas of cultivation and of irrigation systems. Water scarcity means increased utilization of wastewater for irrigation, providing greater possibilities for contamination of fresh produce. RTE-products are increasingly popular, and with removal of a consumer-driven intervention, these have been associated with outbreaks of foodborne parasites [33].

Lack of Awareness from Agencies

Compared with other foodborne pathogens, FBPs are neglected [22], partly due to limited resources and competing priorities. Furthermore, parasitic diseases are often chronic, with long-term sequelae. Echinococcosis, for example, results in morbidity and mortality years, or sometimes decades, after infection. Even epidemics of echinococcosis, for example [34], may remain under the radar. In southern Kyrgyzstan, a large number of cases of alveolar echinococcosis are now being reported [35], possibly related to socioeconomic changes that occurred over 25 years previously that have facilitated parasite transmission. Cystic echinococcosis remains a substantial problem in some EU countries (e.g., Italy and Spain), with hundreds, even thousands, of new cases diagnosed annually [36,37]. Increased efforts are therefore needed to create awareness and to translate research results into policy.

Addressing Current Needs within FBPs in Europe

With a wide spectrum of FBPs, identifying priority research areas is difficult. Priority topics in Europe indicated by the EKE (Figure 2A) were categorized. One major overarching topic (five of the ten needs identified) is methodological issues: improved methods for diagnosis; standardizing existing methods; adopting and adapting newer technologies used for other pathogens to FBPs; identifying appropriate methods for assessing parasite infectivity/viability; and optimizing technologies for inactivating parasites in food.

Tools and Methods for FBPs (Priorities 1–5)

Difficulties in detecting FBPs in food have hampered progress in tackling them, and probably reduced their perceived importance. Thus, development and improvement, including standardization, validation, and harmonization, of tools and methods to address FBPs remain a high research priority. Inadequate knowledge of infection sources compromises our understanding of the prevalence, epidemiology, and effects of interventions. Sophisticated tools, such as multiplex gastrointestinal pathogen panel tests, have indicated that various parasites are more widespread than previously recognized – for example, *Cryptosporidium* [38]. Furthermore, due to the wide spectrum of FBPs, not all can be easily diagnosed. Thus, development of analytical methods, both for diagnosis of infection and for analyzing food samples for contamination with parasites, is key. Such analyses require implementation of sensitive standardized methods. It is not always sufficient to detect an FBP in both patients and a suspected food source, and a robust, standardized, multilocus typing scheme is often needed [39]. Advances in **whole-genome sequencing** (**WGS**) of FBPs has lagged behind that of other pathogens, particularly bacteria [40], and, although ideal, the level of detail provided by WGS may be unnecessary for providing usable epidemiological evidence. When a rapid response is required, such as in outbreak investigations, other typing schemes may be preferable [41].

When FBPs are detected in food, the question arises of whether they are capable of causing infection. For some, such as *Anisakis* larvae, clear traits (motility) indicate viability of infectious stages,

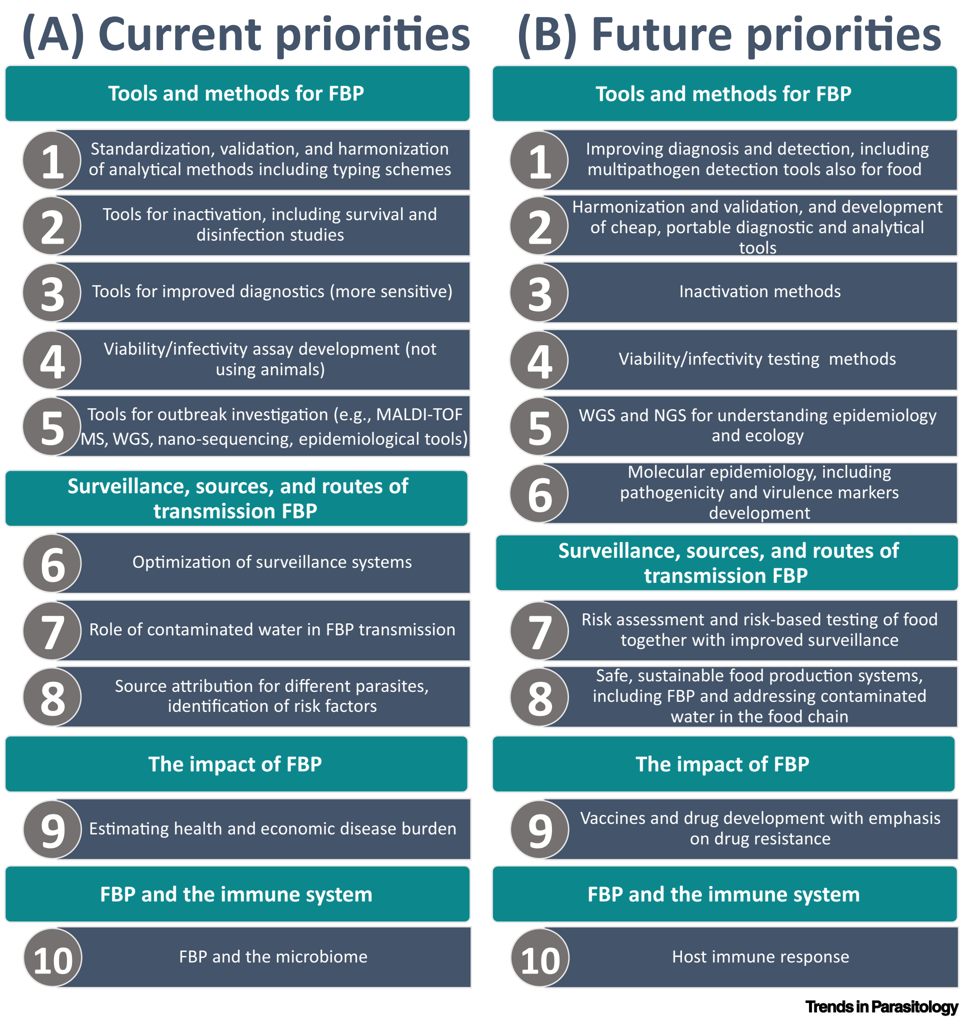

Figure 2. Top Ten Research Priorities for Foodborne Parasitic Diseases. Current (A) and Future (B, in 10 years) priorities for foodborne parasite (FBP) research are identified by the COST Action Euro-FBP using an expert knowledge elicitation (EKE) approach. Abbreviations: **MALDI-TOF MS, matrix-assisted laser desorption/ionization–time of flight mass spectrometry;** NGS, **next-generation sequencing**; WGS, whole-genome sequencing.

and methods have also been developed to account for temporary larval immobility [42]. However, for infectious stages that are not motile, determining their viability is less straightforward. For example, none of the current methodologies are ideal for reliable assessment of the viability of protozoan cysts/oocysts (of *Giardia*, *Cryptosporidium*, and *Toxoplasma*) contaminating fresh produce are infectious to consumers [43]. Such techniques are not only needed for determining whether a FBP contamination event presents an infection risk to consumers, but are also essential for investigating efficacies of control measures. Whereas measures of inactivation for most other foodborne pathogens, viruses, and bacteria are often quantified by log reduction, this is less clear for parasites due to variation regarding units of infection [14].

Furthermore, as FBPs do not replicate during food storage, a two or three log reduction, that may be considered inadequate for bacteria, may be sufficient for FBPs [14,15]. Although FBPs in meat and fish are generally controlled by appropriate freezing or cooking, novel inactivation methods are also relevant [14]. This is even more so for those parasites that are associated with fresh produce (helminth eggs, larvae/metacercaria and protozoan cysts and oocysts) for which temperature-based treatments may affect sensory qualities [15]. In a recent review of three FBPs (*Cryptosporidium, Toxoplasma, Echinococcus*), EFSA [7] recommended development and optimization of inactivation technologies, so this clearly remains an unfulfilled need.

Surveillance, Sources, and Routes of Transmission for FBPs (Priorities 6–8)

Surveillance of FBPs in both humans and animals is important for targeting interventions. However, current surveillance systems in Europe are not harmonized and do not cover all FBPs. Addressing how surveillance can best be optimized is a clear need.

Several FBPs have multiple pathways of infection for humans, of which food is only one. The attributable fraction of human infection with these parasites due to foodborne transmission therefore remains largely unknown due to a lack of empirical data. Echinococcosis, for example, is believed to be substantially transmitted via food, but, because the latent period is so long, the only data for the food-attributable fraction are based on EKE [2,7]. The degree of uncertainty in the estimates are so wide that the attributable fraction is effectively rendered unknown [7]. This indicates a clear need for empirical data on foodborne transmission.

Although drinking water is not a food, water is associated with many aspects of food production. Non-potable irrigation water may be a source of contamination, and wash-water has been implicated in contamination of RTE salads [22]. Bulk washing fresh produce can result in widespread dissemination of a limited, but intense, contamination event. Sanitizers used in salad wash-water are usually intended to reduce bacterial contamination [44], but are probably ineffective against FBPs. Assessing the extent to which water may result in contamination of food with FBPs is worth investigating, such that suitably targeted barriers can be implemented.

The Impact of FBPs (Priority 9)

The global burden of disease due to a number of FBPs has been estimated [2]. However, modelling and EKE were required to fill data gaps, and better estimates and tools are still required [45]. Fewer data are available on the economic burden, although estimates have been made for some countries, for example, the USA [46]. Estimates of economic burden of various zoonotic foodborne diseases, including parasites, are likely to be outcomes of the Global Burden of Animal Disease initiative [47]. Good estimates of the impact (public health outcomes and economic effects) of FBPs will enable calculation of cost-benefits and cost effectiveness of intervention strategies to reduce their burden. Social cost-benefit analyses [48] will also play an important role in determining these strategies.

FBPs and the Immune System (Priority 10)

Our understanding of the human intestinal microbiome [49], containing several trillion microbial cells, is more than a gut feeling, having advanced considerably in recent years. We now have a better understanding of how the intestinal microbiota are intimately associated with many aspects of human physiology and health that were previously considered as functions of our own immune system. Some of these aspects are particularly relevant to FBPs, including the role of the microbiota in immune responsiveness, pathogen adhesion and growth, and pathogen establishment. However, our understanding of how these interactions work for FBPs remains meagre. Recent studies imply that, among FBPs, helminths affect the intestinal microbiome with important consequences [50], indicating that this is an area of special relevance to the control of infections with FBPs. Similarly, for foodborne protozoan parasites, such as *Cryptosporidium* and *Toxoplasma*, recent investigations on their complex interactions with the microbiota and host immune system indicate that infection progression may be modulated by the population composition of the microbiome [51].

Where Can Projections Be Made? Future Research Priorities and Needs for FBPs

Predictions of where research foci will lie in the future can be thwarted by global cataclysms or unexpected breakthroughs. In order to be realistic, we need to assume that, in the short term (10 years), current technological and social changes will continue into the 21st century. Thus, as international markets and commerce expand, new technologies will affect production and preservation of food, and the public will eat more RTE meals outside the home, thereby becoming increasingly dependent on others to ensure the safety of our food. In total, 15 research priorities for the next 10 years were identified by the Euro-FBP COST Action and condensed into ten items (Figure 2B).

Some of these priorities are very similar to those topics identified as being of primary concern today. This reflects the basic needs of diagnosis, detection, validation, harmonization, and identification of markers for characteristics of relevance such as viability and pathogenicity. These questions are not expected to be solved within the next few years. Whatever the problem, surveillance is necessary for detection and defining both scope and magnitude. New technologies enable more sensitive and specific diagnostic methods. In addition, the intention to combine technologies for different pathogens, and potentially indicator organisms, already an attractive goal, may become more feasible. On the basis of credible data, risk-based sampling may become a reality. The development of microbiological criteria has recently been addressed by EFSA [52] for guiding risk assessors regarding microbiological contamination of food, but FBPs were barely considered. Miniaturization, which is already becoming incorporated into other fields for pathogen detection [53], enabling development of cheap, portable diagnostic tools, is in its infancy for FBPs. However, based on current research, particularly on waterborne parasites (e.g., [54]) this may be achievable as a short-term objective. Whether such advances will contribute to the goal of safe, sustainable food production systems remains to be seen. Given the incremental progress towards safe food, consideration of the parasite host is also important. This is not only diagnosis, but also understanding immune mechanisms, and developing vaccination and treatment options. For example, vaccination strategies for some livestock are already described as having an impact on the potential for transmission of both echinococcosis and toxoplasmosis [7]. The potential for FBPs to develop drug resistance should also be considered. Several parasites are already resistant to some drugs, and further understanding of how resistance mechanisms, such as membrane transporters (e.g., ATP-binding cassette transporters), may prevent chemotherapeutic drugs reaching their targets is as likely to be of importance for some FBPs as for other pathogens (e.g., [55]).

Concluding Remarks

History teaches that diseases are, and will remain, an ever-changing problem for public health. In order to address the challenges currently posed by FBPs, and those that will be posed in the future, recognition of their importance is prerequisite, and flexibility, resources, and long-term commitments are necessary. Integrated and complementary activities in each of the current research and future research areas for FBPs are fundamental (Box S1).

International markets and trade are difficult to predict, being affected by political fluctuations and various global events. However, current trends suggest that they will continue to develop in coming years, and new technologies will affect production and preservation. New tools for detecting FBPs in food matrices will need further development and improvement, together with approaches to determine infectivity and viability for FBP transmission stages.

Outstanding Questions

What is needed to raise awareness and to guide funding agencies towards research prioritization for FBPs?

How can research be translated into a message that leads to policy change?

To what extent can diagnostic tools be developed to identify new emerging FBPs?

Which new tools are needed for outbreak investigation?

How can tools for detecting FBPs in food matrices be further developed and improved?

Which approaches should be used for optimizing viability and infectivity tests for FBP transmission stages?

Why are simple detection methods for *Toxoplasma* not developed?

Which inactivation techniques should be further explored for their impact on FBP transmission stages?

Which measures should be implemented to minimize the risk of transmission of FBPs, given the increasing behavioral changes towards new, raw, and exotic foods?

What impacts will changing farming practices have on FBPs?

Why are FBPs so rarely considered along the food-chain, and how can we motivate relevant agencies to improve surveillance?

What impact will climate change have on FBPs?

How will water scarcity impact FBPs?

How can surveillance be best optimized?

How can we improve source attribution for FBPs for which a long incubation period hampers the gathering of relevant information?

Which modelling tools should be further developed, and combined with other impact metrics, for more accurate estimation of the burden associated with FBPs?

The trend for eating more RTE foods and more meals outside the home is also increasing, and new technologies that address not only bacterial pathogens, but also parasites, will be needed to ensure food safety. Determining which measures are most appropriate to implement in order to minimize the risk of FBP transmission needs carefully consideration.

Climate change is a threat and a global concern for many reasons, but its role in FBP transmission should not be underestimated. Lack of water, together with the increased demand for proteins of animal origin, will have a global impact. Furthermore, changing production systems with increased applications of drugs, potentially leading to drug resistance, might further contribute to FBP transmission.

Of particular importance is the lack of rapid, cheap, and easy-to-use diagnostic tools, leading to inadequacy in routine, accurate disease diagnosis and monitoring. This combined with lack of awareness, results in many diseases transmitted via food remaining underestimated, and might be one reason why FBPs are rarely considered along the foodchain.

Creating awareness among stakeholders is essential for motivating relevant agencies to improve surveillance. However, how research findings can be best translated into policy, and thereby create the necessary awareness, remains a partially unresolved issue. Modelling tools can be further developed and combined with other impact metrics for more accurate estimation of the burden associated with FBPs and might provide the necessary evidence and motivation for policy makers, funding bodies, and other relevant stakeholders. Building upon the combined expertise of different agencies of the EU, such as EFSA and ECDC (European Centre for Disease Prevention and Control), along with the trans-European outputs of diverse researchers with interests in FBPs, as exemplified in this COST Action, may provide a roadmap for where research could be usefully focused. A joint European policy on supporting innovation to address some of the technological and research gaps highlighted here might be one approach towards advancing this field of research and ensuring that FBPs do not remain neglected in goal-setting for food safety.

What role does the microbiota have on host immune responsiveness and pathogen establishment regarding FBPs?

What effects will drug resistance have on the transmission of FBPs?

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Supplemental Information

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