### **Control of Fishborne Parasites** in the Food Industry

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#### **SUMMARY**

Fishborne parasites constitute an emerging food safety issue. Whereas the focus in Europe is on nematode larvae (Anisakids), human infections with fishborne trematodes and cestodes are equally prevalent in Asia. Preferences for eating raw or marinated freshwater fish raised where the water may be contaminated with feces, may account for that. Heating ( $\geq 60^{\circ}$ C) and deep-freezing ( $\leq 20^{\circ}$ C), should render fish products safe for consumption as regards nematodes and cestodes; less is known about the resistance to freezing of metacercariae from trematodes. In addition, the potential of traditional, as well as advanced, methods of food processing to serve as critical steps in food production chains has scarcely been explored. The EU COST Action "Euro-FBP: A European Network for Foodborne Parasites" (https:// eurlp.iss.it/) has aimed at reviewing the current status of knowledge regarding fishborne parasites and at pointing out potential deficiencies in control of them in food.

### **OVERVIEW**

Fish constitute an important part of the human diet. The average annual consumption is ca. 20.8 kg per capita worldwide, comparable to that of meat, ca. 35 kg per capita and year (14). Likewise, average annual fish consumption in high-income countries is in the range of 22.8 (United States of America; USA) to 24 (European Union, EU-28) kg per capita. Changes in population and in food preferences make fishborne parasitic diseases no longer an issue primarily for low- and medium-income countries (2). Dorny et al. (3) reviewed the significance of foodborne parasites and concluded that although such fishborne zoonoses were common in Asia, changes in aquaculture, food transport and distribution could increase exposure of consumers in other regions. These authors considered trematodes, cestodes and nematodes as most relevant, and, in principle, this prioritization was confirmed in a ranking of foodborne parasites (10), in which 5 of the 24 top-ranked foodborne parasites globally were associated with marine and freshwater finfish (Anisakidae, Diphyllobothriidae, Heterophyidae, and

Opistorchiidae) or with freshwater crustacea (*Paragonimus* spp.). Not only can risky fish preparation methods and consumption traditions contribute to infection of local inhabitants (*5*), but travelers can acquire a parasitic infection abroad (*4*). In fact, fishborne parasites have changed from a neglected to an emerging food safety issue (*15*).

### Pertinent legislation in the EU and the USA

As the primary responsibility for food safety rests with food business operators (6), these must consider implementation of appropriate, HACCP-based control measures in accordance with the requirements of Regulations EC No. 853/2004 (as amended by Commission Regulation EU No. 1276/2011) and 854/2004 (7, 8). In fact, it is established that fishery products derived from finfish or cephalopod molluscs, if they are placed on the market and intended to be eaten raw or if they have been marinated, salted or treated in another way that is insufficient to kill the viable parasite, must be produced from raw material that has undergone deep freezing. Exceptions from this obligation are as follows: (a) the fishery product undergoes heat treatment before consumption, (b) it has been stored deep-frozen for a sufficient period of time, or (c) it originates from wild catch or aquaculture with some biosecurity precautions (8). Notably, freezing (-20°C for not less than 24 hrs or -35°C for not less than 15 hrs.) and heating regimens (60°C core temperature for not less than 1 min.) have been defined for "parasites other than trematodes." These requirements consider the outcome of a risk assessment conducted by the European Food Safety Authority (9), which focused, on the basis of availability of data, on Anisakis and which concluded that "many traditional marinating and cold smoking methods are not sufficient to kill A. simplex and freezing or heat treatments remain the most effective processes guaranteeing killing. All wild-caught seawater and freshwater fish must be considered at risk of containing any viable parasites of human health concern if these products are to be eaten raw or almost raw. For wild-catch fish, no sea fishing grounds can be considered free of A. simplex." Likewise, in the

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U.S., the food processor has to assess whether a foodborne hazard due to parasites in fish is likely to occur, when "the processor has knowledge or has reason to know that the parasite-containing fish or fishery product will be consumed without a process sufficient to kill the parasites, or where the processor represents, labels, or intends for the product to be so consumed" (16) and must establish appropriate control measures in the form of HACCP (*Fig. 1*).



FIGURE 1. Fish fillets of red gurnard (*Chelidonichthys lucerna*) infested with larvae of the nematode *Pseudoterranova decipiens* (Anisakidae) (arrows). Bar: 1 cm. (Photo: Dutch Reference Laboratory for Parasites, Centre for Zoonotic Diseases and Environmental Microbiology, Bilthoven, The Netherlands.)

### HACCP-based food safety systems as a means to control fishborne parasites

Since parasites, unlike bacteria, will not multiply in post mortem tissues, the control of parasitic stages in fishery products can make use of (a) selection of raw material with low risk of parasitic infection (e.g., farmed Atlantic salmon with appropriate level of biosecurity), (b) detection of parasites and removal of infested tissues or tissues prone to infection (e.g., the bellies in herring in the case of *Anisakis* sp.), or (c) implementation of treatments that ensure inactivation of the infective parasite stages.

For food business operators (FBO), it is essential to know not only which parasites are relevant hazards in fish and fishery products, but also what level of parasite inactivation is required to render food safe for consumption. There are several obstacles in defining such a "performance objective" (12); for example the definition of an infective unit, the infective dose (or a probability of infection) and the way viability and infectivity of parasitic stages are assessed. Consequently, EU legislation defines inactivation temperatures and holding times, but this approach does not take into account synergistic effects of processing techniques, and it is not targeted against parasitic stages of trematodes. It can be argued that it must be sufficient for FBOs to guarantee that EU legal requirements are fulfilled, but since, e.g., trematodes are not dealt with in the regulations, there is a need to assess whether such hazards are relevant and whether there is a critical control point in the processing chain. Given the outbreaks of opisthorchiasis in Italy (1), it is clear that trematodes are also relevant within a European context; it should be noted that in one outbreak, the implicated fish had been frozen at -10°C for 3 days and then marinated before consumption.

### Collating data on survival and inactivation of fishborne parasites: the "Euro-FBP" Action

In order to provide relevant information to the food industry, the EU COST Action FA 1408, "Euro-FBP: A European Network for Foodborne Parasites" (https://www. euro-fbp.org/) collated and critically reviewed information on parasite inactivation by food-processing techniques. Unfortunately, for most parasites, data are too scarce to allow mathematical modelling of viability and decay, especially since (i) interspecies or intraspecies variabilities are not always known, and (ii) standardized methods for assessment of parasites' viability do not always exist or do not allow quantification of reduction. For bacterial pathogens, log reductions are a common way to describe the extent of inactivation. However, one single parasite stage in food may be enough to cause human infection for some species, which calls for dose-response data dealing with exposure and infection, and discriminating between parasite species. Also, in case of complex food processing techniques, the relative contributions of the single factors and the extent of synergy are not well known, so that any deviation from the test conditions would require re-evaluation.

Likewise, knowledge gaps exist for non-traditional fish processing methods, such as high-pressure processing or irradiation. The main processes have recently been reviewed (11), and the underlying set of data has been compiled in an excel sheet. This database will be made publicly available and the reader may refer to the Euro-FBP FA1408 homepage (https://eurlp.iss.it/) for further information.

Available published data suggest that heating to 60–70°C with holding times of a few minutes will lead to a 0.5 to 1.8 log decrease in numbers of viable *Anisakis* larvae. With regard to metacercariae, the few available studies suggest a 60°C core temperature for 15 min. to inactivate metacercariae in flesh from mullet. Likewise, metacercariae seem to withstand deep-freezing well, and although a temperature of -20°C for 24 h will reduce viability in some species, it will not result in complete inactivation. The effect of marination is quite variable, depending on salt and acid concentrations, but several days to weeks will be required to inactivate *Anisakis* larvae. Few data exist on survival of parasites in fermented fish (11).

## TABLE 1. Published data (original research papers) on inactivation of parasites in fishand fish products (11, 13)

Treatments	Thermal treatment	Freezing	Salting, curing, marination, fermentation, drying	High hydrostatic pressure	Irradiation (electron beam or gamma irradiation)
Food commodity					
Fresh fish		An, Cl, Di, He, Op		An	An, Cl, Op, Pa
Frozen raw seafood					
Cooked crustaceans					
Canned seafood					
Cured, smoked and dried seafood			An, Cl		An
Fermented fish products			Op		
Minced fish and surimi products					

Light grey: possible pre-processing treatment of raw materials; Light green: regular part of the process; if scientific studies on the effective control of parasites in a food commodity process step combination (i.e., cell) have been published, the parasite name is inserted in abbreviated form: An: *Anisakis*; Cl: *Clonorchis* metacercariae; Di: *Diphyllobothrium* plerocercoids; He: Heterophyidae; Op: *Opistorchis* metacercariae; Pa: *Paragonimus* metacercariae.

With regard to electron-beam and gamma irradiation, minimum effective doses of 0.1–2.5 kGy have been reported for metacercariae embedded in fish flesh, whereas even a dose of 6 kGy was not effective against *Anisakis* larvae. Concerning high-hydrostatic pressure, 100–300 MPa for 5 min reduced the number of viable *Anisakis* larvae.

Data are scarce on the effect of food processing technology on the survival of fishborne parasites (*Table 1*), and not all studies report the actual extent of inactivation. Because HACCP-based systems address other biological hazards (e.g., bacteria), it is conceivable that some control points are similar for parasites and that the critical process parameters could be adjusted to allow for control of both parasitic and microbial hazards. Admittedly, thermal processes, curing and drying are to some extent addressed in national guides to good practice or food codex standards. Nevertheless, in the course of product development, and because of deliberate or inadvertent changes in processing parameters as is seen with the trend toward minimally processed foods, there is a clear need for more detailed studies on survival of parasites during fish processing, based on harmonized methods for detection and viability assessment of parasites.

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