Manuscript Details

Manuscript number	TIFS_2018_138
Title	Inactivation of parasite transmission stages: Efficacy of treatments on food of animal origin
Article type	Review Article

Abstract

One third of parasitic outbreaks with known source in the US are attributable to food of animal origin (FoAO). Among 24 foodborne parasites ranked by FAO/WHO, 14 are associated with FoAO. Management of these biological hazards is essential to ensure food safety. This constitutes the first systematic review of control measures to inactivate foodborne parasites, including cooking, freezing, curing and combined processes, as well as high-pressure-treatment and irradiation. Wherever possible, the extent of foodborne parasite reduction (expressed as log units) and the methods of assessment of parasite inactivation are reported. Efficacy of freezing and heating depends on parasite species and developmental stage. Cooking at core temperature 60 – 75 °C for 15 – 30 min inactivates parasites in most matrices, but may not be enough to inactivate all parasites; for home cooking, USDA recommends heating meat at 62.8 - 73.9 °C core temperature. Freezing at -21 °C for 1 – 7 days inactivates parasites in meat or fish, but cannot be relied upon in home situations. Parasitic stages are sensitive to 2-5% NaCl, associated with higher osmotic stress, often augmented by lowering pH. Little is known about high pressure- and electron-beam irradiation; gamma radiation at >0.1-0.5 kGy is effective for fish parasites, except Anisakis (10 kGy); doses >0.4-6.5 kGy control meatborne parasites. Literature data are insufficient to model survival as response to treatment. Research on foodborne parasites should be improved towards standardization of experimental approaches for evaluation of inactivation techniques and methods to monitor inactivation.

Keywords	foodborne parasites; inactivation; control measure; meat; fish.
Manuscript region of origin	Europe

Corresponding Author	Frits Franssen
Order of Authors	Frits Franssen, Cédric Gerard, Anamaria Cozma-Petruț, Madalena Vieira Pinto, Anet Režek Jambrak, Neil Rowan, Peter Paulsen, Miroslaw Rozycki, Kristoffer Tysnes, David Rodríguez-Lázaro, Lucy Robertson
Suggested reviewers	Edoardo Pozio, Alvin Gayadhar, Ray Gamble, Giuseppe Arcangeli

Submission Files Included in this PDF

File Name [File Type]

Letter to the Editor Trends Food Sci & Tech.docx [Cover Letter]

Highlights 07032018 FoAO decontamination of parasites.docx [Highlights]

Abstract 07032018 FoAO decontamination of parasites.docx [Abstract]

08032018 FoAO decontamination of parasites-Final.docx [Manuscript File]

To view all the submission files, including those not included in the PDF, click on the manuscript title on your EVISE Homepage, then click 'Download zip file'.

Editor-in-Chief Dr. P. Finglas,

Trends in Food Science & Technology

Bilthoven,

March 7th, 2018

Dear Editor,

Please find enclosed our manuscript entitled ‘Inactivation of parasite transmission stages: Efficacy of treatments on food of animal origin’ for publication in your highly esteemed journal Trends in Food Science & Technology.

Our paper describes the first systematic review of control measures to inactivate parasites in food of animal origin, including cooking, freezing, curing and combined processes, as well as high-pressure-treatment and irradiation. Wherever possible, the extent of foodborne parasite reduction (expressed as log units) and the methods of assessment of parasite inactivation are reported.

We hope that this work will be of interest to Trends in Food Science & Technology.

With kind regards,

Dr. Frits Franssen.

Highlights

- Parasites can be transmitted to people in various foods of animal origin (FoAO)
- Relevant methods for inactivation of transmission stages in FoAO are reviewed
- Methods for evaluating inactivation of foodborne parasites may impact findings
- Key aspects of thermal and non-thermal inactivation methods are provided

Abstract

Background

One third of parasitic outbreaks with known source in the US are attributable to food of animal origin (FoAO). Among 24 foodborne parasites ranked by FAO/WHO, 14 are associated with FoAO. Management of these biological hazards is essential to ensure food safety.

Scope and Approach

This constitutes the first systematic review of control measures to inactivate foodborne parasites, including cooking, freezing, curing and combined processes, as well as high-pressure-treatment and irradiation. Wherever possible, the extent of foodborne parasite reduction (expressed as log units) and the methods of assessment of parasite inactivation are reported.

Key Findings and Conclusions

Efficacy of freezing and heating depends on parasite species and developmental stage. Cooking at core temperature 60 – 75 °C for 15 – 30 min inactivates parasites in most matrices, but may not be enough to inactivate all parasites; for home cooking, USDA recommends heating meat at 62.8 - 73.9 °C core temperature. Freezing at -21 °C for 1 – 7 days inactivates parasites in meat or fish, but cannot be relied upon in home situations. Parasitic stages are sensitive to 2-5% NaCl, associated with higher osmotic stress, often augmented by lowering pH. Little is known about high pressure- and electron-beam irradiation; gamma radiation at >0.1-0.5 kGy is effective for fish parasites, except *Anisakis* (10 kGy); doses >0.4-6.5 kGy control meatborne parasites. Literature data are insufficient to model survival as response to treatment. Research on foodborne parasites should be improved towards standardization of experimental approaches for evaluation of inactivation techniques and methods to monitor inactivation.

Inactivation of parasite transmission stages: Efficacy of treatments on food of animal origin

Franssen Frits[1§], Gerard Cédric[2], Cozma-Petruţ Anamaria[3], Vieira-Pinto Madalena[4], Režek Jambrak Anet[5], Rowan Neil[6], Paulsen Peter[7], Rozycki Miroslaw[8], Tysnes Kristoffer[9], Rodriguez-Lazaro David[10], Robertson Lucy[11]

[§] Corresponding author:

Frits Franssen, Centre for Zoonotic Diseases and Environmental Microbiology, National Institute for Public Health and the Environment, Antonie van Leeuwenhoeklaan 9, 3721 MA Bilthoven, The Netherlands. Tel.: +31 6 524 85662, Email: frits.franssen@rivm.nl

Author address information:

[1] Frits Franssen, Centre for Zoonotic Diseases and Environmental Microbiology, National Institute for Public Health and the Environment, Antonie van Leeuwenhoeklaan 9, 3721 MA Bilthoven, The Netherlands. Email: frits.franssen@rivm.nl

[2] Cédric Gerard, Food Safety Microbiology, Nestlé Research Center, P.O. Box 44, CH-1000, Lausanne, Switzerland. Email: Cedric.Gerard@rdls.nestle.com

[3] Anamaria Cozma-Petruţ, Department of Bromatology, Hygiene, Nutrition, "Iuliu Haţieganu" University of Medicine and Pharmacy, 6 Pasteur Street, 400349 Cluj-Napoca, Romania. Email: anamaria.cozma@umfcluj.ro

[4] Madalena Vieira-Pinto, Department of Veterinary Science, University of Trás-os-Montes e Alto Douro (UTAD), 5001-801 Vila Real, Portugal AND Animal and Veterinary Research Center, UTAD, Portugal Email: mmvpinto@utad.pt

[5] Anet Režek Jambrak, Faculty of Food technology and Biotechnology, University of Zagreb, Pierotti street 6, Zagreb, Croatia. Email: arezek@pbf.hr

[6] Neil Rowan, Centre for Disinfection, Sterilisation and Biosecurity, Athlone Institute of Technology, Dublin Road, Athlone, Co. Westmeath, Republic of Ireland. Email: nrowan@AIT.IE

[7] Peter Paulsen, Institute for Meat Hygiene, Veterinary University Vienna, Veterinärplatz 1, 1210 Vienna, Austria. Email: peter.paulsen@vetmeduni.ac.at

[8] Miroslaw Rozycki, National Veterinary Research Institute in Pulawy (PIWet), Poland. Email: mrozycki@piwet.pulawy.pl

[9] Kristoffer Tysnes, Parasitology, Department of Food Safety and Infection Biology, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Adamstuen Campus, PO Box 8146 Dep., 0033 Oslo, Norway.Email: kristoffer.tysnes@nmbu.no

[10] David Rodriguez-Lazaro, Microbiology Division, Department of Biotechnology and Food Science, Faculty of Science, University of Burgos, Burgos, Spain. Email: drlazaro@ubu.es.

[11] Lucy J. Roberston, Parasitology, Department of Food Safety and Infection Biology, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Adamstuen Campus, PO Box 8146 Dep., 0033 Oslo, Norway. Email: lucy.robertson@nmbu.no

Abstract

Background

One third of parasitic outbreaks with known source in the US are attributable to food of animal origin (FoAO). Among 24 foodborne parasites ranked by FAO/WHO, 14 are associated with FoAO. Management of these biological hazards is essential to ensure food safety.

Scope and Approach

This constitutes the first systematic review of control measures to inactivate foodborne parasites, including cooking, freezing, curing and combined processes, as well as high-pressure-treatment and irradiation. Wherever possible, the extent of foodborne parasite reduction (expressed as log units) and the methods of assessment of parasite inactivation are reported.

Key Findings and Conclusions

Efficacy of freezing and heating depends on parasite species and developmental stage. Cooking at core temperature 60 – 75 °C for 15 – 30 min inactivates parasites in most matrices, but may not be enough to inactivate all parasites; for home cooking, USDA recommends heating meat at 62.8 - 73.9 °C core temperature. Freezing at -21 °C for 1 – 7 days inactivates parasites in meat or fish, but cannot be relied upon in home situations. Parasitic stages are sensitive to 2-5% NaCl, associated with higher osmotic stress, often augmented by lowering pH. Little is known about high pressure- and electron-beam irradiation; gamma radiation at >0.1-0.5 kGy is effective for fish parasites, except *Anisakis* (10 kGy); doses >0.4-6.5 kGy control meatborne parasites. Literature data are insufficient to model survival as response to treatment. Research on foodborne parasites should be improved towards standardization of experimental approaches for evaluation of inactivation techniques and methods to monitor inactivation.

Highlights

- Parasites can be transmitted to people in various foods of animal origin (FoAO)
- Relevant methods for inactivation of transmission stages in FoAO are reviewed
- Methods for evaluating inactivation of foodborne parasites may impact findings
- Key aspects of thermal and non-thermal inactivation methods are provided

Keywords: foodborne, parasite, inactivation, control measure, meat, fish

Contents

1. Introduction and the growing awareness of foodborne parasites

Among all foodborne disease outbreaks reported in the EU between 2007 and 2011, foods of animal origin (FoAO) were associated with 90% of outbreaks, 74% of cases, 65% of hospitalizations, and 54% of deaths (Da Silva Felicio et al., 2015). In USA, appraisal of data from 1998-2008 indicated that approximately 48% of cases of foodborne illnesses were associated with FoAO, 52% of hospitalizations, and 49% of deaths (Painter et al., 2013). However, these data cover the spectrum of infectious agents, and the USA data also include foodborne illnesses associated with chemicals. Data from USA indicates that for foodborne parasitic diseases, the food vehicle is not determined for the majority of cases, but, where it is identified, FoAO account for around one third (Painter et al., 2013). However, these data were derived from reported outbreaks, and, as some foodborne parasites may have a considerable health-related impact, but nevertheless do not often cause outbreaks (e.g., *Toxoplasma*), these data presumably underestimate the human health impacts of the associated food commodities.

Among the 24 foodborne/potentially foodborne parasites listed for risk-ranking by FAO/WHO in 2012 (FAO/WHO, 2014), transmission of 14 of them (58%) can be associated with FoAO. These include parasites associated with both marine and freshwater finfish, including the Anisakidae, Diphyllobothriidae, Heterophyidae, and Opistorchiidae, parasites associated with freshwater crustacea (*Paragonimus* spp.), parasites associated with pork (*Trichinella spiralis*, other *Trichinella* species, *Toxoplasma gondii*, *Taenia solium*, and *Sarcocystis suihominis*), parasites associated with beef (*Taenia saginata*, *Toxoplasma gondii*, and *Sarcocystis bovihominis*), parasites associated with meat from small ruminants (*Toxoplasma gondii*), parasites associated with meat from game animals (*Trichinella* spp. and *T. gondii*), and parasites associated with frog and snake meat (*Spirometra* spp.). In addition, some parasites have been associated with contamination of molluscs that can accumulate excreted transmission stages (e.g. *Giardia duodenalis*), and have also been associated with milk (*Cryptosporidium parvum* and *T. gondii*).

Although certain types of fresh produce are more frequently associated with raw consumption or minimal processing than FoAO, intentional or unintentional under-cooking of FoAO is well recognized. In particular, consumption of raw fish has become a global culinary trend, with the rise in popularity of sushi, sashimi, and traditionally prepared ceviche, and may result in exposure of consumers to fishborne parasites. Although consumption of raw meat occurs in several culinary cultures (e.g., steak tartare from France, *carpaccio* from Italy, *mett* in Germany, *koi soi* in Thailand, *kitfo* from Ethiopia etc.), more common is consumption of rare meat (cooked briefly to a temperature below 60 °C). This may be insufficient to inactivate the transmission stages of pathogens, including some parasites. In addition, meat may be undercooked inadvertently.

Given that some cooking techniques or other preparation of FoAO (e.g., fermentation, drying, freezing, etc.) may be insufficient to inactivate parasite transmission stages, knowledge on the effects of these different procedures at inactivating different parasite transmission stages is of interest, and of particular relevance, given the globalization of the food chain.

Testing for parasitic infections at meat inspection to prevent zoonotic parasites entering the food chain is mandatory for *Trichinella* in Europe (European Commission, 2015). Testing for some other parasites may be relevant, but is not routinely implemented and some parasites are tested for, but with limited sensitivity (e.g., tapeworm cysts). In this review we provide an overview of inactivation techniques in use with the potential to prevent transmission of parasitic infections due to consumption of FoAO. The review does not focus on primary production measures to prevent parasitic infections entering the food chain and does not take into account parasite-derived health hazards other than infection, such as allergic reactions provoked by Anisakidae sp. or toxins associated with *Sarcocystis* species.

2. Current state of knowledge

2.1. Parasites associated with finfish

Anisakiasis, mainly caused by the nematodes *Anisakis* spp. and *Pseudoterranova* sp. has been reported from many countries globally (Audicana & Kennedy, 2008), and *Anisakis simplex sensu strictu* is the most prevalent pathogenic species. Areas where the species occur most frequently are northern waters of the Atlantic Ocean and the Pacific Ocean (EFSA, 2010). *Anisakis pegreffii* occurs less frequently than *A. simplex sensu strictu* and occurs mainly in the Mediterranean Sea and the waters of the southern Atlantic Ocean. *Anisakis simplex Complex* occurs in the southern waters of the Atlantic Ocean and northern Pacific Ocean. *Anisakis typica* has been found in the warm waters of the Atlantic Ocean, whereas *Anisakis schupakovi* is endemic in the Caspian Sea. Parasitoses caused by other Anisakids are less common; contracaecosis cases have been reported from Germany, Australia and Japan, and cases of pseudoterranovosis were reported from Japan, Korea, Iceland, North America and South America (Bilska-Zając et al., 2015; McClelland, 2002). The immune response to anisakid larvae burrowing into the wall of the digestive tract is usually the main cause of the pathology associated with anisakiasis, for which severe abdominal pain is the most obvious manifestation.

Diphyllobothrium latum is present worldwide, but is more common in the northern hemisphere. The main sources of transmission of *D. latum* are salmon, pike, and eel. *D. latum* is endemic in Scandinavia, western Russia, Baltic countries, North America, Chile, and Peru. During the last century, the prevalence of human diphyllobothriosis was highest in Finland and Alaska, but has decreased significantly in these countries. In contrast, diphyllobothriosis has increased in Russia, South Korea, and South America. Several cases have been reported from regions where a disappearance of the disease had been expected, such as the Alpine lake regions in Switzerland, northern Italy, and eastern France, region Haute-Savoie (Scholz, Garcia, Kuchta, & Wicht, 2009). Diphyllobothriosis is generally asymptomatic or associated with abdominal symptoms, but intestinal obstruction has been associated with massive infections and migration of proglottids may cause cholecystitis or

cholangitis; improved nutrition generally means that megaloblastic anaemia is rarely associated with this infection in recent times.

Among fishborne trematodes, the major genera of importance are *Clonorchis* and *Opistorchis*. Liver fluke (*Clonorchis sinensis*) clonorchiasis is endemic in South China, South Korea, Taiwan, and North Vietnam. Opisthorchiasis caused by *Opistorchis viverrini* is endemic in Thailand, Lao, Cambodia and Central Vietnam. *Opisthorchis felineus* is endemic in the Russian Federation, Kazakhstan, Ukraine and East Asia, but sporadic cases have also been observed in Poland and sizeable outbreaks have occurred in Italy. Carp and other cyprinids are the main intermediate hosts of transmission of the parasites to humans (Scholz et al., 2009). For all these fishborne trematodes, infection may present as only mild symptoms, such as dyspepsia and abdominal discomfort, but can have more serious clinical presentation, such as hepatomegaly and liver cirrhosis; the most serious outcome of infection is bile duct cancer (cholangiocarcinoma; CCA).

Intestinal trematodes (e.g. *Heterophyidae*) generally do not present with significant clinical symptoms, compared with liver fluke. However, some species can cause fatal changes in the heart or central nervous system (WHO, 1995). These parasites are mainly observed in Asia, especially Taiwan, Malaysia, Thailand, and Vietnam (Hamed & Elias, 1970).

2.2. Parasites associated with consumption of meat

Trichinella spp. nematodes are prevalent worldwide. At least 149 animal species can be infected by *Trichinella* spp. and transmit the parasite through their muscle tissues. Two clades are recognized in the genus *Trichinella*: encapsulated and non-encapsulated species. Parasites of the genus *Trichinella* are a complex of 12 currently known taxa with a broad geographic range, including, Africa, the Americas, Asia, Australasia, and Europe, and a broad host spectrum encompassing mammals, birds and reptiles (Murrell & Pozio, 2011). The parasite locates intramuscularly; raw or undercooked meat of omnivores (mainly wild boar and pigs) are the main source of infection for humans, but many other animals, including from herbivores, notably horses, that have ingested infected meat, may also be a source of human infection (Pozio, Tamburrini, & La Rosa, 2001). Human trichinellosis has been

199 reported from North America, parts of South America, Central America, parts of Africa, Asia, New
200 Zealand, and Tasmania. The possibility of *Trichinella* occurrence in Australia is currently under
201 investigation (ICT, 2006). The symptoms of trichinosis depend on the stage of infection, with
202 abdominal symptoms associated with invasion of the intestine, fever and inflammation associated
203 with migration of the new larvae about a week after infection, and then rash and myalgia, possibly
204 with heart, lung, or CNS involvement, associated with subsequent encystation of these larvae.
205 The cestode *Taenia solium* (pork tapeworm) is endemic in Eastern Europe (Ukraine, Romania,
206 Serbia), Asia (China, India, Thailand, Malaysia, Laos, Philippines), Africa (Mid and South Africa) and
207 South America. The prevalence of *T. solium* in both pigs and humans varies according to the level of
208 sanitation and eating habits in a region, e.g. in Kenya prevalence of porcine cysticercosis up to 37%
209 has been found (Thomas et al., 2016). Although humans are the definitive host for this parasite,
210 infected through consumption of viable cysticerci in pork, the symptoms of taeniasis, harbouring the
211 adult tapeworm, are relatively mild; more serious is the effect of environmental contamination with
212 the eggs of this tapeworm, as if a human ingests these, then the person can act as an aberrant
213 intermediate host with development of cysticerci throughout the body. Neurocysticercosis is the
214 most serious form of the disease, and can be fatal.
215 In cattle (intermediate hosts) *Taenia saginata* (the beef tapeworm) causes cysticercosis and
216 consumption of in adequately cooked beef may cause taeniasis in humans (definitive hosts), for
217 which symptoms are mild abdominal discomfort and indigestion. This parasite is endemic in Africa,
218 South America, Eastern Europe, the Middle East and South Asia (Bogitsh & Oeltmann, 2013). The
219 worldwide incidence of human infection is low, but in some regions 25% of cattle are estimated to be
220 infected (Eckert, 2005).
221 One of the most common zoonotic parasitic protozoa is *Toxoplasma gondii*. Human toxoplasmosis is
222 present in every country and seropositivity rates range from less than 10% to over 90%. This
223 intracellular parasite has a worldwide distribution and can infect humans, mammals, and birds. In
224 immunocompetent humans, toxoplasmosis is generally a mild illness with non-specific, influenza-like

symptoms although some strains of the parasite are associated with more severe symptoms such as retinochoroiditis; however in the immunocompromised, serious symptoms, such as encephalitis, may occur and may even be fatal. In pregnant women who have not previously been exposed to the infection, the foetus may be aborted or born with significant injuries such as hydrocephalus.

The main sources of infection are meat and other foods, water and the environment (Murrell & Dubey, 1991). Among meat from production animals, the median prevalence in the Netherlands is 30% in sheep, 24% in pork, 13% in cattle, and 7% in equines (Opsteegh, 2011). Seroprevalence reported in farmed goats in Europe varies from 4% to 77%; in non-European countries, seroprevalence ranges from 0% to 40%. The percentage of infected pigs may be as high as 92.7% and as low as 0%; *T. gondii* prevalence in sheep can reach 78% (Jones & Dubey, 2012). It should be noted that the likelihood of detecting *Toxoplasma* antibodies in animals from a free-range farm type is higher than in animals from an enclosed farm.

There are over 130 species of *Sarcocystis*. Infections have been reported worldwide from Africa, Europe, both Americas and Asia. Sarcosporidiosis is often an incidental finding and probably underreported. In humans, the symptoms are generally intestinal, with abdominal pain, self-limiting diarrhoea and nausea. Intestinal *Sarcocystis* in humans varies from 1.1% to 10.4% in Europe, 0.4% to 23.2% in Asia, 0.5% in Australia, and 0% in Argentina (Poulsen & Stensvold, 2014). The prevalence of *Sarcocystis* spp. in adult bovine muscle is close to 100% in most regions of the world (Vangeel et al., 2007). The overall prevalence of *Sarcocystis* in pigs is 3 to 36% worldwide. Prevalence of *Sarcocystis* in pigs in central Europe is approximately 35% for sows and approximately 10% for fattening pigs (Saleque, Juyal, & Bhatia, 1990).

2.3. Occurrence of parasites indirectly transmitted through consumption of contaminated dairy products.

Cryptosporidium spp. are protozoan causative agent of diarrhoea in humans and worldwide, of which thirteen species have been recognized infectious to humans and animals. The diarrhoea tends to be self-limiting, but the lack of effective treatment means that it can have a severe impact on small

children, the malnourished and the immunosuppressed. Sporadic cases and small outbreaks of cryptosporidiosis associated with the consumption of unpasteurized milk and milk products have been reported (Ryan, Hijawi and Xiao, 2018; Putignani & Menichella, 2010). Unpasteurized milk can also act as a source for *Toxoplasma* (Dubey et al., 2014).

3. Key aspects of preventive measures

Parasites transmitted by FoAO span a large group of organisms, with a wide range of different transmission stages. Developing universally applicable measures to prevent infection with these parasites is therefore challenging. The key steps in preventive measures in primary production of FoAO are environmental hygiene, hygienic production, personnel hygiene, facility cleaning and maintenance, and monitoring/surveillance (FAO/WHO, 2016). Other FAO documents describe specific recommendations to prevent animal infection by pathogenic organisms (FAO/WHO, 2004, 2005), e.g., enclosed or controlled housing systems, protection of feed from pests, and ensuring safe drinking water. The OIE Terrestrial Animal Health Code describes effective measures for monitoring and surveillance (OIE, 2012).

Several important parasites transmitted by meat form infectious tissue stages in these animals, for which a main intervention is to prevent production animals from being infected. This has been particularly effective in animals that can be kept strictly indoors, e.g., pigs and poultry, whereas for grazing animals, such as sheep, it may difficult or impossible to avoid exposure. *Trichinella* is now generally absent in meat from pigs kept indoors in many European countries (Pozio, 2014). However, consumption of meat from wild game (e.g., wild boar) and meat from other domestic animals such as horses (Pozio et al., 2001) that can act as hosts for *Trichinella* spp., still poses a risk for human infection. Recent trends in consumer preferences, favouring organic farming and improved animal welfare, have led to changes in pig farming, with an increase in pigs housed outdoors (Park, Min, & Oh, 2017). This may result in increased *Trichinella* exposure of these pigs, and increased human *Trichinella* infection due to pig meat consumption. As sheep are mainly kept outdoors, and restricting

276 the access of cats (definitive host of *T. gondii*) to sheep farms can be difficult or impossible, *T. gondii*
277 is a continuous challenge in sheep production and for food safety.

278 Tissue parasites are also potentially problematic in the aquaculture industry, including farmed and
279 wild caught fish. Anisakidae are mainly a hazard in wild-caught fish. It has been argued that *Anisakis*
280 infection is not a problem in farmed fish production, as these fish do not have access to the parasite's
281 intermediate hosts (crustaceans and smaller fish). Closed breeding facilities for farmed fish have so
282 far not been implemented at a large scale, but may become a future industry standard.

283 **4. Evaluation of inactivation**

284 Bacteria and viruses may be present in vast numbers in or on food, and inactivation after treatment
285 of these organisms is expressed as log reduction. Consequently, reductions by 5 or 6 logs following
286 treatment are regarded as providing a sufficient level of protection. Unlike bacteria and viruses, the
287 infective unit for parasites varies from one to four to tens or hundreds of individuals: the infective
288 unit may be one individual (e.g. amoeba), one egg, or one larval stage (helminths), or four to eight
289 individuals (mature oocysts of coccidians). For parasites that form tissue cysts, one infective unit (the
290 tissue cyst) may contain a few to 1000 individuals per tissue cysts (e.g., *Toxoplasma*). Moreover,
291 parasite transmission stages are often shed in a non-infective form, after which infective stages
292 develop within the egg (helminths, one individual) or within oocysts (coccidian protozoa, four or
293 eight individuals, depending on the genus) under favourable conditions in the environment. Because
294 of this variation in units of infection, a measure for inactivation such as log reduction is not a uniform
295 measure for inactivation of parasites, as it is for bacteria and viruses. The number of parasites on or
296 in foods does not increase during storage, in contrast to bacterial contaminations that may increase
297 to very high numbers. As a result, a two or three log reduction may be considered as marginal for
298 bacteria, but may be highly relevant for parasitic contamination. This may affect the way the food
299 industry evaluates conventional decimal reduction times (D and Z-values) for measuring inactivation
300 performance of parasites.

Transmission stages of most foodborne parasites require an animal host and are not suitable for laboratory cultivation. The gold standard to evaluate parasite (stage) inactivation is method-induced elimination of infectivity in bioassays. Surrogate indicators have been used such as loss of a parasite's ability to proceed to a next developmental phase (e.g. oocyst sporulation), evaluation of motility as determined by microscopy or molecular methods to determine genetic activity.

5. Parasite survival during storage

Parasites do not multiply in or on food during storage and very few studies have investigated survival of parasites in FoAO under storage conditions. The few that are available are described below.

The viability of metacercariae of Heterophyidae in the flesh of mullet was assessed during storage at 9°C (Hamed & Elias, 1970). Under these conditions metacercariae were reported to remain viable for 9 days. Assessment of viability was by microscopy, but as the analytical procedure was not explained in detail, it is difficult to interpret the results.

Cysticerci of the zoonotic cestode, *Taenia solium*, which is transmitted to humans by meat from pigs infected with tissue cysts, have been shown to be viable for up to 30 days when stored at 4 °C (Fan, Ma, Kuo, & Chung, 1998). However, only four samples were analysed at this time point. As the only definitive hosts for *T. solium* are humans, studies such as this rely on visual inspection of scolex evagination and active movement for viability assessment, not infectivity.

Toxoplasma gondii may also survive for prolonged periods during storage. *T. gondii* genotype II tissue cysts in vacuum-packed meat from experimentally infected goats remained infective to mice after 6 weeks of storage at 4 °C (Neumayerová et al., 2014). During the study, sub-samples of meat stored at 4 °C were inoculated into four mice at 7-day intervals for up to 6 weeks. Although not all mice tested positive by PCR at all time points, 4/4 mice tested positive after 6 weeks, and all mice were seropositive by ELISA throughout the study.

6. Conventional processing

6.1 Heat treatment

Heat treatment remains one of the most reliable methods to control parasites in FoAO (Gajadhar, 2015). Table 1 provides an overview of reported data on the efficacy of different heat treatments to inactivate parasites in a variety of food matrices of animal origin.

For *Anisakis* inactivation, heating at ≥ 60 °C at the core of fishery products for at least 1 min to kill the larvae is sufficient (Bier, 1976; EFSA, 2010); consequently, fish fillets 3 cm thick should be heated for 10 min at 60 °C (Wootten, 2001).

For *Heterophyes* in fish, temperatures as high as 100 °C for more than 15 min are required to kill the metacercariae (Hamed & Elias, 1970), whereas isolated metacercariae of *Opisthorchis viverrini* are inactivated when kept at 70 °C for 30 min or at 80 °C for 5 min (Waikagul, J., 1974, cited in: Abdussalam, Käferstein, & Mott, 1995).

Several studies (Table 1) have highlighted the efficacy of microwave heating in killing some parasites in FoAO, like *Anisakis* in Arrowtooth flounder (Adams, Miller, Wekell, & Dong, 1999; Vidacek et al., 2011). Nevertheless, this inactivation method could have some limitations. Heating in standard domestic microwave ovens (2,450 MHz, 700 W) can result in hot and cold spots and the microwaves do not penetrate all areas of the food, depending on thickness (Vidacek et al., 2011). For example, microwave exposures of 1 s (43.2 °C), 2 s (54.0 °C), and 3 s (62.5 °C) partially, but not significantly, reduced the infectivity of *Cryptosporidum parvum* oocysts in oysters for neonatal mice (Collins, Flick, Smith, Fayer, Rubendall, et al., 2005). Moreover, treatments for 2 (54.0 °C) and 3 s (62.5 °C) showed extensive unacceptable changes in oyster meat texture and colour. In previous studies, cooking pork chops to 71 - 82 °C core temperature in the microwave oven (2.9 – 3.1 min) did not prevent *T. spiralis* infection of rats that had been fed larvae that were isolated from cooked pork chops (Kotula, Murrell, Acosta-Stein, Lamb & Douglass, 1983b). *Toxoplasma* cysts in mutton steaks processed in a microwave oven at 65 °C also remained infective (Lunden & Uggla, 1992). As well as the uneven temperature distribution in the food, the short heating time associated with microwave use could

also contribute to incomplete parasite inactivation (Kotula, Murrell, Acosta-Stein, Lamb, & Douglass, 1983b).

Inactivation temperatures for *T. spiralis* may vary from 60 °C for roasted pork (Carlin, Mott, Cash, & Zimmermann, 1969; Kotula et al., 1983a) to 66 °C for pork chops prepared in a conventional oven, convection oven, and flat grill, and 77 °C for char broiler or deep fat fryer (Kotula et al., 1983b). As well as the temperature itself, several studies highlight that heating time is equally important and should be chosen such that the desired temperatures are reached, maintained, and evenly distributed throughout the meat (Kotula et al., 1983a).

According to FDA (2012), *Taenia* cysticerci can be inactivated by cooking whole cuts of beef and pork to at least 62.8 °C (measured in the centre of the thickest part) and then allowing them to rest for at least 3 min (FDA, 2012). Nevertheless, both higher and lower temperature values can also be found in the literature: 60 °C for pork and beef (Murrell and Crompton, 2009) or more than 65 °C for pork (Rodriguez-Canul et al., 2002). Minced meat needs to be cooked to a higher core temperature of at least 71.1 °C, to inactivate not only *Taenia* cysticerci but also bacteria (Rodriguez-Canul et al., 2002).

In order to inactivate *T. gondii* in meat, the US Department of Agriculture recommends that whole cuts of pork, lamb, veal, or beef are cooked to an internal temperature of ≥ 65.6 °C, with a 3-minute rest (USDA, 2017). As stated above, it is important to define time/temperature combinations for heat treatment, since variations may alter the effectiveness of the treatment. As shown by Dubey et al (1990), *T. gondii* cysts were inactivated at 58 °C for 9.5 min, while some cysts were still infective after 64 °C for 3 min (Dubey, Kotula, Sharar, Andrews, & Lindsay, 1990). Also, USDA recommends that minced meat is heated to 71 °C (internal temperature) and poultry to 74 °C (USDA, 2017).

C. parvum oocysts in either water or milk lose infectivity when held at 71.7 °C for 5 sec or more (Harp et al., 1996). Thus, conditions used in commercial pasteurization (71.7°C for 15 s), are sufficient to destroy the infectivity of *C. parvum* oocysts in milk (Harp, Fayer, Pesch, & Jackson, 1996); milk borne cryptosporidiosis outbreaks have been exclusively associated with deliberately unpasteurized milk, or when there had been a failure in pasteurization.

Table 1: Effects of conventional processing on parasites in FoAO. Control measure: Heat treatment.

* n.s.: not stated.

Transmission stage	Condition	Method	Effect	Log reduction	Matrix	Ref
Anisakis						
Larvae	≥60 °C; 1 min 60 °C; 10 min, fillet 3 cm thick 70 °C; 7 min, fillet 3 cm thick	n.s.*	Kills *Anisakis*	n.s.	Fish	Wootten R, 2001
	60 °C; 700-W microwave heating	Larvae viability (with ultraviolet light)	Kills 69% *Anisakis* larvae, initial log population : 1.81	0.51	Fish: Arrowtooth flounder fillets	Adams et al., 1999
			Kills 89% *Anisakis* larvae; initial log population : 1.91	0.96		
	77 °C; 700-W microwave heating	Fluorescence under UV light; Microscopic motility examination under mechanical or glacial acetic acid stimulation	Kills 100% *Anisakis* larvae in food	1.78	Fish: whole fillets of Arrowtooth flounder	
	70 °C; 3 min (microwave heating at maximum 1,000 W)	Motility, emission of fluorescence under UV light, scanning electron microscopy	Kills *Anisakis* (L3)	1.08	Fish: infected hake muscle sandwiches	Vidacek et al., 2011
Heterophyidae						
Metacercariae	50 °C; >180 min 100 °C; >10 min	Microscopic detection metacercariae movement	Kills metacercariae	n.s.	Flesh of mullet	Hamed & Elias, 1970
Sarcocystis spp.						
Sarcocysts	40- 60 °C; 20-25 min (heart muscle)	Bioassay (dogs)	*Sarcocystis levinei* sarcocysts still infective	n.s.	Buffalo heart	Srivastava, Saha, & Sinha, 1986
	65 °C; 20-25 min (heart muscle)	Bioassay (dogs)	*S. levinei* sarcocysts non-infective			
	60 °C; 20min (thigh muscles).	Bioassay (dogs)	*Sarcocystis miescheriana* sarcocysts non-infective		Pork	Saleque et al., 1990
Taenia spp.						
Cysticerci	Cooking to 60 °C	n.s.	Controls *T.solium* and *T.saginata* in meat	n.s.	Pork and beef	Blackburn & McClure, 2009
	> 65 °C Cooking roast pork (cochinita pibil) or pork and beans (frijol con puerco)	In vitro evaluation of metacestode movement and scolex evagination	Damages *T. solium* metacestodes in both cases		Pork	Rodriguez-Canul et al., 2002
Trichinella spp.						
Muscle larvae	≥60 °C (internal temperature, oven cooked)	Larvae viability (after digestion) and bioassay	Inactivates *T. spiralis* larvae in meat	n.s.	Pork loin	Carlin et al., 1969
	49 °C; 6 h 52 °C; 47 min 55 °C; 6 min 60 °C; 2 min	Bioassay (rats)	Destruction of *T. spiralis* infectivity		Pork	Kotula et al., 1983a

	70 °C (core temperature	n.s.	Inactivates *Trichinella* in pork, game and horse meat		Pork, game meat, horse	Blackburn & McClure, 2009
	71.1 °C (core temperature)		Inactivates *Trichinella* in pork and any wild game that may be infected with trichinae		Pork, game meat	Doyle, 2003
	71 - 82 °C; 3 min internal temperature)	Bioassay (rats)	Did not inactivate *T. spiralis* larvae in pork chops		Pork	Kotula AW et al., 1983b
Cryptosporidium						
Oocysts	43.2 °C; 1sec 54 °C; 2 sec (microwave heating)	Bioassay (neonatal mice)	Not effective in reducing the infectivity of C. *parvum*	0.00	Shucked oysters	Collins, Flick, Smith, Fayer, Rubendall, et al., 2005
	62.9 °C for 3 sec; microwave heating		Partially reduces oocysts viability; initial log population : 4.58, log reduction : 0.15	0.15		
	71.7 °C; 5 sec		Inactivates oocysts heat treated in milk	5	Milk	Harp et al., 1996
	71.7 °C; 10 sec					
	71.7 °C; 15 sec (conditions of commercial pasteurization)					
Sarcocystis spp.						
Sarcocysts	65-75 °C; 20-25 min	Bioassay (dogs)	*Sarcocystis levinei* sarcocysts become non-infective to pups	n.s.	Buffalo heart	Srivastava et al., 1986
	70 °C; 15 min		*Sarcocystis miescheriana* sarcocysts become non-infective to pups		Pork (minute pieces)	Saleque et al., 1990
	100 °C; 5 min					
Toxoplasma gondii						
Tissue cysts	65 °C; Microwave oven	Bioassay (mouse)	Not always effective, partial inactivation of cysts	n.s.	Naturally infected sheep	Lunden & Uggla, 1992
	52 °C; 9.5 min (internal temperature) thickness of 2mm	Bioassay (mice)	Does not eliminate infectivity to mice	n.s.	Pork from infected pigs mixed with infected mouse brains and homogenized	Dubey et al., 1990
	58 °C; 9.5 min (internal temperature) thickness of 2mm		Eliminates infectivity to mice			
	> 61 °C; 3.6 min (internal temperature) thickness of 2mm		Eliminates infectivity to mice			
	64 °C; 3 min (internal temperature - thickness of 2mm)		Partial inactivation of Tissue cysts			

6.2. Freezing

Many papers describe varying combinations of time and sub-zero temperatures to inactivate/kill meatborne parasites (Table 2). Factors that influence efficacy of freezing may be determined by parasite-specific factors, and parasite developmental stage or age of larval stage may play a role (e.g., *Taenia saginata* cysticerci) (Hilwig, Cramer, & Forsyth, 1978). Parasites such as *Trichinella* vary

382 in freeze susceptibility between species (EFSA, 2004; Pozio et al., 2006), with some species,
383 particularly those from Arctic areas, being particularly freeze-resistant. Additionally, combinations of
384 parasite species and host species play a role for *Trichinella* spp. other than *T. spiralis* (Hill et al., 2009;
385 Lacour et al., 2013). Process-specific variables may also influence the efficacy of freezing (e.g.,
386 thickness of meat cuts, stacking of meat cuts (ICT, 2006), freezing of whole or half carcasses
387 (WHO/FAO/OIE, 2005), packed products in boxes or crates (ICT, 2006)).

388 Primary literature concerning freeze inactivation of *Diphyllobothrium* spp. is scarce; however,
389 isolated *Diphyllobothrium* spp. plerocercoids have been inactivated by freezing at -10 °C, which
390 prevented infection of golden hamsters (Salminen, 1970).

391 *Anisakis* spp. in fish have been inactivated at -15 °C for days to -40 °C for hours (Deardorff & Throm,
392 1988; McClelland, 2002). *Anisakis* spp. inactivation was evaluated by observing larval movement
393 after physical stimulation. However, moving larvae after freeze-treatment were considered
394 moribund. Subsequent sub-zero storage after freezing is therefore advised to inactivate anisakidae
395 larvae completely (Deardorff & Throm, 1988).

396 *Clonorchis sinensis* in fish and fishery products are considered to be inactivated at -10 to -20 °C for 5-
397 20 days (EFSA, 2010). However, *Clonorchis sinensis* metacercariae in fish, frozen at -12 °C for 10-18
398 days or at -20 °C for 5-7 days remained viable and infective in bioassays using rats and rabbits. Only
399 20 days of freezing at -12 °C or 3 days of freezing at -20 °C followed by thawing and another freeze
400 treatment for 4 days at -20 °C eliminated infectivity in rabbit and rat bioassays (Fan, 1998).

401 Freeze-treatment of fish fillet of mullet for 30h at -10 °C or -20 °C is not effective for inactivating
402 *Heterophyes* metacercariae (Hamed & Elias, 1970). At temperatures below -20 °C for two to 32 h, the
403 viability of *Opisthorchis* spp. in fish has been markedly, but not completely, reduced (Fattakhov,
404 1989).

405 *Taenia solium* in pork is inactivated by freeze treatment at -5 °C for 4 days, at -15 °C for 3 days, and at
406 -24 °C for 1 day, as shown by *in vitro* culture assay (Sotelo, Rosas, & Palencia, 1986), whereas

inactivation of *Taenia saginata* in beef requires freezing at -5 °C to -25 °C for 10-15 days (Hilwig et al., 1978).

Freezing to inactivate *Trichinella* spp. other than *T. spiralis* in pork, game, and horse meat, cannot be relied upon. *T. spiralis* and *T. britovi* in experimentally infected wild boars, 24 weeks post infection, were inactivated by freezing at -21 °C for one week as determined by mouse bioassay. Note that log reduction has been calculated from infectivity index data (number of *Trichinella* larvae recovered/number inoculated), as no parasite counts were available (Lacour et al., 2013). However, frozen wild boar meat from a naturally *T. britovi*-infected animal (3 larvae per gram), kept at -35 °C for one week, caused clinical trichinellosis in six people (Gari-Toussaint et al., 2005). Moreover, *Trichinella nativa*, associated with human trichinellosis after consumption of walrus meat or bear meat, was found to be infective by bioassay after naturally infected walrus or bear meat was stored frozen at -20 °C for up to 20 months (Leclair et al., 2004) or 4 months respectively (Hill et al., 2005). In contrast, *T. nativa* muscle larvae in experimentally infected pig meat were inactivated by freezing during 106 h at -17.7 °C (0 °F), as were larvae of *T. spiralis*, *T. britovi*, *T. murelli* and *T. pseudospiralis*, determined by bioassay (mice) (Hill et al, 2009). The International Commission on Trichinellosis (ICT) recommends freezing *T. spiralis* in pork at -21 °C for 7 days for inactivation, but freeze inactivation of *Trichinella* in bulk packages may need lower temperatures or longer exposure time (e.g., -29 °C for 6 days to -15 °C for 30 days), depending on meat thickness and stacking height in industrial freezers (ICT, 2006).

Toxoplasma gondii in pork, mutton, and other meat is completely inactivated by freezing at -7 to -13 °C for 2-4 days (Dubey, 1988; Kotula, 1991; Kuticic & Wikerhauser, 1996; Lunden & Uggla, 1992). After freezing at -2 °C for 24 h, *Sarcocystis levinei* tissue cysts in buffalo meat remained infective to dogs, but freezing of beef, buffalo and pork at -4 to -20 °C for 2-4 days renders *Sarcocystis* spp. inactive (Srivastava et al., 1986; Saleque et al., 1990).

Parasites such as *Cryptosporidium parvum* and *Cyclospora cayetanensis* may play a role as foodborne pathogens through faecal contamination of milk and other dairy products. Oocysts of these protozoan parasite species have been spiked into dairy products to evaluate their freeze inactivation, mimicking ice cream production. Freezing at -15 °C for 2 days inactivated oocysts of both *Cryptosporidium parvum* and *Cyclospora cayetanensis* in milk matrix (Deng & Cliver, 1999; Sathyanarayanan & Ortega, 2006), see Table 2. Freeze treatment of *Cyclospora* oocysts at -15 °C for 1 day was not effective at preventing oocyst sporulation (Sathyanarayanan & Ortega, 2006).

Table 2: Effects of conventional processing on food borne parasites. Control measure: Freezing.

Transmission stage	Condition	Method	Effect	Log reduction	Matrix	Ref
Anisakis						
Larvae	-35 °C; 15 h; followed by -18 °C; 24 h	Movement after stimulation with dissection needle	Kills *Anisakis* larvae, 6/3545 survived after 1 h of freezing; after 24 h no larvae survived	2.77	Fish: Sockeye salmon and canary rockfish	Deardorff & Throm, 1988
Clonorchis sinensis						
Metacercariae	-12 °C; 18 days	Bioassay (rats)	Metacercariae only marginally inactivated	0.00	Fish	Fan, 1998
	-12 °C; 10 days	Bioassay (rats)	Metacercariae survival	0.00		
	-20 °C, 7 days	Bioassay (rats)	100% of rats infected by metacercariae	0.00	Fish	
	-12 °C, 20 days	Bioassay (rabbits)	Eliminates infectivity for rabbits; 160 metacercariae inoculated, no flukes recovered	2.20		
	-20 °C for 3 days, thawing, and refreezing for 4 days	Bioassay (rats)	Eliminates infectivity for rats; 400 metacercariae inoculated, no flukes recovered	2.60		
Diphyllobothrium spp.						
Plerocercoid larvae	-10; 5 min	Bioassay (golden hamster)	Inactivates plerocercoids	n.s.	Fish fillets (isolated plerocercoids)	Salminen, 1970
Heterophyidae						
Metacercariae	-10 °C or -20 °C; 30 h	Motility	Inefficient, metacercariae can survive	0.00	flesh of mullet	Hamed & Elias, 1970
Opistorchis spp.						
Metacercariae	-28 °C; 20 h -35 °C; 8 h -40 °C; 2 h	n.s.	Viability markedly reduced, but not completely inhibited	n.s.	Fish	Fattakhov, 1989
Sarcocystis fusiformis						
Sarcocysts	-20 °C; 3 days	Bioassay (cats)	Complete loss of infectivity	n.s.	Beef	Gestrich & Heydorn, 1974
Sarcocystis spp.						
Sarcocysts	-2 °C; 24 h	Bioassay (dogs)	Meat containing sarcocysts still infective	0.00	Buffalo heart	Srivastava et al., 1986
	-4 °C; 48 h		Inactivates *S. levinei* sarcocysts	n.s.		
	-4 °C; 2 days		Inactivates *S. miescheriana* sarcocysts	3.1	Pork	Saleque et al., 1990
	-20 °C; 1 day			3.1		
Taenia saginata						
Cysticerci	-5 °C; 360 h -10 °C; 216 h ≤ -15 °C; 144 h	*In vitro* viability assay	Inactivates *T.saginata* cysticerci	n.s.	Beef carcasses	Hilwig et al., 1978
Taenia solium						
Tissue cysts	0 °C or above	*In vitro* culture assay	Does not affect parasite survival in culture	0.00	Pork	Sotelo et al., 1986)

	-15 °C; 3 days		Inactivates cysts	2.24		
	-24 °C; 1 day			2.25		
	-5 °C; 4 days			2.50		
Trichinella spp.						
Muscle larvae	-21 °C; 7 days	Bioassay (mice)	Inactivates *T. britovi* muscle larvae	1.50	Wild boar meat	Lacour et al., 2013
	-20 °C; up to 20 months	Bioassay (guinea pigs)	Does not inactivate *T. nativa* muscle larvae	n.s.	Walrus meat	Leclair et al., 2004
	-20 °C; 4 months	Bioassay (pigs)	Does not inactivate *T. nativa* muscle larvae	n.s.	Bear meat	Hill et al., 2005
Muscle larvae	-21 °C; 7 days	Bioassay (mice)	Inactivates *T. spiralis* muscle larvae	2.19	Wild boar meat	Lacour et al., 2013
Toxoplasma gondii						
Tissue cysts	-12 °C; 3 days	Bioassay (cats)	Inactivates tissue cysts	n.s.	Meat: Experimentally infected pigs	Dubey, 1988
	-7 °C to -12 °C; 4 days	Bioassays (cats and/or mice)			Pork	Kuticic & Wikerhauser, 1996
	-20 °C for 54h and thawed overnight at 4 °C	Bioassay (mice)			Mutton	Lunden & Uggla, 1992
	-8 °C; 3 days -7 °C; 17 days,	Bioassay (mice)			Pork	Kotula , 1991
Cryptosporidium parvum						
Oocysts	Ice cream mixing, freezing and hardening at -20 °C for 24 h	Exclusion of fluorochrome propidium iodide	Inactivation of oocysts	3.90	Dairy products: ice cream matrix	Deng & Cliver, 1999
Cyclospora cayetanensis						
Oocysts	-15 °C; 24 h	Oocyst sporulation	No inactivation of oocyst sporulation	0.00	Dairy substrates	Ortega & Sanchez, 2010
	-15 °C; 2 days		Inactivates oocysts	2.00	Dairy products: diluted milk substrate	Sathyanarayanan & Ortega, 2006
					Dairy products: milk matrix	
					Dairy products: whipped cream matrix	

438

439 *6.3. Curing and combined processes*

440 Some traditionally applied food processing techniques, such as marination, fermentation, smoking
441 etc., have parasite-inactivating potential, often as a result of a combination of several mechanisms,
442 possibly acting synergistically. Table 3 gives an overview of the effects of combined processes for
443 inactivation of *Anisakis* larvae in fish and *Trichinella* larvae in meat products.

444 Both drying and addition of salt reduce the amount of available water and increase osmotic pressure,
445 which is detrimental for all living cells. Marination can be defined as treatment of meat or fish with
446 brines containing salt, organic acids, and, occasionally, essential oils. Fermentation is an enzyme-
447 driven breakdown of the main constituents of flesh, most notably the degradation of carbohydrates
448 to lactic acid. The resultant acidification and consumption of oxygen have major immediate effects
449 (Ockerman, 2017). In dry- and semidry fermented meats, drying with weight losses up to 30 % are
450 achieved by drying procedures subsequent to the fermentation process. The application of smoke
451 causes the deposition of carbonyls, phenanthrene, and other compounds on the food surface.

452 Smoking at room temperature is unlikely to exert antiparasitic effects, whereas in hot-smoking,
453 elevated temperatures may inactivate parasites.

454 Marination of fish is a traditional processing method with some effect on nematode larvae. As
455 regards composition of brine, ranges in NaCl and acetic acid of 5 – 20%, and 2.6 – 40%, respectively,
456 have been studied (Table 3). With increasing salt concentrations, time to inactivation decreases
457 (AESAN, 2007; CEVPM, 2005; Karl, 1998; Karl, Roepstorff, Huss, & Bloemsma, 1994), but is still in the
458 range of more than one week. In herring, a NaCl content of 20% NaCl in the fish tissue water phase
459 resulted in a 1 log reduction of *Anisakis* larvae motility within 14 days, and a >2 log reduction in 28
460 days (Karl & Leinemann, 1989). In contrast, when the fish tissue water phase contained 15% NaCl,
461 the reduction was less than 1 log after 21 days.

462 Even for dry-salted herring, 20 days of storage is recommended in order to ensure inactivation of
463 *Anisakis* larvae (CEVPM 2005). Marination in vinegar (6% acetic acid) for 4-24 h is considered
464 insufficient to inactivate larvae (AESAN, 2007), and recommended procedures comprise marinating
465 for 31 days in brine with 2.5% NaCl and 6% acetic acid or 6% NaCl and 12% acetic acid for 13 days.

466 Essential oils have proven antibacterial properties, and there is evidence that such substances can
467 have some inactivation properties for parasites also. Since these substances are lipophilic, their
468 addition to aqueous marinades is less promising than to vegetable oil. Giarratana et al. (2014) were
469 able to inactivate third stage larvae of *Anisakis* in 5 and 10% solutions of essential oils of *Thyme*
470 *vulgaris* (containing mostly thymol, linalool, and pinens) in sunflower seed oil for 14 and 7 h,
471 respectively (Giarratana, Muscolino, Beninati, Giuffrida, & Panebianco, 2014). Inactivation was
472 assessed by motiliy and electron microscopy observations of structural damages of the cutis. Even
473 when this anti-*Anisakis* effect might be delayed in a fish flesh matrix, there should be ample time
474 during the time periods of food distribution and display in the shelves before it reaches the
475 consumer. Control relies not only on process parameters, but on rejection of infested carcasses,
476 routine removal of predilection sites and/or use of deep frozen fish for processing. As *Anisakis*

antigens with allergenic potential may persist despite deep-freezing, margination, and simulated gastric digestion (Solas et al., 2009), removal of infested carcasses or parts thereof may be the safer way to control this parasite in fish.

Salting is effective at inactivating intermediate stages of trematodes in fish. Inactivation of *Clonorchis sinensis* metacercariae in heavily-salted freshwater fish (3 g NaCl / 10g fish) at 6 °C took at least 8 days (Fan, 1998). Inactivation of *Opisthorchis* metacercariae in fish flesh salted with 13.6% NaCl was observed after 24 h (Kruatrachue, Chitramvong, Upatham, Vichasri, & Viyanant, 1982), whereas 20% NaCl for 5 h was less effective (Tesana, 1986). In fermented fish, inactivation was influenced by the duration of both cold storage of the fish and the fermentation time (Onsurathum et al., 2016).

Trichinella in pork and *Anisakis* in fish are the most relevant meatborne and fishborne nematodes, respectively. For *Trichinella*, most studies refer to *T. spiralis*, although other species might occur in meat. Zimmermann (1971) studied salt content, drying time and temperature and concluded that 28 days curing with 40 g NaCl/kg, plus re-salting at day 14, followed by 7 days drying at 37 °C or above would render *Trichinella* larvae non-infectious (bioassay in mice) (Zimmermann, 1971). The procedure was not safe when drying was performed at room temperature. Drying temperatures of 37 °C are however, not common in European dry ham production. In a German study, pork with 400-700 larvae/g was cured by injection or immersion and stored at 10 °C (Lötzsch & Leistner, 1979); depending on the type of ham, no infectivity was demonstrated in mouse bioassay at day 10 of storage (a_W 0,904; pH 5.6) or 29 (a_W 0.921; pH 5.6). Lötzsch and Leistner (1979) reviewed previous studies on *Trichinella* inactivation and reported that in fermented sausages and dry-cured ham, the larvae would be inactivated within 7-28 days (corresponding to a water: NaCl ratio of 4.7-19.8) and 90 days (corresponding to a water: NaCl ratio of 15.0 – 20.0), respectively (Lötzsch & Leistner, 1979). In their own experiments, the survival of *T. spiralis* in fermented sausages made with 2.8% nitrite curing salt and 0.5% sugar added was assessed, and also in dry-cured as well as brine-injected and dried hams. Although the number of infectious larvae declined markedly within the first four days,

502 the water and/or NaCl content were less reliable indicators for product safety than water activity.
503 The time to loss of infectivity ranged from 6 – 14 days in various types of fermented sausages, with
504 NaCl content from 3.2 – 3.8%. Since inactivation was observed at a_W of 0.93 – 0.95 for fermented
505 sausages and 0.90 – 0.92 for dried hams, it was suggested that a_W of 0.90 and 0.87 could be used as
506 threshold levels for fermented sausage and dried hams, respectively. Thus, a hypothesis generated
507 previously, that loss of infectivity in fermented sausages occurs when a_W values around 0.93 – 0.94
508 are reached during ripening (Hill et al., 2016; R. Lötzsch and Rödel, 1974), was supported. However,
509 there are also raw sausages with no fermentation or only short-term fermentation, such as
510 “Teewurst” or “Mettwurst” types. In Teewurst (2.8% nitrite-curing-salt) sausages containing 950
511 larvae/g, 21 days of ripening were required for loss of infectivity (bioassay in mice), corresponding to
512 water activity of ca. 0.949 and pH of 5.3 (Lötzsch and Rödel, 1974), whereas in the same product with
513 200 larvae/g, 14 days of ripening (a_w ca. 0.944; pH 5.3) were sufficient. Nöckler and Kolb (2000)
514 studied starter culture spiked with around 200 larvae per gram sausage batter manufactured with
515 lower content of nitrite-curing-salt (2%). The number of viable larvae decreased markedly between
516 the 4th and 7th day after manufacture. Loss of motility of digested larvae and of infectivity in mice
517 were observed from the 9th day onwards. Although these studies indicate that Teewurst sausages
518 would be a safe product after 9 – 14 days of storage with respect to *Trichinella*, such products are
519 usually placed on the market and consumed before this period. Some outcomes of studies on
520 combined processes are given in Table 3. In sum, a water activity of 0.92 is reported as the limit for
521 survival of *Trichinella* larvae (species not specified), which corresponds to dry rather than semi-dry to
522 fresh fermented sausages (Ockerman, 2017). Control of this parasite for fermented meats can also be
523 achieved by the use of deep-frozen meat for production. However, in many countries there is focus
524 on processing of *Trichinella*-free pork into fermented or dried meats, with pig production systems of
525 adequate biosecurity level ensuring a lack of *Trichinella* in the pork.

526 Rodriguez-Canul et al. (2002) reported inactivation of *Taenia solium* cysts in pork salted with 70-105
527 g /kg and left overnight at ca. 30 °C (Rodriguez-Canul et al., 2002). The authors observed structural

528 changes in the cyst and inability of the scolex to evaginate. They attributed this inactivation to
529 changes in osmotic pressure rather than to the pH decline from about 6.0 to 5.3. For cysts of *T.*
530 *saginata* in beef, a water activity of 0.98 is regarded as the limit for survival (Ockerman, 2017).

531 Protozoan parasite stages in meat and fish flesh are sensitive to salt concentration. *Toxoplasma*
532 tissue cysts in muscle of mice were inactivated within one day at 2.5% NaCl (Pott et al., 2013). Nitrite-
533 curing salt (99.5 % NaCl with 0.5% $NaNO_2$) proved more effective than NaCl alone. In contrast,
534 *Toxoplasma* tissue cysts have a high pH tolerance: at lower pH (pH 5 and 6 compared to pH 7)
535 however, infectivity was not reduced with exposure for 24 to 26 days at 4 °C. This finding was
536 regarded as relevant, not only for fresh meats, but also for fermented meats where the pH can be in
537 the order of 5.0. In cured-dried and cured-cooked meats, the pH is typically at 6 or above, but the
538 infectivity of tissue cysts in loin has been demonstrated to decrease rapidly with exposure to 2%
539 NaCl. *Toxoplasma* tissue cysts in pork loin that was injected with brine to give 2% NaCl or 1.4%
540 sodium- or potassium lactate in the loin (injection volume 10% of loin weight) followed by storage for
541 7 days at 4 °C, were not infectious when the pork was fed to cats (Hill, Sreekumar, Gamble, & Dubey,
542 2004). Moreover, it was shown that inactivation of cysts (assessed via bioassay) in pork loins with
543 addition of 2% sodium chloride or 1.4% potassium or sodium lactate occurs at 4 °C within the first 8 h
544 after treatment (D. E. Hill et al., 2006). In contrast, infectivity of positive controls (infected, but
545 injected with 0.85% NaCl only) was demonstrated at least partially, even after 45 days of storage.
546 Sodium triphosphate and sodium diacetate, both common compounds in meat enhancers, had no
547 effect. A study on processing of mutton (Lunden & Uggla, 1992) indicated that in meat cured for 64 h
548 at 4 °C with 30 – 50g sodium chloride and 25 – 40g sucrose for 200 – 360 g of meat, cysts lost
549 infectivity. Also, warm-smoking at above 50 °C for 24 – 48 h inactivated *Toxoplasma* tissue cysts in
550 brine-injected mutton (as assessed via bioassay in mice). The survival and infectivity of *Toxoplasma*
551 tissue cysts in ham from experimentally infected pigs after the standard curing process required for
552 Parma ham (storage for 12, 14 and 16 months and typical average NaCl contents from 4.2 – 6.2%)
553 was recently assessed (Genchi et al., 2017). Bioassay in mice and *in vitro* culture followed by PCR

were used to determine infectivity and viability. None of the mice became infected and the *in vitro* culture/PCR did not provide evidence that the *Toxoplasma* were viable after the curing process (Genchi et al, 2017). Water activity (a_w) below 0.95 and/or pH below 5.3, are recognized as being detrimental to *Toxoplasma* tissue cysts (Ockerman, 2017).

Table 3: Effects of combined processes on the infectivity of *Anisakis* and *Trichinella* larvae in meat and fish products. Control measure: Marination, Pickling, Smoking, Fermentation, Salting.

Transmission stage	Condition	Evaluation method	Effect	Log reduction	Matrix	Ref
Anisakis						
Larvae	Marination in 2.6% acetic acid and 5–6% salt for 12 weeks Marination in 2.6% acetic acid and 8–9% salt for 6 weeks	n.s.	Inactivates *Anisakis* larvae	n.s.	Fish (herring)	Doyle, 2003
	6% acetic acid (v/v) (vinegar); 12% salt for 13 days, 4 °C 10% acetic acid; 12% NaCl for 5 days 20% acetic acid; 12% NaCl for 3 days 30% acetic acid; 12% NaCl for 3 days 40% acetic acid; 12% NaCl for 2 days	Movement; determination of stress protein levels; bioassay (rat)	Inactivates *Anisakis* larvae	1.78	Fish: anchovies	Sanchez-Monsalvez et al., 2005
	Storage in brine with 6.3% salt and 3.7% acetic acid in the aqueous phase of the fish for 28 days	Motility	Inactivates *Anisakis* larvae	n.s.	Fish: herring	Karl et al., 1994
	5% NaCl; >17 weeks 6-7% NaCl; 10 - 12weeks	Motility	Inactivates *Anisakis* larvae	n.s.	Fish: Herring	Karl et al., 1994
	6% acetic acid, 10% NaCl for 24 h followed by the addition of sunflower seed oil and refrigeration at 4 °C for 13 days	Motility	Inactivates *Anisakis* larvae	n.s.	Fish: Sardines	Arcangeli, 1996
	Pickled herring; 28 days	Motility in 1% acetic acid and staining	Inactivates *Anisakis* larvae	≥2.60	Fish, anchovies	H Karl & Leinemann, 1989
Clonorchis sinensis						
metacercariae	3 g NaCl / 10 g fish flesh; 8 days	Bioassay (rat)	Inactivation of metacercariae	n.s.	Freshwater fish	Fan, 1998
Opistorchis viverrini						
metacercariae	7.5% NaCl, glutinous rice; keeping fish 3 days refrigerated plus 4 days fermentation time at room temperature	Bioassay (hamster)	metacercariae non-infectious	n.s.	Fermented fish (*pla-som*)	Onsurathum et al., 2016
Taenia solium						
metacestodes	70-105g NaCl/ kg meat at 30 °C	microscopy	Inactivation overnight	n.s.	pork	Ockerman, 2017
Trichinella spp.						
Larvae	2.8% nitrite-curing salt; initial larva count 1090/g	Examination of digested larvae and bioassay (mice)	Larvae lose motility between days 7-10; no larvae recovered from mice fed with salami ripened for 10 or more days (a_w ca. 0.942; pH 5.4)	n.s.	Raw pork sausage	Lötzsch & Rödel, 1974
	2.8% nitrite-curing salt; initial larva count 530 /g		Larvae lose motility between days 4-7; no larvae recovered from mice fed with cervelat ripened for 10 or more days (a_w ca. 0.932; pH 5.4)			
	2.8% nitrite-curing salt; initial larva count 200/g		Kills *Trichinella* larvae in 55-75 mm diameter salami ripened for 6 days (a_w ca. 0.931/ 0.944; pH 5.7/5.4			
	2.8% nitrite-curing salt; initial larva count 200/g		Kills *Trichinella* larvae in 55 - 75 mm diameter cervelat ripened for 7 -9 days (a_w ca. 0.948; pH 5.4/5.2)			
	2.8% nitrite-curing salt; initial larva count 800/g		Kills *Trichinella* larvae in 75 mm diameter Mailänder Salami ripened for 11 days (a_w ca. 0.939; pH 5.1)			
	storage at 10 °C; initial larva count 400-700/g		Kills *Trichinella* larvae in dry-cured ham in 21 (aW 0,948; pH 5.5) to 57 (a_w 0.922; pH 5.6) days, according to ham type			

Toxoplasma gondii						
Tissue cysts	2% NaCl	Bioassay (mice)	Viable at day 8	n.s.	Muscle from mice in tissue culture medium	Lötzsch & Rödel, 1974
	2.5 and 3% NaCl		Inactivation within one day			
	25 nitrite-curing salt (99.5% NaCl, 0.5% $NaNO_2$)		Inactivation within 4 days			
	2% NaCl or 1.4% sodium- or potassium lactate in the loin; 7 days	Bioassay (cats)	Inactivation of tissue cysts		Pork loin	Hill et al., 204
	30-50 g NaCl; 64 h	Bioassay (mice)	Inactivation of tissue cysts		Mutton meat	Lunden & Uggla, 1992
	4.2-6.2% NaCl; 12, 14 and 16 months		Inactivation of tissue cysts		Parma ham	Genchi et al., 2017

7. Advanced methodologies

7.1. High pressure processing (HPP)

High pressure processing (HPP) is a non-thermal processing technique that has been successfully implemented in the food industry to treat food without being heated or deformed. Food products that are HPP treated are usually vacuum-packaged and placed in a pressurized vessel. Water is used as compression medium during treatment and pressure is kept constant for a set amount of time. Typically, a pressure range from 200 to 800 MPa is used. Time, temperature, decompression time and liquid temperature vary, depending on product and food composition. During HPP, pressure is transmitted uniformly and instantly with little variation in temperature, independent of food shape or size (Rendueles et al., 2011). In general, temperature increases approximately 3 °C per 100 MPa pressure increase, depending on food composition. HPP may be used as an alternative inactivation treatment for foods that are preferably consumed raw, like oysters, for which temperature treatment is not applicable or desirable.

Table 4 provides an overview of the efficacy of high HPP on parasites in fish, meat, and oysters. *Anisakis* larvae in Nile perch filets are killed at a pressure of 200 MPa for 10 min at a temperature between 0 and 15 °C using motility as an indicator of larval death. To inactivate all *Anisakis* larvae, pressures can be lowered to 140 MPa, but simultaneously, treatment time has to increase to one

hour. However, most larvae treated for more than 10 min at pressures over 120 MPa were dead, using autofluorescence as indicator of larval death (Molina-Garcia & Sanz, 2002). Anisakis larvae in mackerel filets were completely inactivated at 300 Mpa for 5 min (Brutti, Rovere, Cavallero, et al., 2010)

Cryptosporidium parvum oocysts have been HPP treated at pressures of 305 – 550 MPa for ≥180 sec, which reduced numbers of infected mice significantly, but could not prevent infection of mouse pups in a bioassay (Collins, Flick, Smith, et al., 2005).

T. spiralis isolated from infected pork were significantly inactivated using hydrodynamic pressure (Hydrodyne process, method for tenderising meat or fish using explosion induced shock waves in water), although the pressure generated (55 to 60 MPa) did not eliminate the infectivity to mice as determined by bioassay (Gamble, Solomon, & Long, 1998b). *Toxoplasma gondii* tissue cysts in ground pork were successfully inactivated using 300 – 400 MPa for 30 sec, whereas 100 and 200 MPa were ineffective (Lindsay, Collins, Holliman, et al. 2006).

Table 4: Advanced methodologies. Control measure: High Pressure Processing.

Transmission stage	Condition	Evaluation method	Effect	Log reduction	Matrix	Ref
Anisakis						
Larvae	140 MPa; 1 h	Motility tests, methylene blue fluorescence	Inactivates *Anisakis* larvae	Low numbers used; log reductions cannot be calculated	Fish: Nile perch fillet	Molina-Garcia & Sanz, 2002
	150 MPa; 30 min					
	200 MPa; 10 min					
	170 MPa; 3 x 2 min					
	180 MPa; 2 x 2 min					
	190 MPa; 15 min					
	100 MPa; 5 min	Motility test	8% larval inactivation	Low numbers used; log reductions cannot be calculated	Fish: Mackerel filet	Brutti et al., 2010
	200 MPa; 5 min		97% larval inactivation			
	300 MPa; 5 min		100% larval inactivation			
Cryptosporidium						
Oocysts	4.0x10^8Pa; 180 sec HHP	Bioassay (mice)	Reduction of infected mice by 40%	Low numbers used; log reductions cannot be calculated	Shellfish: Oysters	Collins et al., 2005
	3.7x10^8Pa; 180 sec HHP		57%			
	4.8x10^8Pa; 180 sec HHP		57%			
	3.05x10^8Pa; 180 sec HHP		48%			
	5.5x10^8Pa; ≥180 sec HHP		≥65%			
Trichinella spp.						
Larvae	55 to 60 MPa	Bioassay (mice)	Does not inactivate *Trichinella spiralis*	n.s.	Pork	Gamble et al., 1998b
Toxoplasma gondii						
Tissue cysts	300 MPa; 30 sec	Bioassay	Inactivates tissue cysts	n.s.	Meat: Ground pork	Lindsay et al., 2006
	400 MPa; 30 sec					

590 *7.2. Electron beam irradiation*

591 Electron beam (E-Beam) is a process used for microbial inactivation that utilizes high-energy
592 electrons, produced by electric energy in electron accelerators. Electrons produced are accelerated
593 to close to the speed of light, and the resulting high energies (up to 12 million electron volts) are
594 capable of uniformly penetrating food materials. Foodstuffs are typically placed on pallets for large
595 throughput and the dose received is controlled by manipulating the beam current, the beam
596 scanning length along with the under-beam conveyor speed (McFadden et al., 2017; Murray et al.,
597 2015).

598 Collins et al (2005, Table 5) examined the efficacy of E-Beam irradiation on the viability of *C. parvum*
599 oocysts in Eastern Oysters (*Crassostrea virginica*), artificially contaminated with the Beltsville strain
600 of *C. parvum* (Collins, Flick, Smith, Fayer, Rubendall, et al., 2005). Contaminated oysters were treated
601 in a commercial e-beam facility and the effects of the treatments evaluated by feeding the processed
602 oyster tissues to neonatal mice. Infective dose was approximately 10^5 oocysts per gram tissue.
603 Significant reductions ($P<0.05$) in infectivity were observed for in-shell and shucked oysters treated
604 with e-beam irradiation at doses of 1.0, 1.5, or 2 kGy. A dose of 2 kGy completely eliminated *C.*
605 *parvum* infectivity and did not adversely affect the visual appearance of the oysters.

606 Collins and co-workers showed that e-beam electrons have a limited penetration depth of about 5
607 cm or less, much below that of X-rays that have significantly higher penetration depth (60–400 cm)
608 depending on the energy used (Collins, Flick, Smith, Fayer, Rubendall, et al., 2005). However, this
609 limited penetration was appropriate for the size of oysters used in the study and is suitable for
610 treating similarly sized oysters. Thus, irradiation doses equal to or more than 2.0 kGy may be used in
611 a commercial process to eliminate *C. parvum* in fresh oysters, shucked or in shell (Table 5). However,
612 researchers reporting on the use of E-Beam for effective bacterial sterilization of food products have
613 observed changes in meat tenderization, colour, and flavour at 2 kGy (Yim et al., 2015). These effects
614 were pronounced with aging and when combined with elevated storage temperature. How E-Beam

615 destroys parasites at the cell and molecular level is currently unresolved, but studies focusing on
616 bacterial inactivation demonstrated that DNA is the principal cellular target that affects viability after
617 exposure to E-Beam treatments (Shehata, Gomaa, & Helal, 2011).

618 *7.3. Gamma irradiation*

619 The inactivation effect of gamma irradiation is quite diverse, as reflected in the huge variation of the
620 observed minimum effective dose (MED) and directly related to the type of parasite, the parasite
621 stage and food product assayed (Table 5). While *Trichinella* radio sensitivity is high and MED of 0.3
622 KGy for *Trichinella* spp. can result in pork products being free of viable larvae, the MED observed for
623 several fishborne or other aquatic foodborne parasites varied from 0.1 KGy for *Clonorchis* sp. larvae
624 to 10 kGy for *Anisakis simplex* larvae in fish.

625 The radio resistance of *A. simplex* is high; doses as high as 1 kGy do not reduce the infectivity of third
626 stage larvae, and even higher doses (2-10 kGy) only produce a reduction in penetration ability and
627 infectivity in rats, but not in rabbits (Chai, Hong, & Lee, 1991). When salted fish products were
628 assayed, similar results were observed (Van Mameren & Houwing, 1968); doses as high as 6 kGy
629 were not totally effective for larvae in salted herring. Although the number of larvae was reduced,
630 substantial numbers of nematodes survived.

631 The radio resistance of trematodes varies depending on the parasite species and whether the
632 treatment is applied to meat or another matrix. The radio resistance of *Clonorchis sinensis* varied
633 significantly depending on the mode of the treatment. Irradiation of *C. sinensis* metacercariae at 0.1
634 KGy lead to 99% inactivation of parasites (Lee, Park, Sohn, Hong, & Chai, 1989). The metacercariae of
635 *C. sinensis* were three-fold less susceptible to gamma irradiation when encysted in the flesh of fish in
636 comparison to when they were isolated from the fish; i.e. the MED for metacercariae in fish was 0.15
637 and 0.05 kGy, respectively, whereas this was 0.02 KGy when metacercariae were isolated from the
638 fish (Chai, Hong, & Lee, 1991, Park & Yong, 2003). A similar situation was observed for *Paragonimus*
639 *westermani*; the MED in metacercariae in crab was 25 times higher than that for metacercariae

isolated from the crustacean (2.5 kGy vs 0.1 kGy) (Song, Duan, Shou, & Zhu, 1993). Thus, it is the higher dose that is of practical application. However, identical MED (0.1 kGy) were observed when *Opisthorchis viverrini* metacercariae were submitted to gamma irradiation in fish or after isolation from fish (Sornami, IMPand, & Bundisting, 1993).

The radio sensitivity of *Trichinella* in meat depends on the species and origin (Kasprzak et al., 1993). The MED needed to inactivate parasites in heavily contaminated pork carcasses is 0.3 kGy (Brake et al., 1985; Kasprzak et al., 1993; Murrell & Dubey, 1993). The US FDA approved irradiation for the control of *T. spiralis* in pork under Regulation 21 CFR 179 in 1985, allowing treatments of 0.3 kGy as minimum and 1 kGy as maximum.

The MED varied significantly for *Taenia* cysticerci (*Cysticercus bovis*); while 3.7 kGy is required to inactivate *Taenia saginata* cysticerci in beef meat and 6 kGy for a complete inactivation (Alabay, Emre, Çerçi, Ersen, & Mutluer, 1993; Geerts, De Borchgrave, Brandt, & Kumar, 1993), doses of 0.2 to 0.6 KGy produce an irreversible effect on the viability and development of *Taenia solium* adult worms, affecting the viability of the cells in the neck region to divide and form new proglottids (Verster, Du Plessis, & Van Den Heever, 1976). Similar doses (0.5-0.7 KGy's) inhibit the infectivity of cysticerci, but higher doses (6.5 kGy) are needed for a complete inactivation of cysticerci (De Aluja, Nunez, & Villalobos, 1993).

Studies of inactivation of *Toxoplasma gondii* by gamma irradiation in meat demonstrated that intermediate irradiation doses (0.4-0.45 kGy) can significantly reduce the infectivity of bradyzoites and tissue cysts in pork products (Dubey, Brake, Murrell, & Fayer, 1986; Murrell & Dubey, 1993). Song et al (1993), and several independent studies have obtained similar MEDs for tissue cysts in pork products. However, some authors have observed differences in radio resistance between *T. gondii* strains, ranging MEDs from 0.4 to 0.7 kGy (Wikerhauser, Kuticic, Razem, Orsanic, & Besvir, 1993).

665

Table 5: Advanced methodologies. Control measure: E-beam and gamma irradiation.

Transmission stage	Condition	Evaluation method	Effect	Log reduction	Matrix	Ref
E-BEAM IRRADIATION						
Cryptosporidium parvum						
Oocysts	From 1 to 1.5 kGy	Bioassay (neonatal mice)	No significant reduction in oocyst viability	0.10 (1 KGy) to 0.25 (1.5 KGy)	In shell Oysters	Collins, Flick, Smith, Fayer, Rubendall, et al., 2005
	From 1 kGy to 2 KGy		Infectivity reduction from 47-57% (1 KGy) to 100% (2 kGy)	0.32 (1 KGy) to 1.78 (2 KGy)	Oysters (in shell and shucked)	
GAMMA IRRADIATION						
Anisakis simplex						
Larvae	3-6 kGy	Visual inspection	6 kGy : reduction on the number of larvae but still substantial numbers of nematodes survived	n.s.	Fish: salted herring	Van Mameren & Houwing, 1968
Clonorchis sinensis						
Metacercariae	0.01-0.20 kGy (^{137}Cs ; ^{60}Co)	Bioassay (albino rats, guinea pigs)	MED was 0.15 kGy; complete control of the infectivity. The LD_{50} was established at 0.05 kGy	n.s	Fish	Chai et al., 1991; Lee et al., 1989
Opisthorchis viverrini						
Metacercariae	0.05-0.1 kGy (^{60}Co)	Bioassay (hamsters, rabbits, cats)	MED: 0.1 kGy	n.s	Fish	Sornami et al., 1993
Paragonimus westermani						
Metacercariae	0.05-0.1 kGy (60Co)	Bioassay (albino mice)	MED : 2.5 kGy		Crab (*Potamon* spp.)	Song, Duan, et al., 1993
Trichinella spp.						
Larvae	0.1-0.8 kGy (^{60}Co, ^{137}CS)	Bioassay (rats)	MED: 0.5 kGy 0.15 to 0.3 kGy block production of larval progeny 0.3-0.6 kGy inactivates *Trichinella* larvae	n.s.	Pork	Kasprzak et al., 1993; Murrell & Dubey, 1991
Taenia spp.						
Cysticerci	1-6 kGy (^{137}Co)	Bioassay (gerbils)	MED: 3.7 KGy; total inactivation of *Taenia saginata*	n.s.	Meat	Alabay et al., 1993
	0.2-0.6 kGy (^{60}Co)	Bioassay (Human volunteers)	MED: 0.5 kGy		Cooked meat previously frozen	Geerts et al., 1993
	0.2-1.40 kGy	Bioassay (golden hamsters)	MED : 0.60 kGy *Taenia solium*		Pork meat	Verster et al., 1976
	0.5-11 kGy (^{60}Co)	Bioassay (golden hamsters)	MED: 6.5 kGy *Taenia solium* 0.5-0.7 kGy does not kill cysticerci but inhibits infection		Pork meat	De Aluja et al., 1993
Toxoplasma gondii						
Tissue cysts	0.1-1 kGy (^{60}Co)	Bioassay (NIH mice)	MED: 0.55 kGy Elimination of infection	n.s.	Pork products	Song, Yuan, et al., 1993
	0.1-0.5 kGy (^{137}Cs, ^{60}Co)	Bioassay (cat)	MED : 0.5 kGy At 0.25 kGy: elimination of infection in cats, at 0.4 kGy 10,000-fold reduction of infectivity in mice and cat; 0.5 kGy no detectable infective *Toxoplasma* in mice		Pork	Dubey et al., 1986
	0.4-0.7 kGy (^{60}Co)	Bioassay (cats, mice)	Complete inactivation depending on *T. gondii* isolate			Wikerhauser et al., 1993

666

667 ## 8. Future trends

668 Trends in the production and trade of meat are changing and becoming more extensive, in addition
669 to a growing global population, and increasing meat and dairy consumption per capita (Henchion,
670 McCarthy, Resconi, & Troy, 2014). Detailed data on the import and export of different food
671 commodities of animal origin and live animals are available on the FAOSTAT pages
672 (http://www.fao.org/faostat/en/#home), and data compiled by the meat industry indicate that in

terms of exports, India, Brazil, Australia and the United States accounted for over 60% of the world's beef exports in 2016.

Whether these changes in trade may present an increased threat to importing countries and consumers due to foodborne parasites is not clear. However, a review of the potential iMPact of globalization on spread of foodborne parasites (Robertson, Sprong, Ortega, van der Giessen, & Fayer, 2014), noted that the incidence of bovine cysticercosis increased from 4% to 38% when mass importation of live cattle to Israel began. This could obviously have knock on effects to human infection. Furthermore, multiple liver cysts of *E. granulosus* were detected in slaughtered cattle in The Netherlands in 2007; these cattle had been imported from Romania where *E. granulosus* is endemic. This parasite had been eradicated in The Netherlands in the 1950s, and a risk-based slaughterhouse strategy was introduced to maintain the free status of the Dutch livestock. The same review (Robertson et al., 2014) also comments on the enormous expansion in the aquaculture industry in recent decades - rising from around 30 million tons annually in the 1990s to over 76 million tons today. Transport of live fish and shellfish between countries has not only contributed to the spread of economically threatening diseases in the aquaculture industry, but may also result in the spread of zoonotic parasites. One example is the introduction of rainbow trout and brown trout to lakes in Argentina and Chile. These later became exposed to *Diphyllobothrium latum* eggs by contamination of the lake water with faeces from infected humans, resulting in diphyllobothriosis becoming endemic in some locations (Chai, Murrell, & Lymbery, 2005).

In addition to globalization being responsible for the movement of people, animals, parasites and food commodities, food traditions involving undercooking of fish and meat are also spreading and may result in the likelihood of transmission of foodborne parasites in FoAO. For example, the trend of eating meat from exotic wildlife in some countries, has resulted in bush meat (meat from wild animals hunted in Africa and Asia) being increasingly imported into exclusive restaurants in Europe and the USA. Such imports may not only represent a threat to the wildlife species, but also to naïve

698 populations being exposed to new or unexpected meatborne pathogens. Another growing food
699 trend is that of sushi, sashimi, ceviche, and carpaccio. All these dishes include the consumption of
700 raw FoAO, and although may not result in the establishment of a parasite in a particular location,
701 may be more likely to result in infection of the consumer. Although anisakiasis still tends to be mostly
702 associated with Japan, the comparative rates of infection in Europe and USA (Chai et al, 2005)
703 indicate that raw fish consumption is no longer particularly associated with that culture.

704 Against this backdrop of globalization trends being drivers for the spread of foodborne parasitic
705 diseases in foods of animal origin, improvements in our knowledge technologies and knowledge
706 regarding diagnostics, tracing, and inactivation methodologies provides a balance. Use of more
707 sensitive diagnostics, such as multiplex PCR, enables infections to be identified before they can
708 disseminate further; tracing systems, such as the trans-European network, TRAde Control and Expert
709 System (TRACES) (http://ec.europa.eu/food/animal/ diseases/traces/), enable worldwide traceability
710 of animal and animal product movement, and, along with other systems, provide, theoretically, the
711 opportunity to be able to determine the origins and histories of different animals and food derived
712 from them. Moreover, novel inactivation methodologies can be used to complement the traditional
713 methods of heating, freezing, pickling, salting etc., to ensure that even if FoAO contains parasitic
714 stages, they are not infectious and cannot be transmitted to the consumer. Research on foodborne
715 parasites should be improved towards standardization of experimental approaches for the evaluation
716 of inactivation methods and methods to monitor inactivation.

717 Ensuring that the balance tips towards sustainable agriculture and food supply, public and veterinary
718 health is one of the challenges to be met in the coming years of an increasingly urbanized and
719 growing population.

720 **9. Conclusions**

721 Based on our extensive literature review, information on the relevant effects of different removal
722 and inactivation techniques on parasites in FoAO has been assimilated. The efficacy of time-

temperature combinations for freezing and heating procedures is influenced by parasite species and developmental stage, but in general, heating to 60 – 75 °C for 15 – 30 min or freezing at -21 °C for 1 – 7 days inactivates parasites in meat or fish, as determined using bioassays. USDA recommends heating meat at a core temperature of 62.8 - 73.9 °C or freezing at -18 °C to inactivate parasites in meat or fish, but freezing cannot be relied upon for total inactivation in home situations. Industrial pasteurization of fluids (15 sec 71.7 °C) or fish and crabs (175 - 65 min 85 – 92.2 °C) is effective for control of parasites in milk and parasites in fish.

Meat- and fishborne parasitic stages are generally sensitive to NaCl contents of 2 – 5%, associated with higher osmotic stress and often augmented by lowering pH (fermentation or organic acids). "Safe" pH and water activity limits have been established for fermented and marinated products. Other inactivation technologies that are relevant include high pressure treatment and E-beam to inactivate parasites in animal origin matrices, but little information is available in the literature. The minimal effective dose for gamma radiation ranges >0.1 – 0.5 kGy for fish parasites except *Anisakis* (10 kGy) and >0.4 – 6.5 kGy for meatborne parasites. Literature data are currently not sufficient to model survival as response to treatment. Changes in culinary preferences, food trade, and spread of parasites may create new risky commodities.

With strong drivers for the spread of foodborne parasites through FoAO, the information provided here may be useful for informing the food industry. In addition, this information could be used for underpinning decision making regarding technologies and approaches for inactivating parasites in FoAO and thereby protecting consumers.

Moreover, research on foodborne parasites should be improved towards standardization of experimental approaches for the evaluation of inactivation methods.

Declaration of interests

The authors declare that they have no conflict of interests.

747

748 **Acknowledgements**

749 This work was performed in collaboration within the framework of the European Network for

750 Foodborne Parasites in Europe (EURO-FBP), COST Action FA 1408.

References

Abdussalam, M., Käferstein, F. K., & Mott, K. E. (1995). Food safety measures for the control of foodborne trematode infections. *Food Control*, *6*(2), 71-79. doi: 10.1016/0956-7135(95)98910-S

Adams, A. M., Miller, K. S., Wekell, M. M., & Dong, F. M. (1999). Survival of *Anisakis simplex* in microwave-processed arrowtooth flounder (*Atheresthes stomias*). *Journal of Food Protection*, *62*(4), 403-409.

AESAN. (2007). Sobre medidas para reducir el riesgo asociado a la presencia de *Anisakis*. *Informe del Comité Científico de la Agencia Española de Seguridad Alimentaria y Nutrición (AESAN)*.

Alabay, B. M., Emre, Z., Çerçi, H., Ersen, S., & Mutluer, B. (1993). Inhibition of viability and infectivity of *Cysticercus bovis* by irradiation of meat. *Proc. final research co- ordination meeting on Use of Irradiation to Control Infectivity of Food-borne Parasites*, *Mexico City*, *Vienna*(IAEA), 15–22.

Arcangeli, G., Bicchieri, Gamberini, Presicce,. (1996). Prove sperimentali sulla vitalità di larve del genere *Anisakis* in semiconserve ittiche. *Industria Conserve*, *71*, 502-507.

Audicana, M. T., & Kennedy, M. W. (2008). *Anisakis simplex*: from obscure infectious worm to inducer of immune hypersensitivity. *Clin Microbiol Rev*, *21*(2), 360-379, table of contents. doi: 10.1128/CMR.00012-07

Bier, J. W. (1976). Experimental anisakiasis: cultivation and temperature tolerance determinations. *J. Milk Food Technol.*, *39*, 132-137.

Bilska-Zając, E., Różycki, M., Chmurzyńska, E., Karamon, J., Sroka, J., Kochanowski, M., . . . Cencek, T. (2015). Parasites of Anisakidae Family—Geographical Distribution and Threat to Human Health *Journal of Agricultural Science and Technology*, *A5*, 146-152. doi: 10.17265/2161-6256/2015.01.010

Bogitsh, B., & Oeltmann, T. (2013). Human Parasitology (Fourth Edition). *ISBN: 978-0-12-415915-0*. doi: 10.1016/B978-0-12-415915-0.01001-3

Brake, R. J., Murrell, K. D., Ray, E. E., Thomas, J. D., Muggenburg, B. A., & Sivinski, J. S. (1985). Destruction of *Trichinella spiralis* by low dose irradiation of infected pork. *J. Food Safety*, *7*, 127-143.

Brutti, A., Rovere, P., Cavallero, S., D'Amelio, S., Danesi, P., Arcangeli, C. (2010) Inactivation of Anisakis simplex larvae in raw fish using high hydrostatic pressure treatments. Food Control 21, 331-333.

Carlin, A., Mott, C., Cash, D., & Zimmermann, W. (1969). Destruction of *Trichinella* larvae in cooked pork roasts. *Journal of food science*, *34*, 210-212.

784 CEVPM. (2005). Etude des conditions de destruction des larves d'*Anisakis simplex* dans le hareng salé
785 au sel sec destiné à la fabrication de harengs saurs traditionnels. *Centre d'Expérimentation et*
786 *de valorisation des Produits de la Mer*.
787 Chai, J., Hong, S., & Lee, S. (1991). Effects of gamma irradiation on the survival and development of
788 *Clonorchis sinensis* metacercariae. *Final FAO/IAEA Research Co-ordination Meeting on Use of*
789 *Irradiation to Control Infectivity of Food-Borne Parasites. Vienna: IAEA.*, 33-41.
790 Chai, J., Murrell, D., & Lymbery, A. (2005). Fish-borne parasitic zoonoses: status and issues. *Int J*
791 *Parasitol.*, *35*(11-12), 1233-1254.
792 Collins, M. V., Flick, G. J., Smith, S. A., Fayer, R., Croonenberghs, R., O'Keefe, S., & Lindsay, D. S.
793 (2005). The effect of high-pressure processing on infectivity of *Cryptosporidium parvum*
794 oocysts recovered from experimentally exposed Eastern oysters (*Crassostrea virginica*). *J*
795 *Eukaryot Microbiol*, *52*(6), 500-504. doi: 10.1111/j.1550-7408.2005.00059.x
796 Collins, M. V., Flick, G. J., Smith, S. A., Fayer, R., Rubendall, E., & Lindsay, D. S. (2005). The effects of E-
797 beam irradiation and microwave energy on Eastern Oysters (*Crassostrea virginica*)
798 experimentally infected with *Cryptosporidium parvum*. *J Eukaryot Microbiol*, *52*(6), 484-488.
799 doi: 10.1111/j.1550-7408.2005.00056.x
800 Da Silva Felicio, M. T., Hald, T., Liebana, E., Allende, A., Hugas, M., Nguyen-The, C., McLauchlin, J.
801 (2015). Risk ranking of pathogens in ready-to-eat unprocessed foods of non-animal origin
802 (FoNAO) in the EU: initial evaluation using outbreak data (2007-2011). *Int J Food Microbiol*,
803 *195*, 9-19. doi: 10.1016/j.ijfoodmicro.2014.11.005
804 De Aluja, A. S., Nunez, F., & Villalobos, A. N. M. (1993). Use of gamma irradiation to prevent
805 infectivity of metacestodes of *Taenia solium* in pork. *Proc. final research co-ordination*
806 *meeting on Use of Irradiation to Control Infectivity of Food-borne Parasites, Mexico City 24–*
807 *28 June 1991. Vienna: IAEA.*, 23–32.
808 Deardorff, T. L., & Throm, R. (1988). Commercial blast-freezing of third-stage *Anisakis simplex* larvae
809 encapsulated in salmon and rockfish. *J Parasitol*, *74*(4), 600-603.
810 Deng, M. Q., & Cliver, D. O. (1999). *Cryptosporidium parvum* studies with dairy products.
811 *International Journal of Food Microbiology*, *46*(2), 113-121. doi: 10.1016/S0168-
812 1605(98)00187-1
813 Doyle, M. E. (2003). foodborne parasites - a review of the scientific literature. *FRI Briefings - Food*
814 *research institute - University of Wisconsin - Madison*.
815 Dubey, J. (1988). Long-term persistence of *Toxoplasma gondii* in tissues of pigs inoculated with *T.*
816 *gondii* oocysts and effect of freezing on viability of tissue cysts in pork. *Am J Vet Res*, *49*(6),
817 910-913.

Dubey, J., Verma, S., LR, F., Oliveira, S., Cassinelli, A., Ying, Y., Jones, J. (2014). Detection and Survival of *Toxoplasma gondii* in Milk and Cheese from Experimentally Infected Goats. *Journal of Food Protection*, *77*(10), 1747-1753. doi: 10.4315/0362-028X.JFP-14-167

Dubey, J. P., Brake, R. J., Murrell, K. D., & Fayer, R. (1986). Effect of irradiation on the viability of *Toxoplasma gondii* cysts in tissues of mice and pigs. *Am J Vet Res*, *47*(3), 518-522.

Dubey, J. P., Kotula, A. W., Sharar, A., Andrews, C. D., & Lindsay, D. S. (1990). Effect of high temperature on infectivity of *Toxoplasma gondii* tissue cysts in pork. *J Parasitol*, *76*(2), 201-204.

Eckert, J. (2005). Helminths. *In: Kayser, F.H., Bienz, K.A., Eckert, J., and Zinkernagel, R.M. (Eds.) Medical Microbiology. Stuttgart: Thieme, 560–562.*

EFSA. (2004). Opinion of the Scientific Panel on Biological Hazards on “the suitability and details of freezing methods to allow human consumption of meat infected with *Trichinella* or *Cysticercus*”. *The EFSA Journal*, *142*, 1-51.

EFSA. (2010). Opinion of the Scientific Panel on Biological Hazards on"Risk assessment of parasites in fishery products". *EFSA Journal 8*(4), 1543.

European-Commission. (2015). Commission Regulation (EC) No 2015/1375 of 10 august 2015 laying down specific rules for *Trichinella* in meat. *Official Journal of the European Union*, *212*, 7-34.

Fan, P. C. (1998). Viability of metacercariae of *Clonorchis sinensis* in frozen or salted freshwater fish. *International Journal for Parasitology*, *28*(4), 603-605. doi: 10.1016/S0020-7519(97)00215-4

Fan, P. C., Ma, Y. X., Kuo, C. H., & Chung, W. C. (1998). Survival of *Taenia solium* cysticerci in carcasses of pigs kept at 4 C. *J Parasitol*, *84*(1), 174-175.

FAO/WHO. (2004). CAC/RCP 54-2004 - Code of Practice on Good Animal Feeding *Codex Alimentarius*.

FAO/WHO. (2005). CAC/RCP 58-2005 - Code of Hygienic Practice for Meat *Codex Alimentarius*.

FAO/WHO. (2014). Multicriteria-based ranking for risk management of food-borne parasites. *[Food and Agriculture Organization of the United Nations/World Health Organization] - Microbiological Risk Assessment Series, N° 23 Rome*.

FAO/WHO. (2016). CAC/GL 88-2016 - Guidelines on the application of general principles of food hygiene to the control of foodborne parasites. *Codex Alimentarius*.

Fattakhov, R. G. (1989). [Low-temperature regimens for the decontamination of fish of the larvae of *Opisthorchis*]. *Med Parazitol (Mosk)*(5), 63-64.

FDA, U. (2012). Handbook of Foodborne Pathogenic Microorganisms and Natural Toxins. *Bad Bug Book, 2012. Second edition online*.

Gajadhar, A. A. E. (2015). Foodborne Parasites in the Food Supply Web: Occurrence and Control. *Woodhead Publishing*.

852 Gamble, H. R., Solomon, M. B., & Long, J. B. (1998a). Effects of hydrodynamic pressure on the
853 viability of *Trichinella spiralis* in pork. *J Food Prot*, *61*(5), 637-639.
854 Gamble, H. R., Solomon, M. B., & Long, J. B. (1998b). Effects of hydrodynamic pressure on the
855 viability of *Trichinella spiralis* in pork. *Journal of Food Protection*, *61*(5), 637-639.
856 Gari-Toussaint, M., Tieulie, N., Baldin, J., Dupouy-Camet, J., Delaunay, P., Fuzibet, J. G., Marty, P.
857 (2005). Human trichinellosis due to *Trichinella britovi* in southern France after consumption
858 of frozen wild boar meat. *Euro Surveill*, *10*(6), 117-118. doi: 550 [pii]
859 Geerts, S., De Borchgrave, J., Brandt, J., & Kumar, V. (1993). Susceptibility of *Taenia saginata*
860 metacestodes to gamma irradiation and shelf-life extension of the treated meat. *Proc. final*
861 *research co-ordination meeting on Use of Irradiation to Control Infectivity of Food-borne*
862 *Parasites*, *Mexico City 24–28 June 1991*, *Vienna*(IAEA), 49–54.
863 Genchi, M., Vismarra, A., Mangia, C., Faccini, S., Vicari, N., Rigamonti, S., Fabbi, M. (2017). Lack of
864 viable parasites in cured 'Parma Ham' (PDO), following experimental *Toxoplasma gondii*
865 infection of pigs. *Food Microbiol.*, *66*, 157-164. doi: 10.1016/j.fm.2017.04.007
866 Gestrich, R., & Heydorn, A. O. (1974). [Studies on the survival time of *Sarcocystis* in the meat of
867 slaughter animals]. *Berl Munch Tierarztl Wochenschr*, *87*(24), 475-476.
868 Giarratana, F., Muscolino, D., Beninati, C., Giuffrida, A., & Panebianco, A. (2014). Activity of *Thymus*
869 *vulgaris* essential oil against *Anisakis* larvae. *Experimental Parasitology*, *142*, 7-10.
870 Hamed, M. G. E., & Elias, A. N. (1970). Effect of food-processing methods upon survival of the
871 trematode *Heterophyes* sp. in flesh of mullet caught from brackish Egyptian waters. *Journal*
872 *of Food Science*, *35*(4), 386-388.
873 Harp, J. A., Fayer, R., Pesch, B. A., & Jackson, G. J. (1996). Effect of pasteurization on infectivity of
874 *Cryptosporidium parvum* oocysts in water and milk. *Applied and Environmental Microbiology*,
875 *62*(8), 2866-2868.
876 Henchion, M., McCarthy, M., Resconi, V. C., & Troy, D. (2014). Meat consumption: trends and quality
877 matters. *Meat Science*, *98*(3), 561-568.
878 Hill, D. E., Benedetto, S. M., Coss, C., McCrary, J. L., Fournet, V. M., & Dubey, J. P. (2006). Effects of
879 time and temperature on the viability of *Toxoplasma gondii* tissue cysts in enhanced pork
880 loin. *J Food Prot*, *69*(8), 1961-1965.
881 Hill, D. E., Forbes, L., Zarlenga, D. S., Urban, J. F., Jr., Gajadhar, A. A., & Gamble, H. R. (2009). Survival
882 of North American genotypes of *Trichinella* in frozen pork. *J Food Prot*, *72*(12), 2565-2570.
883 Hill, D.E., Gamble, H.R., Zarlenga, D.S., Coss, C. & Finnigan, J. (2005) *Trichinella nativa* in a black bear
884 from Plymouth, New Hampshire. *Vet Par* 132: 143 – 146.

885 Hill, D. E., Luchansky, J., Porto-Fett, A., Gamble, H. R., Fournet, V. M., Dawkins-Cooper, D. S., Dubey, J.
886 P. (2016). Curing conditions to inactivate *Trichinella spiralis* muscle larvae in ready-to-eat
887 pork sausage. *Food and Waterborne Parasitology*. doi: 10.1016/j.fawpar.2017.06.001
888 Hill, D. E., Sreekumar, C., Gamble, H. R., & Dubey, J. P. (2004). Effect of commonly used enhancement
889 solutions on the viability of *Toxoplasma gondii* tissue cysts in pork loin. *J Food Prot*, *67*(10),
890 2230-2233.
891 Hilwig, R. W., Cramer, J. D., & Forsyth, K. S. (1978). Freezing times and temperatures required to kill
892 cysticerci of *Taenia saginata* in beef. *Veterinary Parasitology*, *4*(3), 215-219. doi:
893 10.1016/0304-4017(78)90048-1
894 ICT. (2006). Recommendations on Methods for the Control of *Trichinella* in Domestic and Wild
895 Animals Intended for Human Consumption. *ICT (International Commission on Trichinellosis)*.
896 Jones, J. L., & Dubey, J. P. (2012). Foodborne toxoplasmosis. *Clinical Infectious Diseases*, *55*(6), 845-
897 851. doi: 10.1093/cid/cis508
898 Karl, H., & Leinemann, M. (1989). Survival of nematode larvae at the production of salted herring [In
899 German]. *Archiv für Lebensmittelhygiene*, *20*, 104-120.
900 Karl H, L. M. (1998). Survival of nematode larvae in the production of salte herring
901 (Überlebensfähigkeit von Nematodenlarven bei der Herstellung von gesalzenen
902 Heringserzeugnissen) [In German]. *Archiv für Lebensmittelhygiene*, *20*, 104-120.
903 Karl, H., Roepstorff, A., Huss, H. H., & Bloemsma, B. (1994). Survival of *Anisakis* larvae in marinated
904 herring fillets. *International Journal of Food Science & Technology*, *29*(6), 661-670. doi:
905 10.1111/j.1365-2621.1994.tb02107.x
906 Kasprzak, W., Pozio, E., Rauhut, W., Nowosad, P., Gustowska, L., & Z., G. (1993). Effect of low dose
907 irradiation on *Trichinella* isolates. *Proc. final research co-ordination meeting on Use of*
908 *Irradiation to Control Infectivity of Food-borne Parasites*, *Mexico City 24–28 June 1991*,
909 *Vienna*(IAEA), 55–72.
910 Kotula, A. W., Sharar A.K., Andrews C. D. , Shen S. K. , and Lindsay D.S. (1991). Effect of Freezing on
911 Infectivity of *Toxoplasma Gondii* Tissue Cysts in Pork. *Journal of Food Protection*, *54*(9), 687-
912 690. doi: http://dx.doi.org/10.4315/0362-028X-54.9.687
913 Kotula, A.W., Murrell, K.D., Acosta-Stein, L., Lamb, L., & Douglass, L. (1983b). Destruction of
914 *Trichinella spiralis* during cooking. *J Food Sci*, *48*, 765-768.
915 Kotula, A. W., Murrell, K. D., Acosta-Stein, L., Lamb, L., & Douglass, L. (1983a). *Trichinella spiralis*:
916 effect of high temperature on infectivity in pork. *Exp Parasitol*, *56*(1), 15-19.
917 Kruatrachue, M., Chitramvong, Y. P., Upatham, E. S., Vichasri, S., & Viyanant, V. (1982). Effects of
918 physico-chemical factors on the infection of hamsters by metacercariae of *Opisthorchis*
919 *viverrini*. *Southeast Asian Journal of Tropical Medicine and Public Health*, *13*(4), 614-617.

Kuticic, V., & Wikerhauser, T. (1996). Studies of the effect of various treatments on the viability of *Toxoplasma gondii* tissue cysts and oocysts. *Curr Top Microbiol Immunol*, *219*, 261-265.

Lacour, S. A., Heckmann, A., Mace, P., Grasset-Chevillot, A., Zanella, G., Vallee, I., Boireau, P. (2013). Freeze-tolerance of *Trichinella* muscle larvae in experimentally infected wild boars. *Vet Parasitol*, *194*(2-4), 175-178. doi: 10.1016/j.vetpar.2013.01.049

Lecleir, D., Forbes, L.B., Suppa, S., Proulx, J.F. & Gajadhar, A.A. (2004) A preliminairy investigation of the infectivity of *Trichinella* larvae in traditional preparations of walrus meat. Parasitol Res 93: 507 – 509. doi: 10.1007/s00436-004-1179-4

Lee, S. H., Park, Y. H., Sohn, W. M., Hong, S. T., & Chai, J. Y. (1989). The effects of gamma irradiation on the survival and development of *Clonorchis sinensis* metacercariae. *Kisaengchunghak Chapchi*, *27*(3), 187-195.

Lindsay, D. S., Collins, M. V., Holliman, D., Flick, G. J., & Dubey, J. P. (2006). Effects of high-pressure processing on *Toxoplasma gondii* tissue cysts in ground pork. *J Parasitol*, *92*(1), 195-196. doi: 10.1645/GE-631R.1

Lötzsch, R., Leistner, L. (1979). Überleben von *Trichinella spiralis* in Rohwurst und Rohschinken in Abhängigkeit von der Wasseraktivität (aW Wert). *Fleischwirtschaft*, *69*, 231-233.

Lötzsch, R., Rödel, W. (1974). Untersuchungen über die Lebensfähigkeit von *Trichinella spiralis* in Rohwürsten in Abhängigkeit von der Wasseraktivität. *Fleischwirtschaft 64*, 1203-1208.

Lunden, A., & Uggla, A. (1992). Infectivity of *Toxoplasma gondii* in mutton following curing, smoking, freezing or microwave cooking. *Int J Food Microbiol*, *15*(3-4), 357-363.

McClelland, G. (2002). The trouble with sealworms (*Pseudoterranova decipiens* species complex, Nematoda): a review. *Parasitology*, *124 Suppl*, S183-203.

McFadden, E., Costa-Ramos, A. L., Bradley, D., Vrain, O., McEvoy, B., & Rowan, N. J. (2017). CoMParative studies on the novel sterilisation of Irish retailed infant milk formula using electron beam and pulsed light treatments. *International Journal of Science, Environment and Technology*, *12*(6), 4375-4377.

Molina-Garcia, A. D., & Sanz, P. D. (2002). *Anisakis simplex* larva killed by high-hydrostatic-pressure processing. *J Food Prot*, *65*(2), 383-388.

Murray, K. A., Kennedy, J. E., Barron, V., McEvoy, B., Vrain, O., Ryan, D., Higginbotham, C. L. (2015). Effects of electron beam irradiation on the property behaviour of poly(ether-block-amide) blended with various stabilisers. *Radiation Physics and Chemistry*, *110*, 24-37. doi: 10.1016/j.radphyschem.2015.01.009

Murrell, D. and Crompton, D. (2009) Foodborne helminth infections. In: Blackburn, C., & McClure, P. J. (Eds.), *Foodborne Pathogens: Hazards, Risk Analysis and Control, 2nd Edition Elsevier*.

954 Murrell, D., & Dubey, J. P. (1991). Epidemiology and control of trichinellosis and toxoplasmosis. *Final*
955 *FAO/IAEA Research Co-ordination Meeting on Use of Irradiation to Control Infectivity of Food-*
956 *Borne Parasites*, *International Atomic Energy Agency*, 73-79.
957 Murrell, D., & Dubey, J. P. (1993). Epidemiology and control of trichinellosis and toxoplasmosis. *Proc.*
958 *final research co-ordination meeting on Use of Irradiation to Control Infectivity of Food-borne*
959 *Parasites*, *Mexico City 24–28 June 1991*, *Vienna*(IAEA), 73-79.
960 Murrell, D., & Pozio, E. (2011). Worldwide Occurrence and IMPact of Human Trichinellosis, 1986–
961 2009. *Emerg Infect Dis.*, *17*(12), 2194–2202. doi: 10.3201/eid1712.110896
962 Neumayerová, H., Jurankova, J., Salakova, A., Gallas, L., Kovarcik, K., & Koudela, B. (2014). Survival of
963 experimentally induced *Toxoplasma gondii* tissue cysts in vacuum packed goat meat and dry
964 fermented goat meat sausages. *Food Microbiol*, *39*, 47-52. doi: 10.1016/j.fm.2013.11.001
965 Nöckler, K., Kolb, H. (2000). Survival of *Trichinella spiralis* in raw sausage production.
966 *Fleischwirtschaft*, *80*, 102-105.
967 Ockerman, H., Basu, L (2017). Technology of fermented meat products. *In: Zdolec N (ed): Fermented*
968 *meat products: Health aspects. CRC Press*, *Boca Raton – London*, *New York*, 15-48.
969 OIE. (2012). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. *OIE Website*, *1 and 2*
970 (7th edition). http://www.oie.int/manual-of-diagnostic-tests-and-vaccines-for-terrestrial-
971 animals/
972 Onsurathum, S., Pinlaor, P., Haonon, O., Chaidee, A., Charoensuk, L., Intuyod, K., Pinlaor, S. (2016).
973 Effects of fermentation time and low temperature during the production process of Thai
974 pickled fish (pla-som) on the viability and infectivity of *Opisthorchis viverrini* metacercariae.
975 *Int J Food Microbiol*, *218*, 1-5. doi: 10.1016/j.ijfoodmicro.2015.11.001
976 Opsteegh, M. (2011). *Toxoplasma gondii* in animal reservoirs and the environment. *Dissertation*
977 *Utrecht University*, *Faculty of Veterinary Medicine*, *Utrecht*, *the Netherlands*.
978 Ortega, Y. R., & Sanchez, R. (2010). Update on *Cyclospora cayetanensis*, a food-borne and
979 waterborne parasite. *Clin Microbiol Rev*, *23*(1), 218-234. doi: 10.1128/CMR.00026-09
980 Painter, J. A., Hoekstra, R. M., Ayers, T., Tauxe, R. V., Braden, C. R., Angulo, F. J., & Griffin, P. M.
981 (2013). Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities
982 by using outbreak data, United States, 1998-2008. *Emerg Infect Dis*, *19*(3), 407-415. doi:
983 10.3201/eid1903.111866
984 Park, G. M., & Yong, T. S. (2003). Effects of gamma-irradiation on the infectivity and chromosome
985 aberration of *Clonorchis sinensis*. *The Korean Journal of Parasitology*, *41*(1), 41-45.
986 Park, H., Min, B., & Oh, S. (2017). Research trends in outdoor pig production - A review. *Asian-*
987 *Australas J Anim Sci.*, *30*(9), 1207-1214. doi: 10.5713/ajas.17.0330. Epub 2017 Jul 17.

Pott, S., Koethe, M., Bangoura, B., Zoller, B., Daugschies, A., Straubinger, R. K., Ludewig, M. (2013). Effects of pH, sodium chloride, and curing salt on the infectivity of *Toxoplasma gondii* tissue cysts. *J Food Prot*, *76*(6), 1056-1061. doi: 10.4315/0362-028X.JFP-12-519

Poulsen, C., & Stensvold, C. (2014). Current status of epidemiology and diagnosis of human sarcocystosis. *J Clin Microbiol.*, *52*(10), 3524-3530. doi: 10.1128/JCM.00955-14

Pozio, E. (2014). Searching for *Trichinella*: not all pigs are created equal. *Trends Parasitol*, *30*(1), 4-11. doi: 10.1016/j.pt.2013.11.001

Pozio, E., Kapel, C. M., Gajadhar, A. A., Boireau, P., Dupouy-Camet, J., & Gamble, H. R. (2006). *Trichinella* in pork: current knowledge on the suitability of freezing as a public health measure. *Euro surveillance : bulletin européen sur les maladies transmissibles = European communicable disease bulletin*, *11*(11).

Pozio, E., Tamburrini, A., & La Rosa, G. (2001). Horse trichinellosis, an unresolved puzzle. *Parasite*, *8*, S263-S265. doi: 10.1051/parasite/200108s2263

Putignani, L., & Menichella, D. (2010). Global Distribution, Public Health and Clinical IMPact of the Protozoan Pathogen *Cryptosporidium*. *Interdisciplinary Perspectives on Infectious Diseases*, *vol. 2010*, *Article ID 753512*. doi: 10.1155/2010/753512

Rendueles, E., Omer, M., Alvseike, O., Alonso-Calleja, C., Capita, R., & Prieto, M. (2011). Microbiological food safety assessment of high hydrostatic pressure processing: A review. *Food Science and Technology*, *44*, 1251-1260. doi: 10.1016/j.lwt.2010.11.001

Robertson, L. J., Sprong, H., Ortega, Y. R., van der Giessen, J. W., & Fayer, R. (2014). IMPacts of globalisation on foodborne parasites. *Trends Parasitol.*, *30*(1), 37-52.

Rodriguez-Canul, R., Argaez-Rodriguez, F., Pacheco De La Gala, D., Villegas-Perez, S., Fraser, A., Craig, P. S., Dominguez-Alpizar, J. L. (2002). *Taenia solium* metacestode viability in infected pork after preparation with salt pickling or cooking methods common in Yucatán, México. *Journal of Food Protection*, *65*(4), 666-669.

Ryan, U., Hijawi, N., and Xiao, L. (2018) Foodborne cryptosporidiosis. *Int J Parasitol*. 48(1): 1 – 12. Doi: 10.1016/j.ijpara.2017.09.004.

Saleque, A., Juyal, P. D., & Bhatia, B. B. (1990). Effect of temperature on the infectivity of *Sarcocystis miescheriana* cysts in pork. *Vet Parasitol*, *36*(3-4), 343-346.

Salminen, K. (1970). The infestiveness of heat and cold exposed *Diphyllobothrium latum* plerocercoids on golden hamster. *Acta Vet Scand*, *11*(2), 247-253.

Sanchez-Monsalvez, I., de Armas-Serra, C., Martinez, J., Dorado, M., Sanchez, A., & Rodriguez-Caabeiro, F. (2005). A new procedure for marinating fresh anchovies and ensuring the rapid destruction of *Anisakis larvae*. *J Food Prot*, *68*(5), 1066-1072.

Sathyanarayanan, L., & Ortega, Y. (2006). Effects of temperature and different food matrices on *Cyclospora cayetanensis* oocyst sporulation. *Journal of Parasitology*, *92*(2), 218-222. doi: 10.1645/GE-630R.1

Scholz, T., Garcia, H., Kuchta, R., & Wicht, B. (2009). Update on the Human Broad Tapeworm (Genus *Diphyllobothrium*), Including Clinical Relevance. *Clin Microbiol Rev.*, *22*(1), 146–160. doi: 10.1128/CMR.00033-08

Shehata, M. M. K., Gomaa, F. A. M., & Helal, Z. H. (2011). Effects of gamma and electron beam irradiation on viability and DNA elimination of *Staphylococcus aureus*. *Archives of Clinical Microbiology*, *2*(6). doi: 10:3823/244

Solas, M., Garcia, M., de las Heras, C., Rodriguez-Mahillo, A., Gonzalez-Munoz, M., Moneo, I., Tejada, M. (2009). *Anisakis Simplex* Antigens in Fresh and Frozen-thawed Muscle of Anchovies in Vinegar. *Food Science and Technology International*, *15*, 139-148.

Song, C., Duan, Y., Shou, G., & Zhu, H. (1993). Studies on the use of 60Co irradiation on the infectivity of *Paragonymus westermani* metacercariae. *Proc. final research co-ordination meeting on Use of Irradiation to Control Infectivity of Food-borne Parasites, Mexico City 24–28 June 1991. Vienna: IAEA.*, 99-104.

Song, C., Yuan, X., Shen, L., Gan, X., & Ding, J. (1993). The effect of cobalt-60 irradiation on the infectivity of *Toxoplasma gondii*. *Int J Parasitol.*, *23*(1), 89-93.

Sornami, S., IMPand, P., & Bundisting, C. (1993). Irradiation of fish to control the infectivity of the liver fluke *Opisthorchis viverrini*. *Proc. final research co- ordination meeting on Use of Irradiation to Control Infectivity of Food-borne Parasites*, *Mexico City*, *Vienna: IAEA.*, 115-128.

Sotelo, J., Rosas, N., & Palencia, G. (1986). Freezing of infested pork muscle kills cysticerci. *JAMA*, *256*(7), 893-894.

Srivastava, P. S., Saha, A. K., & Sinha, S. R. (1986). Effects of heating and freezing on the viability of sarcocysts of *Sarcocystis levinei* from cardiac tissues of buffaloes. *Vet Parasitol*, *19*(3-4), 329-332.

Tesana, S., Kaewkes, S. & Phinlaor, S. (1986). Infectivity and survivorship of *Opisthorchis viverrini* metacercariae in fermented fish. *Journal of Parasitology of the Tropical Medical Association Thailand*, *9*, 21-30.

Thomas, L. F., Stevenson Harrison, L., Toye, P., Anson de Glanville, W. A., Cook, E. A. J., Njeri Wamae, C., & Fèvree, E. M. (2016). Prevalence of *Taenia solium* cysticercosis in pigs entering the food chain in western Kenya. *Tropical Animal Health and Production*, *48*(1), 233–238.

USDA (2017) Safe minimum internal temperature chart (approached 15-2-2017). https://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-fact-sheets/safe-food-handling/safe-minimum-internal-temperature-chart/ct_index.

Van Mameren, J., & Houwing, H. (1968). Effect of irradiation on *Anisakis larvae* in salted herring. *In: Elimination of Harmful Organisms from Food and Feed by Irradiation (International Atomic Energy Agency (IAEA) ed.)*, *IAEA*, *Vienna*. , 73–80.

Vangeel, L., Houf, K., Chiers, K., Vercruysse, J., D'Herde, K., & Ducatelle, R. (2007). Molecular-based identification of *Sarcocystis hominis* in Belgian minced beef. *J Food Prot*, *70*(6), 1523-1526.

Verster, A., Du Plessis, T. A., & Van Den Heever, L. W. (1976). The effect of gamma radiation on the cysticerci of *Taenia solium*. *Onderstepoort Journal of Veterinary Research*, *43*(1), 23-26.

Vidacek, S., De Las Heras, C., Solas, M. T., Garcia, M. L., Mendizabal, A., & Tejada, M. (2011). Viability and antigenicity of *Anisakis simplex* after conventional and microwave heating at fixed temperatures. *J Food Prot*, *74*(12), 2119-2126. doi: 10.4315/0362-028X.JFP-11-108

WHO. (1995). Control of foodborne trematode infections. *http://apps.who.int/iris/bitstream/10665/41544/1/WHO_TRS_849_(part1).pdf*.

WHO/FAO/OIE. (2005). Guidelines for the surveillance, prevention and control of taeniosis/cysticercosis - K.D. Murrel Editor. *OIE Website*.

Wikerhauser, T., Kuticic, V., Razem, D., Orsanic, L., & Besvir, J. (1993). Irradiation to control infectivity of *Toxoplasma gondii* in murine brains and edible porcine tissues. *Proc. final research co-ordination meeting on Use of Irradiation to Control Infectivity of Food-borne Parasites*, *Mexico City*, *Vienna: IAEA.*, 133-136.

Wootten R, C. D. (2001). Round Worms in Fish. *FAO - (Torry Advisory Note No. 80 : Round Worms in Fish, Ministry Of Agriculture, Fisheries And Food)*.

Yim, D. G., Jo, C., Kim, H. J., Cha, J. S., Kim, H. C., & Nam, K. C. (2015). Combined effect of irradiation and ageing condition on physicochemical and microbial quality of Hanwoo eye of round. *Korean Journal for Food Science of Animal Resources*, *35*(3), 406-412. doi: 10.5851/kosfa.2015.35.3.406

Zimmermann, W. J. (1971). Salt cure and drying-time and temperature effects on viability of *Trichinella spiralis* in dry-cured hams. *J. Food Sci.* , *36*, 58-62.