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Review

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

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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 for food safety.

Scope and approach: Control measures to inactivate the 12 most relevant parasites in FoAO are evaluated, including cooking, freezing, curing, and traditionally applied food-processing techniques, as well as high-pressure treatment and irradiation.

Key findings and conclusions: How inactivation is determined may affect results, however efficacy of freezing and heating depends on parasite species and developmental stage, as well as temperature and time conditions. Cooking at core temperature 60–75 °C for 15–30 min inactivates parasites in most matrices. Freezing at −21 °C for 1–7 days generally inactivates parasites in FoAO, but cannot be relied upon in home situations. Parasitic stages are sensitive to 2–5% NaCl, often augmented by lowering pH. Gamma irradiation at > 0.1–0.5 kGy is effective for fish parasites, except *Anisakis* (10 kGy); > 0.4–6.5 kGy control meatborne parasites. More research is needed to investigate and improve irradiation technologies using sustainable energy sources. Literature data are diverse and insufficient to model survival as response to treatment. Research on foodborne parasites should be improved to standardize experimental approaches for evaluation of inactivation techniques and methods to monitor inactivation.

1. Introduction and the growing awareness of foodborne parasites

In 2010, parasitic infections were estimated to cause 91.1 million cases of human disease and 51,909 deaths globally per year; excluding enteric protozoa, 48% of cases were foodborne, of which ascariasis and toxoplasmosis were the most common parasitic diseases (Torgerson, 2015). However, there were considerable regional differences and low-income countries suffered the highest disease burden.

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Among foodborne disease events 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). Appraisal of foodborne illness data from USA between 1998 and 2008, indicated that FoAO were associated with approximately 48% of cases, 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.

The food vehicle is unknown for most foodborne parasitic diseases in the USA, but where identified, FoAO account for around one third (Painter et al., 2013). Some foodborne parasites may have a considerable health-related impact, but do not often cause outbreaks (e.g. *Toxoplasma*). As data were derived from outbreaks, human health impacts from foodborne parasites are probably underestimated.

Among 24 (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 (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 ceviche, and since fish that is commonly eaten raw may contain infective parasites, e.g. *Anisakis simplex* (Mo et al., 2014), this may result in increased exposure of consumers to fishborne parasites (Robertson, 2018). 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 transmission stages of pathogens, including some parasites. In addition, meat may be inadvertently undercooked.

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 (Dorny, 2009).

The present study intends to provide a comprehensive overview of the 12 most relevant parasites in FoAO that have been ranked globally and regionally for Europe (FAO/WHO, 2014; Bouwknegt et al., 2018). Although it is clear that the highest burden from foodborne parasitic infections is in lower income countries, our focus is directed towards foodborne parasites of greatest relevance in European countries, as an output from a Eurocentric COST Action (see acknowledgements). Nevertheless, treatments that are effective in Europe, will also be effective elsewhere, so the assessment of treatments is of global relevance.

Many of the more recent review papers and recommendations by food safety organisations (e.g. EFSA, FDA, ANSES and others) are based on the same, sometimes old, original papers, with more recent studies lacking for many parasites.

Testing for parasitic infections at meat inspection to prevent zoonotic parasites entering the food chain is mandatory for *Trichinella* in Europe, according to Regulation EU 2015/1375 (European-Commission, 2015). Testing for some other parasites may be relevant, but may not be 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 with the potential to prevent transmission of parasitic infections due to consumption of FoAO. This review 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. Reference inclusion criteria

A non-systematic literature review was used to gather scientific publications, reports, and official documents relevant for this article. Original papers were included that quantified effects of methods for parasite inactivation over a wide range of topics; different parasites, different matrices, different inactivation methods, and different ways of assessing inactivation.

With such a breadth of cover, ensuring reference quality is difficult. Should only references answering to our highest quality requirements be included (i.e., recent papers providing detailed quantification of parasite inactivation determined by bioassay), some parasites, matrices, and different methods (e.g. heat inactivation of Heterophyidae, freeze inactivation of *Opisthorchis* spp., all inactivation methods for Anisakidae), would have no reference material and therefore would not be included at all. On the other hand, references that have been founding papers in their field (e.g., Kotula et al. (1983) and Kotula et al. (1990) for *Trichinella* control), provide time/temperature combinations for complete inactivation of *Trichinella* in pork, but lack quantitative details regarding parasite inactivation. Such information could be used to model inactivation as part of a QMRA, to reflect consumer behaviour. In the example of *Trichinella*, not all consumers will cook their meat to safe time/temperature combinations, which will result in partial inactivation of muscle larvae. Moreover, legal requirements exist for some parasites, and official authorities conduct tests, like for *Trichinella*; whereas for others, it is the sole responsibility of the food business operators to establish a risk-based limit and to adjust their control measures accordingly.

3. Current state of knowledge

The reader is referred to Annex 7 of the FAO/WHO multi-criteria based ranking for risk management of foodborne parasites (FAO/WHO, 2014), for a comprehensive overview of parasite biology, geographical distribution, disease in humans, relevance for trade and impact on economically vulnerable populations, concerning the parasites of FoAO included in the current review.

4. Key aspects of preventive measures

Many different parasites may be transmitted by FoAO, 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).

Several important parasites transmitted by meat form infectious tissue stages in animals, for which a main intervention is to prevent food production animals from being infected. This has been particularly effective in animals that can be kept confined, e.g. pigs and poultry, whereas for grazing animals, such as sheep, it may be difficult or impossible to avoid exposure. *Trichinella* is now generally absent in meat from pigs kept indoors in many European countries (Pozio, 2014). Recent trends in consumer preferences, favouring organic production

and improved animal welfare, have led to changes in pig farming, with an increase in pigs raised outdoors (Park, Min, & Oh, 2017). This may result in greater *Trichinella* exposure of these pigs, and thereby increased human *Trichinella* infection. As sheep are mainly kept outdoors, and restricting the access of cats (definitive host of *T. gondii*) to sheep farms can be impossible, *T. gondii* is a continuous challenge in sheep production and for food safety.

Tissue parasites are also potentially problematic in the aquaculture industry, including farmed and wild-caught fish. Anisakidae are mainly a hazard in wild-caught fish. It has been argued that *Anisakis* infection is not a problem in farmed fish production, as these fish have minimal access to the parasite's intermediate hosts (crustaceans and smaller fish). Nevertheless, 0.7% *Anisakis pegreffii* infestation was reported recently in farmed sea bass from the Mediterranean Sea (Cammilleri, 2018) and *Anisakis simplex* has been found in farmed salmon (Mo, 2015). Although closed breeding facilities may reduce exposure of farmed fish to parasite infective stages such as *Anisakis* spp., it has not yet been implemented at a large scale, but may become a future industry standard.

5. Evaluation of inactivation

Unlike bacteria and viruses, the infective unit for parasites may be one individual (e.g. amoeba), one egg or one larval stage (helminths), or four to eight individuals (mature oocysts of coccidians). For parasites that form tissue cysts, one infective unit (the tissue cyst) may contain a few to 1000 individuals per tissue cyst (e.g. *Toxoplasma*). Because of this variation in units of infection, using the standard log reduction measure for inactivation, as commonly applied for bacteria and viruses, is not a uniform measure for inactivation of individual parasites. However, log reduction may still be used mentioning the unit of infection (e.g. tissue cyst, cyst, oocyst, egg). Parasites on or in foods do not grow or replicate during storage, unlike bacterial contaminations that may increase to very high numbers. As a result, a two or three log reduction that may be considered marginal for bacteria, may be highly relevant for parasitic contamination.

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. In recent decades, use of experimental animals has become controversial, and in more recent studies, infection experiments have been replaced by surrogate indicators. Such indicators may be loss of a parasite's ability to proceed in development (e.g. oocyst sporulation), evaluation of motility or morphological integrity as determined by microscopy, or molecular methods to evaluate genetic activity (Rousseau et al., 2018), which should be validated in relation to the gold standard.

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

Metacercariae of trematodes seem more tolerant to heat, since 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 at 70 °C for 30 min or at 80 °C for 5 min (Waikagul, J., 1974, cited in: Abdussalam, Käferstein, & Mott, 1995). Metacercariae of *Ascocotyle* were inactivated by heating at ≥60 °C for 15 min (Novo Borges, Corrêa Lopes, & Portes Santos, 2018).

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). However, heating in standard domestic microwave ovens (2450 MHz, 700 W) may not penetrate all areas of the food, resulting in hot and cold spots, and thus some parasites may evade inactivation (Vidacek et al., 2011). *Toxoplasma* cysts in mutton steaks processed in a microwave oven at 65 °C remained infective (Lunden & Uggla, 1992) and microwave exposures for 1–3 s (43.2–62.5 °C) partially, but not significantly, reduced infectivity of *Cryptosporidum parvum* oocysts in oysters for neonatal mice, but treatment above 43.2 °C caused unacceptable changes in oyster meat texture and colour (Collins, Flick, Smith, Fayer, Rubendall, et al., 2005).

Larvae isolated from pork chops cooked at 71–82 °C core temperature in the microwave oven (2.9–3.1 min) did not prevent *T. spiralis* infection of rats (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, Murrell, Acosta-Stein, Lamb, & Douglass, 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).

Several studies highlight that heating duration is as important as temperature, and should be chosen such that desired temperatures are reached, maintained, and evenly distributed throughout the meat (Kotula et al., 1983a). Heat inactivation of *T. spiralis* in pork was tested at a range for both time (2 min–6 h) and temperature (49–63 °C) on 2 mm thick pieces of experimentally infected pork (Kotula et al., 1983a). In that study, the intrinsic freeze sensitivity for *T. spiralis* in pork was defined by linear regression as $\mathrm{Log}(t) = 17.3 - 0.302T$, where t represents time (hours) and T temperature (°C). By this equation, it is possible to define time/temperature combinations to which pork should be exposed for complete *T. spiralis* inactivation (Table 1). Notably, these time - temperature combinations refer to the conditions in the core of the meat piece. More recently, Franssen et al. (2018, under review) developed a heat-inactivation model based on experimental data including bioassay in mice. According to this model, consumer cooking of portions of pork for a total time of 15 min would expose *Trichinella* muscle larvae to 60 °C during 10 min, inactivating 99% (*T. britovi*) or 96% (*T. spiralis*) of *Trichinella* larvae.

Based on the work of Kotula et al. (1983a), *Taenia* cysticerci can be inactivated by cooking whole cuts of beef and pork to at least 62.8 °C core temperature and subsequent rest for at least 3 min (FDA, 2012). Nevertheless, both higher and lower temperature values can also be found in the literature (Table 1).

The US Department of Agriculture recommends that whole cuts of pork, lamb, veal, or beef are cooked to an internal temperature of 62.8 °C, with a 3-min rest to inactivate *T. gondii* in meat (Jones & Dubey, 2012), based on the work of Kotula et al. (1983a). Dubey, Kotula, Sharar, Andrews, and Lindsay (1990) exposed 20 g samples of *Toxoplasma gondii* infected and spiked pork, compressed to 2 mm in thickness, to temperatures ranging from 49 to 67 °C for 0.01–96 min. Parasite inactivation was evaluated by bioassay in mice. *T. gondii* tissue cyst inactivation was characterised in that study as $\mathrm{Log}(t) = 7.918–0.146T$. Following this equation, time/temperature combinations to which *T. gondii* in pork should be exposed for complete parasite inactivation were provided, e.g. > 61 °C for 3.6 min (Dubey et al., 1990). However, in their experiments, *T. gondii* tissue cysts survived 64 °C for 3 min once and therefore, Jones and Dubey (2012) advised that whole cuts of pork, lamb, veal, or beef, should be cooked to an internal temperature of at least 65.6 °C, with a 3-min rest.

C. parvum oocysts in either water or milk lose infectivity when held at 71.7 °C for 5 s or more, indicating that conditions used in commercial pasteurization (71.5-72 °C for 15 s) are sufficient to inactivate *C. parvum* oocysts in milk (Harp, Fayer, Pesch, & Jackson, 1996); milkborne

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

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.[a]	Kills Anisakis	n.s.	Fish	Wootten, 2001
	60 °C; 700-W microwave heating	Larvae viability (with ultraviolet light)	Kills 69% Anisakis larvae, initial log population: 1.81 Kills 89% Anisakis larvae; initial log population: 1.91	0.51 0.96	Fish: Arrowtooth flounder fillets	Adams et al. (1999)
	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 1000 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 and Elias (1970)
	≥60 °C; 15 min	Metacercariae movement				Novo Borges et al. (2018)
Sarcocystis spp.						
Sarcocysts	40–60 °C; 20–25 min (heart muscle)	Bioassay (dogs)	Sarcocystis levinei sarcocysts still infective	n.s.	Buffalo heart	Srivastava et al. (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, Juyal, and Bhatia (1990)
Taenia spp.						
Cysticerci	Cooking to 60 °C	n.s.	Controls T.solium and T.saginata in meat	n.s.	Pork and beef	Murrell and Crompton (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 and 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 et al. (1983b)
Cryptosporidium						
Oocysts	43.2 °C; 1sec 54 °C; 2 s (microwave heating)	Bioassay (neonatal mice)	Not effective in reducing the infectivity of C. parvum	0.00	Shucked oysters	Collins et al. (2005)
	62.9 °C for 3 s; microwave heating		Partially reduces oocysts viability; initial log population: 4.58, log reduction: 0.15	0.15		
	71.7 °C; 5 s 71.7 °C; 10 s 71.7 °C; 15 s (conditions of commercial pasteurization)		Inactivates oocysts heat treated in milk	5	Milk	Harp et al. (1996)

(*continued on next page*)

Table 1 (*continued*)

Transmission stage	Condition	Method	Effect	Log reduction	Matrix	Ref
Sarcocystis spp.						
Sarcocysts	65–75 °C; 20–25 min	Bioassay (dogs)	Sarcocystis levinei sarcocysts become non-infective to pups	n.s.	Buffalo heart	Srivastava, Saha, and Sinha (1986)
	70 °C; 15 min 100 °C; 5 min		Sarcocystis miescheriana sarcocysts become non-infective to pups		Pork (minute pieces)	Saleque et al. (1990)
Toxoplasma gondii						
Tissue cysts	65 °C; Microwave oven	Bioassay (mouse)	Not always effective, partial inactivation of cysts	n.s.	Naturally infected sheep	Lunden and Uggla (1992)
	52 °C; 9.5 min (internal temperature) thickness of 2 mm	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 2 mm		Eliminates infectivity to mice			
	> 61 °C; 3.6 min (internal temperature) thickness of 2 mm		Eliminates infectivity to mice			
	64 °C; 3 min (internal temperature - thickness of 2 mm)		Partial inactivation of Tissue cysts			

[a] n.s.: not stated.

cryptosporidiosis outbreaks have been exclusively associated with unpasteurized milk.

6.2. Freezing

Table 2 gives an overview of freezing to inactivate parasites in FoAO.

Anisakis spp. in fish have been inactivated in a blast freezer at −35 °C for ≥15 h or at −20 °C for at least 24 h (Deardorff & Throm, 1988; McClelland, 2002). *Anisakis* spp. inactivation was evaluated by observing larval movement after physical stimulation. Some larvae seen to be moving after freeze-treatment at −35 °C for 1 h were considered moribund. Subsequent sub-zero storage after freezing is recommended for complete inactivation of anisakidae larvae (Deardorff & Throm, 1988).

Trematode metacercariae appear to be more resistant to freezing temperatures, although not many studies have been performed to date. *Clonorchis sinensis* in fish and fishery products are considered to be inactivated at −10 to −20 °C for 5–20 days (EFSA, 2010). However, *C. sinensis* metacercariae in fish that had been frozen at −12 °C for 10–18 days or at −20 °C for 5–7 days remained viable and infective in bioassays using rats and rabbits. Only 20 days of freezing at −12 °C or 3 days of freezing at −20 °C followed by thawing and another freeze treatment for 4 days at −20 °C eliminated infectivity in rabbit and rat bioassays (Fan, 1998). Freeze-treatment of mullet fillets for 30 h at −10 or −20 °C is not effective at inactivating *Heterophyes* metacercariae (Table 2). At temperatures below −20 °C for 2–32 h, the viability of *Opisthorchis* spp. in fish has been markedly, but not completely, reduced (Table 2). Although anecdotal evidence, an outbreak of opisthorchiasis in Italy was due to consumption of infected fish that had been frozen in a household freezer at −10 °C for 3 days (Armignacco, Caterini, Marucci, Ferri, et al., 2008).

In contrast, larval stages of cestodes appear more sensitive to freeze treatment, although primary literature is scarce; one paper describes inactivation of isolated *Diphyllobothrium* spp. plerocercoids (Table 2). *Taenia solium* cysticerci in pork are inactivated by freezing at −24 to −5 °C for 1–4 days, whereas inactivation of *Taenia saginata* cysticerci in beef requires freezing at −5 to −25 °C for 10–15 days (Table 2).

Freeze inactivation of *T. spiralis* in pork was tested at a wide range for both time (1 s–182 days) and sub-zero temperatures (−1 to −193 °C) on 2 mm thick pieces of experimentally infected pork (Kotula et al., 1990). In that study, the intrinsic freeze sensitivity for *T. spiralis* in pork was defined by linear regression as $\mathrm{Log}(t) = 5.98 + 0.40T$, where t represents time (hours) and T temperature (°C). Using this equation, time/temperature combinations have been defined to which *T. spiralis* in pork should be exposed for complete parasite inactivation (Table 2). Note that the time needed to reach the desired temperature in pork must be determined for each situation and should be added to the calculated inactivation time. Based on the work of Kotula et al. (1990), the International Commission on Trichinellosis (ICT) recommends freezing at −21 °C for 7 days for complete inactivation of *T. spiralis* in pork. However, freeze inactivation of *Trichinella* in bulk packages may need lower temperatures or longer exposure times (e.g. −29 °C for 6 days to −15 °C for 30 days) to ensure safety, depending on meat thickness and stacking height in industrial freezers (ICT, 2006). These recommendations have been included in EU recommendation 2015/1375 (European-Commission, 2015), laying down specific rules on official controls for *Trichinella* in meat regarding freeze treatment, and its previous version (EU Recommendation 2075/2005).

Inactivation studies on *Trichinella* spp. in other matrices than pork are less elaborated and limited in number. *T. spiralis* and *T. britovi* in experimentally infected wild boars, 24 weeks post infection, were inactivated by freezing at −21 °C for 1 week as determined by mouse bioassay (Lacour et al., 2013). Freezing to inactivate *Trichinella* species other than *T. spiralis* in pork, game, and horse meat, cannot be relied upon. Frozen wild boar meat from a naturally *T. britovi*-infected animal

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 and 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 °C; 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 and 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 and 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, Cramer, and Forsyth (1978)
Taenia solium	0 °C or above		Does not affect parasite survival in culture	0.00		
Tissue cysts		In vitro culture assay			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, Gamble, Zarlenga, Coss, and Finnigan (2005)
	−10 °C; 4 days, excluding cooling down time −15 °C; 57 min, excluding cooling down time −20 °C; 1 min, excluding cooling down time	Bioassay (mice)	Inactivates *T. spiralis* muscle larvae	n.s.	Pork	Kotula et al. (2010)
	−21 °C; 7 days	Bioassay (mice)	Inactivates *T. spiralis* muscle larvae	2.19[a]	Wild boar meat	Lacour et al. (2013)

(*continued on next page*)

Table 2 (*continued*)

Transmission stage	Condition	Method	Effect	Log reduction	Matrix	Ref
Toxoplasma gondii						
Tissue cysts	−12 °C; 3 days	Bioassay (cats)	Inactivates tissue cysts	n.s.	Meat: Experimentally infected pigs	Dubey et al. (1988)
	−7 °C to −12 °C; 4 days	Bioassays (cats and/or mice)			Pork	Kuticic and Wikerhauser (1996)
	−20 °C for 54 h and thawed overnight at 4 °C	Bioassay (mice)			Mutton	Lunden and Uggla (1992)
	−8 °C; 3 days −7 °C; 17 days,	Bioassay (mice)			Pork	Kotula, Sharar, Andrews, Shen, and Lindsay (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 and Cliver (1999)
Cyclospora cayetanensis						
Oocysts	−15 °C; 24 h	Oocyst sporulation	No inactivation of oocyst sporulation	0.00	Dairy substrates	Ortega and Sanchez (2010)
	−15 °C; 2 days		Inactivates oocysts	2.00	Dairy products: diluted milk substrate Dairy products: milk matrix Dairy products: whipped cream matrix	Sathyanarayanan and Ortega (2006)

[a] 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).

Table 3
Effects of enzymatic and chemical preservation on the infectivity of parasite stages in meat and fish products. Control measure: Marination, Pickling, Smoking, Fermentation and 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, .Roepstorff, Huss, and Bloemsma (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	Karl and Leinemann (1989)
	21% NaCl; 15 days	Motility and scanning electron microscopy	Inactivates Anisakis pegreffi larvae	n.s.	Fish, anchovies	Anastasio et al. (2016)
	13% NaCl brine; 5 °C; 24 h plus dry salt maturation at 5° for 15 days	Motility	Inactivates Anisakis larvae	n.s.	Fish, cod	Smaldone et al. (2017)
Clonorchis sinensis metacercariae Opistorchis viverrini	3 g NaCl/10 g fish flesh; 8 days	Bioassay (rat)	Inactivation of metacercariae	n.s.	Freshwater fish	Fan (1998)

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Table 3 (*continued*)

Transmission stage	Condition	Evaluation method	Effect	Log reduction	Matrix	Ref
metacercariae	13.6% NaCl, 48 h	Bioassay (hamster)	Reduced infectivity of metacercariae	n.s.	Fermented fish	Kruatrachue et al. (1982)
	20% NaCl, 5 h	Bioassay	metacercariae non-infectious	n.s.	Fermented fish	Tesana, Kaewkes, and Phinlaor (1986)
	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 (plasom)	Onsurathum et al. (2016)
Taenia solium metacestodes	7–10.5% NaCl at 30 °C	microscopy	Inactivation overnight	n.s.	pork	Ockerman and Basu (2017)
Trichinella spp. Larvae	2.8% nitrite-curing salt (99.5% NaCl, 0.5% $NaNO_2$); 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 and 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 *T. spiralis* 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 *T. spiralis* 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 *T. spiralis* larvae in 75 mm diameter Mailänder Salami ripened for 11 days (a_W ca. 0.939; pH 5.1)			
	≥1.3% NaCl; pH 4.6; initial larva count 181 - 447/g		Kills *T. spiralis* larvae in 8 days (a_w 0.935–0.961)		Cured pork sausage	Hill et al. (2017)
	≥1.3% but < 1.8% NaCl; 4.6 < pH ≤ 5.2; initial larva count 386–390/g		Kills *T. spiralis* larvae in 11 days (a_w 0.965)			
	≥1.8% but < 2.8% NaCl; 4.6 < pH ≤ 5.2; initial larva count 181–447/g		Kills *T. spiralis* larvae in 9 days (a_w 0.943–0.961)			
	≥2.8% NaCl; 4.6 < pH ≤ 5.2; initial larva count 322 -323		Kills *T. spiralis* larvae in 8 days (a_w 0.935–0.945)			
	storage at 10 °C; initial larva count 400–700/g		Kills *T. spiralis* 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		Dry ham	Lötzsch and Leistner (1979)
	6% NaCl cure, then storage at 10 °C; total 75 days; 8.5 kg initial ham weight; initial larva count 390–440/g		Infective in rat bioassay		Dry ham	Lin et al., 1990a; Lin et al., 1990b
	6% NaCl cure, then storage at 10 °C; total 90 days; 8.5 kg initial ham weight; initial larva count 390–440/g		Kills *T. spiralis* larvae			
	6% NaCl cure, then storage at 10 °C; total 90 days; 11.1 kg initial ham weight; initial larva count 380–525/g				Dry ham	
	6% NaCl cure, then storage at 23.9 °C; total 5 days; 11.1 kg initial ham weight; initial larva count 380–525/g					
	4.4% NaCl cure, storage at 23.9 °C; total 23 days; 11.1 kg initial ham weight; initial larva count 600/g				Bag-cured ham	
Toxoplasma gondii Tissue cysts	2% NaCl	Bioassay (mice)	Viable at day 8	n.s.	Muscle from mice in tissue culture medium	Lötzsch and Rödel (1974)
	2.5% and 3% NaCl		Inactivation within one day			
	2.5% nitrite-curing salt		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
	3–5% NaCl; 64 h	Bioassay (mice)	Inactivation of tissue cysts		Mutton meat	Lunden and Uggla (1992)
	4.2–6.2% NaCl; 12, 14 and 16 months		Inactivation of tissue cysts		Parma ham	Genchi et al. (2017)

(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. In contrast, *T. nativa* muscle larvae in experimentally infected pig meat were inactivated by freezing for 106 h at −17.7 °C (0 °F), as determined by mouse bioassay (Table 2).

Toxoplasma gondii in pork, mutton, and other meat is completely inactivated by freezing at between −7 and −13 °C for 2–4 days (Table 2). 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. tissue cysts inactive (Table 2).

Parasites such as *C. 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 *C. parvum* and *Cyclospora cayetanensis* in milk matrices (Table 2).

More work is needed to evaluate techniques such as rapid chilling to very low temperatures, which is commonly used in food processing industries.

6.3. Enzymatic and chemical preservation

Parasites in FoAO do not grow during storage, but they are able to survive for days to weeks under cold storage conditions (> 0 °C) in meat or fish flesh (Hamed & Elias, 1970; Fan, Ma, Kuo, & Chung, 1998; Neumayerová et al., 2014). This means that production of ready-to-eat-foods from animal origin at the production plant must ensure parasite absence or inactivation. Some traditionally applied food-processing techniques, such as marination, fermentation, smoking etc., have parasite-inactivating potential, often as a result of a combination of several mechanisms, occasionally acting synergistically. Table 3 gives an overview of the effects of enzymatic and chemical inactivation of foodborne parasites in meat and fish products. Both drying and addition of salt reduce the amount of available water and increase osmotic pressure, which is detrimental for all living cells. Marination can be defined as treatment of meat or fish with brines containing salt, organic acids, and, occasionally, essential oils. Fermentation is an enzyme-driven breakdown of the main constituents of flesh, most notably degradation of carbohydrates to lactic acid. The resultant acidification and oxygen consumption have major immediate effects (Ockerman & Basu, 2017).

Marination of fish is a traditional processing method with some effect on nematode larvae. As regards composition of brine, ranges in NaCl and acetic acid of 5–20%, and 2.6–40%, respectively, have been studied (Table 3). With increasing salt concentrations, time to inactivation decreases (AESAN, 2007; CEVPM, 2005; Karl, 1998; Karl, Roepstorff, Huss, & Bloemsma, 1994), but is still in the range of more than one week. In herring, an NaCl content of 20% in the fish tissue water phase resulted in a 1 log reduction in *Anisakis* larvae motility within 14 days, and a > 2 log reduction in 28 days (Karl & Leinemann, 1989). In contrast, when the fish tissue water phase contained 15% NaCl, the reduction was less than 1 log after 21 days. In cod, a combination of brine salting (13% NaCl) at 5 °C for 24 h, in combination with dry-salting for another 14 days, inactivated *Anisakis* larvae (Smaldone, Marrone, Palma, Sarnelli, & Anastasio, 2017).

Even for dry-salted herring, 20 days of storage is recommended in order to ensure inactivation of *Anisakis* larvae (CEVPM, 2005). For dry-salted anchovies, 15 days of storage inactivated *Anisakis pegreffii* at a salt concentration of 21% in the anchovies fillets (Anastasio et al., 2016). Also Marination in vinegar (6% acetic acid) for 4–24 h is considered insufficient to inactivate larvae (AESAN, 2007), and recommended procedures comprise marinating for 31 days in brine with 2.5% NaCl and 6% acetic acid or 6% NaCl and 12% acetic acid for 13 days.

Essential oils have proven antibacterial properties, and there is evidence that such substances can inactivate parasites. Giarratana, Muscolino, Beninati, Giuffrida, and Panebianco (2014) were able to inactivate third stage larvae of *Anisakis* in 5 and 10% solutions of essential oils of *Thyme vulgaris* (containing mostly thymol, linalool and pinens) in sunflower seed oil with 14 and 7 h, respectively (Giarratana et al., 2014). Anisakis L3 larvae were inactivated after 2 h in 1% and 5% solutions of essential oils of *Tagetes minuta* (containing mostly β-ocimene, limonine and (Z)-tagetone) in saline solution, and after 4 h in 0.1–5% essential oil in an industrial marinating solution (water and vinegar 1:1, with 3% NaCl and 1% citric acid), but not in sunflower oil (Giarratana et al., 2017). Inactivation was assessed by motility and electron-microscopic observation of structural damages of the cutis in both studies. Even when this anti-*Anisakis* effect might be delayed in a fish flesh matrix, there should be ample time during the time periods of food distribution and display in the shelves before it reaches the consumer.

Inactivation of *Clonorchis sinensis* metacercariae in heavily-salted freshwater fish (3 g NaCl/10 g 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). As could be expected, among the traditional salted fish products in Thailand, those salted and stored for 2–3 months (Sithithaworn & Haswell-Elkins, 2003) have the least risk to contain viable metacercariae.

Due to the highly variable conditions for above described methods to inactivate parasites in fish, EU Regulation (EC) 853/2004 and its amendment (EC) 1276/2011 demand that fishery products intended for raw consumption, cold smoking preparation (< 60 °C), or processing by marinating and/or salting, must be frozen at −20 °C in all parts of the product for at least 24 h or at −35 °C for at least 15 h to inactivate other parasites than trematodes (European-Commission, 2004, 2011). As shown in section 6.2 and Table 2, trematodes require exposure to freezing during longer time periods for complete inactivation.

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). The procedure was not safe when drying was performed at room temperature. Lin et al. (1990b) studied *Trichinella* survival in dry- (hind legs of 8.5–11.1 kg initial weight) and bag-cured (11.1 kg initial weight) hams. Pork contained 300–525 larvae/g. The dry curing process included covering the ham with a cure mix (40 g NaCl/kg ham and additional 20 g NaCl/kg at day 10) for 28–39 days (according to ham weight) at 2.2 °C, followed by rinsing and an equalisation period to allow even distribution of salt in the muscle, whereas for bag curing, hams with 11.1 kg weight were salted with 44 g NaCl/kg and then stored in wrapped condition (Lin et al., 1990a). Dry hams were stored at 10–32.2 °C for up to 90–11 days; storage at 10 °C was only effective after 90 days, whereas this was considerably shorter when hams were stored at 23.9 °C (Table 3).

European dry ham production generally relies on low aging temperatures. 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 day 29 (a_W 0.921; pH 5.6). The time to loss of infectivity of *T. spiralis* in fermented sausages made with 2.8% nitrite curing salt and 0.5% sugar, ranged from 6 to 14 days with NaCl content from 3.2 to 3.8% in the finished

products (Table 3). Since inactivation was observed at a_W of 0.93–0.95 for fermented 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 threshold levels for fermented sausage and dried hams, respectively (Lötzsch & Leistner, 1979).

The United States Department of Agriculture (USDA) requires that cured pork products are produced with pork that tested negative for *Trichinella* muscle larvae at meat inspection, or have been produced according to validated procedures (Hill et al., 2017). To determine *Trichinella* muscle larva inactivation in cured ready-to-eat dry type sausage, Hill et al. (2017) performed a validation study monitoring five parameters during curing: salt/brine concentration, a_w, pH, temperature and time, using experimentally *Trichinella* infected pork for the production of batter. In their experiments, pH ranged 4.6–5.2 and salt varied between 1.3% and 2.8%. Loss of infectivity of *T. spiralis* in fermented sausages was determined by mouse bioassay after 0–11 days. From these experiments, key conditions could be defined for the production of cured dry sausages that simultaneously inactivated *T. spirals* muscle larvae. These included NaCl concentrations > 1.3% and fermentation to pH ≤ 5.2 for complete *T. spiralis* inactivation after 7–10 days post-stuffing (Hill et al., 2017, Table 3). However, there are also raw sausages with no fermentation or only short-term fermentation, such as “Teewurst” or “Mettwurst” types. For Teewurst sausages (2.8% nitrite-curing-salt) containing 950 larvae/g, 21 days of ripening were required for loss of infectivity (bioassay in mice), corresponding to a_W of around 0.949 and pH of 5.3 (Lötzsch & Rödel, 1974), whereas in the same product with 200 larvae/g, 14 days of ripening (a_w ca. 0.944; pH 5.3) were sufficient. Nöckler and Kolb (2000) studied larval survival in sausage batter starter culture manufactured with lower content of nitrite-curing-salt (2%). The number of viable larvae decreased markedly between the 4th and 7th day after manufacture. Loss of motility of digested larvae and of infectivity in mice were observed from the 9th day onwards. Although these studies indicate that Teewurst sausages with respect to *Trichinella* would be a safe product after 9–14 days of storage, such products are usually placed on the market and consumed before this period. In sum, a_W of 0.92 is reported as the limit for survival of *Trichinella* larvae (species not specified), which corresponds to dry, rather than semi-dry to fresh, fermented sausages (Ockerman & Basu, 2017). Control of this parasite for fermented meats can also be achieved by the use of industrially deep-frozen meat for production or from pig production systems of adequate biosecurity level to ensure a lack of *Trichinella* in the pork.

Rodriguez-Canul et al. (2002) reported inactivation of *Taenia solium* cysts in pork salted with 70–105 g/kg and left overnight at ca. 30 °C. The authors observed structural changes in the cyst and inability of the scolex to evaginate. They attributed this inactivation to changes in osmotic pressure rather than to the pH decline from about 6.0 to 5.3. For cysts of *T. saginata* in beef, a water activity of 0.98 is regarded as the limit for survival (Ockerman & Basu, 2017).

Protozoan parasite stages in meat and fish flesh are sensitive to salt concentration. *Toxoplasma* tissue cysts in muscle of mice were inactivated within 1 day at 2.5% NaCl (Pott et al., 2013). Nitrite-curing salt (99.5% NaCl with 0.5% $NaNO_2$) proved more effective than NaCl alone. In contrast, *Toxoplasma* tissue cysts have a high pH tolerance: at lower pH (pH 5 and 6 compared to pH 7), infectivity was not reduced with exposure for 24–26 days at 4 °C. This finding was regarded as relevant, not only for fresh meats, but also for fermented meats where the pH can be around 5.0. In cured-dried and cured-cooked meats, the pH is typically at 6 or above, but the infectivity of tissue cysts in loin has been demonstrated to decrease rapidly with exposure to 2% NaCl. *Toxoplasma* tissue cysts in pork loin that was injected with brine to give 2% NaCl or 1.4% sodium- or potassium lactate (injection volume 10% of loin weight) followed by storage for 7 days at 4 °C, were not infectious when the pork was fed to cats (Hill, Sreekumar, Gamble, & Dubey, 2004). Moreover, it was shown that inactivation of cysts (assessed via bioassay) in pork loins held at 4 °C with addition of 2% sodium chloride or 1.4% potassium or sodium lactate occurs within the first 8 h after treatment (Hill et al., 2006). In contrast, infectivity of positive controls (infected, but injected with 0.85% NaCl only) was demonstrated at least partially, even after 45 days of storage. Sodium triphosphate and sodium diacetate, both common compounds in meat enhancers, had no effect. A study on processing of mutton (Lunden & Uggla, 1992) indicated that in meat cured for 64 h at 4 °C with 30–50 g sodium chloride and 25–40 g sucrose for 200–360 g of meat, cysts lost infectivity. Also, warm-smoking at above 50 °C for 24–48 h inactivated *Toxoplasma* tissue cysts in brine-injected mutton (as assessed via bioassay in mice). The survival and infectivity of *Toxoplasma* tissue cysts in ham from experimentally infected pigs after the standard curing process required for Parma ham (storage for 12, 14 and 16 months and typical average NaCl contents from 4.2 to 6.2%) 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). Thus, a_w below 0.95 and/or pH below 5.3 are recognized as being detrimental to survival of *Toxoplasma* tissue cysts (Ockerman & Basu, 2017).

7. High pressure and irradiation

With the survival of some parasites under the conditions of traditional inactivation methods for FoAO, such as freezing and curing, there is interest in alternative approaches. However, data are relatively limited, and there is a clear need for further testing.

7.1. High pressure processing (HPP)

High pressure processing (HPP) is a non-thermal processing technique that uses a liquid compression medium and constant pressure to treat vacuum-packaged food products. Typically, a pressure range from 200 to 600 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.

Table 4 provides an overview of the efficacy of HPP on parasites in fish, meat, and oysters, although only a limited number of parasites has been investigated. *Anisakis* larvae in fish filets were killed at a pressure of 200–300 MPa for 5–10 min at a temperature between 0 and 15 °C using motility as an indicator of larval death. *Cryptosporidium parvum* oocysts have been HPP treated at pressures of 305–550 MPa for ≥180 s, which significantly reduced infectivity to mouse pups in a bioassay, but could not totally prevent infection.

Significant inactivation of *T. spiralis* larvae isolated from infected pork using hydrodynamic pressure (Hydrodyne process, method for tenderising meat or fish using explosion induced shock waves in water) has been reported, although the pressure generated (55–60 MPa) did not eliminate the infectivity to mice as determined by bioassay. *Toxoplasma gondii* tissue cysts in ground pork were successfully inactivated using 300–400 MPa for 30 s, whereas 100 and 200 MPa were ineffective (Table 4).

7.2. Electron beam irradiation

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

Collins et al. (2005) examined the efficacy of E-Beam irradiation on the viability of the Beltsville strain *C. parvum* oocysts as artificial contaminants of Eastern Oysters (*Crassostrea virginica*), by feeding E-beam treated oyster tissues to neonatal mice. A dose of 2 kGy completely eliminated *C. parvum* infectivity and did not adversely affect the visual appearance of the oysters (Table 5).

7.3. Gamma irradiation

The inactivation effect of gamma irradiation is quite diverse, as reflected in the huge variation of the observed minimum effective dose (MED) and directly related to the type of parasite, the parasite stage, and food product assayed (Table 5).

The radio resistance of *A. simplex* is high; doses as high as 2–10 kGy, on isolated *Anisakis* larvae in physiological salt produced a reduction in penetration ability and infectivity in rats (up to 70% worm recovery rate), but a dose of 2 kGy was not fully effective to prevent infection in rabbits (up to 25% recovery rate; Chai, Hong, & Lee, 1991). When salted fish products were assayed, similar results were observed; doses as high as 6 kGy were not effective for larvae in salted herring, with substantial numbers surviving the treatment (Table 5).

The radio resistance of trematodes varies depending on parasite species and whether the treatment is applied to meat or another matrix. Metacercariae of *Clonorchis sinensis* were three-fold less susceptible to gamma irradiation when encysted in the flesh of fish than when they were isolated from the fish; i.e. the MED for metacercariae in fish was 0.15 kGy, but 0.02 KGy when metacercariae had been isolated from the fish (Table 5). A similar situation was observed for *Paragonimus westermani;* the MED for metacercariae in crab was 25 times higher than that for metacercariae isolated from the crustacean (2.5 kGy vs 0.1 kGy). Thus, the higher dose is required for practical application. However, identical MED (0.1 kGy) were required for inactivating *Opisthorchis viverrini* metacercariae in fish or after isolation from fish (Table 5).

The MED needed to inactivate *Trichinella* in heavily contaminated pork carcasses is 0.3 kGy (Table 5). 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 for *Taenia saginata* cysticerci in beef varied significantly, ranging from 3.7 to 6.5 kGy. Doses of 0.2–0.6 KGy on *Taenia solium* cysticerci produce an irreversible effect on the development of the adult worms, affecting the viability of the cells in the neck region (Table 5).

Studies of inactivation of *T. gondii* by gamma irradiation in meat demonstrated that intermediate irradiation doses (0.1–1 kGy) significantly reduce the infectivity of bradyzoites and tissue cysts in pork products. However, differences in radio resistance between *T. gondii* strains have been observed, with MEDs ranging from 0.4 to 0.7 kGy (Table 5).

8. Future perspectives

Bioassay is regarded the gold standard for evaluation of treatment efficacy of parasites in food. However, 65% of the people questioned in a 2016 UK survey accepted the use of experimental animals for medical research, but, at the same time, 35% of respondents think that experimental animal use should be banned on animal welfare grounds and 75% agreed that more needs to be done to search for alternatives to experimental animal use (Clemens & Leaman, 2016). Alternative methods to evaluate parasite inactivation include morphological examination of structure integrity, evaluation of movement after mechanical stimulation, or *in vitro* essays to evaluate parasite development into the next life stage. However, sensitivity and specificity of alternative indicators remain to be determined in most cases and more research is needed to evaluate such indicators in comparison with the gold standard. Future efforts to modify and advance treatment methods may benefit from next-generation sequencing (NGS) and bioinformatics regarding (absence of) gene expression, although finding anti-parasitic targets using NGS would be more relevant. Free availability and open access data that come with NGS will improve standardization and harmonization of research efforts.

Although low doses of irradiation were long ago found to be

Table 4
Effects of high pressure and irradiation on foodborne parasites. Control measure: High Pressure Processing.

Transmission stage	Condition	Evaluation method	Effect	Log reduction	Matrix	Ref
Anisakis						
Larvae	140 MPa; 1 h 150 MPa; 30 min 200 MPa; 10 min 170 MPa; 3 x 2 min 180 MPa; 2 x 2 min 190 MPa; 15 min	Motility tests, methylene blue fluorescence	Inactivates Anisakis larvae	Low numbers used; log reductions cannot be calculated	Fish: Nile perch fillet	Molina-Garcia and Sanz (2002)
	100 MPa; 5 min 200 MPa; 5 min 300 MPa; 5 min	Motility test	8% larval inactivation 97% larval inactivation 100% larval inactivation	Low numbers used; log reductions cannot be calculated	Fish: Mackerel filet	Brutti et al. (2010)
Cryptosporidium						
Oocysts	400 MPa; 3 min 370 MPa; 3 min 480 MPa; 3 min 305 MPa; 3 min 550 MPa; ≥3 min	Bioassay (mice)	Reduction of infected mice by 40% 57% 57% 48% ≥65%	Low numbers used; log reductions cannot be calculated	Shellfish: Oysters	Collins et al. (2005)
Trichinella spp.						
Larvae	55–60 MPa	Bioassay (mice)	Does not inactivate *Trichinella spiralis*	n.s.	Pork	Gamble, Solomon, and Long (1998)
Toxoplasma gondii						
Tissue cysts	300 MPa; 30 s 400 MPa; 30 s	Bioassay	Inactivates tissue cysts	n.s.	Meat: Ground pork	Lindsay, Collins, Holliman, Flick, and Dubey (2006)

Table 5
Effects of high pressure and irradiation on foodborne parasites. 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 et al. (2005)
	From 1 kGy to 2 KGy		Infectivity reduction from 47 to 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 and 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, Park, Sohn, Hong, & Chai, 1989
Opisthorchis viverrini						
Metacercariae	0.05–0.1 kGy (^{60}Co)	Bioassay (hamsters, rabbits, cats)	MED: 0.1 kGy	n.s.	Fish	Sornami, Impand, and Bundisting (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 0.15–0.3 kGy block production of larval progeny 0.3–0.6 kGy inactivates Trichinella larvae	n.s.	Pork	Brake et al., 1985; 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, Emre, Çerçi, Ersen, and Mutluer (1993)
	0.2–0.6 kGy (^{60}Co)	Bioassay (Human volunteers)	MED: 0.5 kGy		Cooked meat previously frozen	Geerts, De Borchgrave, Brandt, and Kumar (1993)
	0.2–1.40 kGy	Bioassay (golden hamsters)	MED: 0.60 kGy Taenia solium		Pork meat	Verster, Du Plessis, and Van Den Heever (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, Nunez, and Villalobos (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 et al., 1993; Murrell & Dubey, 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, Brake, Murrell, and Fayer (1986)
	0.4–0.7 kGy (^{60}Co)	Bioassay (cats, mice)	Complete inactivation depending on T. gondii isolate			Wikerhauser, Kuticic, Razem, Orsanic, and Besvir (1993)
	0.1–1 kGy (^{60}Co)	Bioassay (NIH mice)	MED: 0.55 kGy Elimination of infection	n.s.	Pork products	Song et al., 1993; Murrell & Dubey, 1993

effective at inactivating at least seven genera of parasites, this methodology has barely been considered for use in controlling foodborne parasites in FoAO. This is partly due to considerable controversy concerning the safety of irradiated food. Fierce opposition against the use of irradiation from consumer groups in Europe is often based on old information and plays on consumer fear (Roberts, 2014). Although irradiation is increasingly used for treatment of various foods globally, its use in the European Union is limited and even decreasing; strict legislation only permits irradiation treatment of dried herbs and frog legs (Feliciano, 2018).

E-beam is a potential methodology that circumvents the need of radioactive isotopes, but E-beam electrons have a limited penetration depth of, at most, 5 cm, considerably below that of X-rays (penetration depth 60–400 cm, depending on the energy used) (Collins, Flick, Smith, Fayer, Rubendall, et al., 2005). However, this limited penetration has been proven appropriate for some foods (e.g. oysters). E-beam irradiation doses ≥2.0 kGy may be used in commercial processes, but irradiation at 2 kGy changes meat tenderization, colour, and flavour (Yim et al., 2015). In industrialized regions especially, such as the US and Europe, a trend towards more critical consumer attitudes regarding sustainability of food products and production methods is ongoing, with increasing demands for freshness and “naturalness” of foods, thereby excluding additives and human intervention (e.g. irradiation, but also freezing) (Román, Sánchez-Siles, & Siegrist, 2017). This calls for better communication and more research to investigate and improve irradiation technologies. Using sustainable energy sources may improve public acceptance of irradiation treatment of foods. Indeed, evaluation of electrical equipment to generate E-beam and X-ray irradiation to replace Cobalt-60 use for irradiation of foods is ongoing (Feliciano, 2018).

High pressure processing (300 MPa for 0.5–5 min) may be used to inactivate Anisakidae larvae in fish and *Toxoplasma* tissue cysts in (minced) meat, but *Cryptosporidium* oocysts in oysters appear to be highly resistant to HPP. Also here, more research is needed to evaluate applicability of HPP to inactivate parasites in FoAO.

Research on foodborne parasites should be improved towards standardization of experimental approaches to evaluate inactivation methods, but also towards standardization of methods to monitor inactivation. Literature data are diverse and are currently generally insufficient for modelling survival as a response to treatment. Although inactivation effects vary considerably between parasites and methods, modelling may help to determine minimal effective treatment parameters to ensure food safety for FoAO.

9. Conclusions

Based on our extensive literature review, information on the effects of different inactivation techniques on 12 most relevant 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 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). Literature on high pressure treatment and E-beam to inactivate parasites in animal origin matrices is scant. 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 insufficient for modelling survival as response to treatment.

This assimilation of data clearly shows that research on foodborne parasites should be improved, and efforts should be directed towards sustainable novel inactivation methods for parasites in FoAO and standardization of experimental approaches for the evaluation of inactivation methods.

Declaration of interests

The authors declare that they have no conflict of interests.

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