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Abstract: Herring is the third most commercialized fish species in the EU and a common host of Anisakis spp. larvae. The aim of this study was to assess the occurrence, distribution and viability of Anisakis spp. larvae in different kind of ready to eat (RTE) herring products. One hundred and thirty-five products, consisting of 50 smoked whole specimens and 85 filleted products were collected in Italy from 2016 to 2018. Viscera and muscle of whole herrings were visually inspected and separately submitted to artificial digestion. Filleted products were also visually inspected and digested. Natural and UV light were used for examining the residual material. Nematodes larvae viability was assessed, then they were counted, collected and identified to genus level by optical microscopy. In addition, the cox2 gene was targeted for the identification of part of the larvae. Anisakis spp. larvae were found in 56 products (41.5%) and overall 1715 larvae were collected (range 0-172 larvae/product). Most of the larvae (1559, 91%) were found in the viscera of 49 of the 50 whole herrings (98%). A statistically significant difference ($p<0.001$) was observed between the positivity rate and larval density of the remaining 156 larvae found at muscle level, as 149 larvae were found in the muscle of 31 whole herrings (positivity rate 62%, 0.022 larval density/g), while only 7 larvae were found in the 85 filleted products (positivity rate 7%, 0.001 larval density/g). Larvae were molecularly identified as A. simplex. Although all the larvae were dead, the high level of contamination of whole herrings on the market poses some issue related to the presence of a potentially hazardous defect. In particular, the significant difference between infection levels of muscle tissue of whole and filleted herrings, likely due to differences in the production process, results in different risk of exposure to parasitic antigens. Therefore, a better management of the herring supply chain is required to improve the whole quality and to protect consumers' health.

Research Data Related to this Submission

There are no linked research data sets for this submission. The following reason is given:

Occurrence, distribution and viability of *Anisakis* spp. larvae in various kind of marketed herring products in Italy.

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Abstract

Herring is the third most commercialized fish species in the EU and a common host of *Anisakis* spp. larvae. The aim of this study was to assess the occurrence, distribution and viability of *Anisakis* spp. larvae in different kind of ready to eat (RTE) herring products. One hundred and thirty-five products, consisting of 50 smoked whole specimens and 85 filleted products were collected in Italy from 2016 to 2018. Viscera and muscle of whole herrings were visually inspected and separately submitted to artificial digestion. Filleted products were also visually inspected and digested. Natural and UV light were used for examining the residual material. Nematodes larvae viability was assessed, then they were counted, collected and identified to genus level by optical microscopy. In addition, the *cox2* gene was targeted for the identification of part of the larvae. *Anisakis* spp. larvae were found in 56 products (41.5%) and overall 1715 larvae were collected (range 0-172 larvae/product). Most of the larvae (1559, 91%) were found in the viscera of 49 of the 50 whole herrings (98%). A statistically significant difference ($p<0.001$) was observed between the positivity rate and larval density of the remaining 156 larvae found at muscle level, as 149 larvae were found in the muscle of 31 whole herrings (positivity rate 62%, 0.022 larval density/g), while only 7 larvae were found in the 85 filleted products (positivity rate 7%, 0.001 larval density/g). Larvae were molecularly identified as *A. simplex*. Although all the larvae were dead, the high level of contamination of whole herrings on the market poses some issue related to the presence of a potentially hazardous defect. In particular, the significant difference between infection levels of muscle tissue of whole and filleted herrings, likely due to differences in the production process, results in different risk of exposure to parasitic antigens. Therefore, a better management of the herring supply chain is required to improve the whole quality and to protect consumers' health.

Keywords

Semi-preserved seafood products, anisakids, visible parasites, smoked herrings, marinated herrings, canned herrings

1. Introduction

Anisakid nematodes represent one of the major parasitological risk to humans from the consumption of fish (EFSA, 2010). In fact, *Anisakis* spp. have an indirect life cycle which takes place in an aquatic environment, involving marine mammals and fish-eating birds as definitive hosts, crustaceans as first intermediate hosts, and fish or cephalopods as intermediate or paratenic hosts. The consumption of raw or undercooked seafood harbouring live third stage larvae usually represents the source of human infection (Mattiucci & Nascetti, 2008; Mattiucci et al., 2018). Therefore, if fishery products are intended to be consumed raw, a preventive freezing treatment should be conducted (Reg. (EC) No 853/2004 and further modifications). However, several anisakid antigens are thermostable (Audicana & Kennedy, 2008; Caraballo & Coronado, 2018; Gonzalez- Munoz, Rodriguez- Mahillo, & Moneo, 2010; Rodríguez-Mahillo, Gonzalez-Munoz, de las Heras, Tejada, & Moneo, 2010) and, although the allergenic potential of dead larvae is still discussed (Audicana & Kennedy, 2008; Daschner, Cuéllar, & Rodero, 2012), it is generally agreed that, after sensitisation via infection by live larvae, allergy can be elicited by exposure to allergen alone from killed parasites (EFSA, 2010). In addition, visible parasites, dead or alive, represent a defect (Codex Stan 244-2004; Codex Alimentarius, 2012) and may cause disgust in consumers. Food Business Operators (FBOs) should perform regular checks to avoid the commercialization of products obviously contaminated, which are unfit for human consumption (Reg. (EC) No 178/2002). Therefore, the acquisition of data on the occurrence and distribution of such parasites in the edible parts (mostly flesh) of fish is highly needed and also recommended by EFSA (EFSA, 2010) as an essential source of data for a proper risk analysis.

The Atlantic herring (*Clupea harengus*), distributed in various stocks in the Atlantic Ocean, is a frequent host of *Anisakis simplex*, which is commonly known as the "herring worm" (EFSA, 2010). This term derives from the first confirmed human cases of anisakiasis described in the Netherlands around 1960, which were associated to the consumption of a traditional Dutch specialty made of very lightly salted herrings ("*green herring"*) (Van Thiel, 1962; Van Thiel, Kuipers, & Roskam, 1960). After this, other cases due to the consumption of raw or semi-preserved herring products have been described, mainly in Central Europe (Gall, Reddy, & Regenstein, 2000; Kapral et al., 2009; Lorenz, 1982; Ozcan, Avcu, Pauwels, Mortelé, & De Backer, 2012; Plath et al., 2001).

The publication of the first studies on *Anisakis* spp. in herrings (Van Thiel, 1962; Van Thiel, Kuipers, & Roskam, 1960) lead to the execution of numerous other scientific investigations, in order to better characterize the basic biological knowledge including other possible intermediate/paratenic hosts of this parasite as well as the distribution and the potential zoonotic risk (Berland, 2003).

While the available literature provides a very detailed picture regarding the presence of *Anisakis* spp. in fresh herring (Bao et al., 2017; Campbell et al., 2007; Huang, 1988; Levsen, Lunestad, & Berland, 2005; Levsen et al., 2018; Smith & Wotten, 1975; Tolonen & Karlsbakk, 2003), studies on processed products, both on the detection of parasites (Hauck, 1977; Khalil, 1969; Lagoin, 1980; Levsen & Lunestad, 2010; Panebianco & Lo Schiavo, 1985; Panebianco & Lo Schiavo, 1987; Szostakowska, Myjak, Wyszynski, Pietkiewicz, & Rokicki, 2005; see Table 1 for details) and experimentally investigating the parasites' viability (Grabda, 1974; Grabda, 1983; Priebe, Jendrusch, & Haustedt, 1973), are fewer and generally older.

The Atlantic herring has a long history of commercial exploitation (http://www.gma.org/herring/harvest_and_processing/default.asp). It is the main fish species landed in the European Union in terms of volume, accounting for 15% of the total catches, covering 110% of the European needs. It is also the third most commercialized species among member states, and it

is sold frozen (39% of the total), fresh (35%) or differently processed (dried, smoked, salted, canned) (26%) (EUMOFA, 2017). As regards Italy, a recent survey on fish consumption highlighted that herring products are regularly bought by 13% of the consumers (Greenpeace-Ixè Institute, 2016). Besides the most traditional whole cured and smoked products, such as golden and silver herrings, many kinds of ready to eat products (RTE) made of fillets (smoked, marinated, smoked and marinated and canned) are increasingly appreciated and can be easily purchased in the commercial circuit, fulfilling the growing consumers' request for similar products (authors' note).

The presence of *Anisakis* spp. larvae in RTE products made of anchovies was investigated in a previous study (Guardone et al., 2018) showing a high level of contamination in salted products. Therefore, considering the high prevalence values found for herrings in a preliminary study on different RTE seafood products (Guardone et al., 2016a), the need to update the level of risk associated to the consumption of processed herring products (semi-preserved and preserved) and the increased mediatic attention towards this kind of parasites, the present study aims to investigate the occurrence, distribution and viability of *Anisakis* spp. larvae in various kind of products made of herring sold on the Italian market.

2. Materials and Methods

2.1 Sampling

One hundred and thirty-five herring products were sampled from February 2016 to July 2018 in Tuscany (Northern Italy) in different supermarkets of a national chain and partly at the Border Inspection Point (BIP) of Livorno-Pisa. Samples were selected according to their representativity on the market. Sampled products consisted of 50 smoked whole herrings (2 sub-categories: 25 golden and 25 silver herrings) and 85 filleted products (3 sub-categories: 25 smoked, 30 smoked and marinated, 30 canned) (Fig. 1). The samples were then transported to the FishLab, Department of Veterinary Sciences, University of Pisa, where they were registered and photographed. The

following information were retrieved from the label, when available: name of the food, brand, commercial and scientific denomination of the species, catch area and net weight.

2.2. Parasitological analysis

2.2.1 Analytical procedure. Whole herrings were weighted and gutted. The viscera and the abdominal cavity were visually inspected for the detection of anisakid larvae. Then, the muscle part of whole herrings was manually filleted, weighted and visually inspected. As regards the filleted products, the whole content of the packet was weighted and inspected, including the marinating liquid or sauces, when present, which were carefully removed and visually examined. The visual examination was carried out as described in Commission Regulation (EC) No. 2074/2005, in order to detect the presence of visible endoparasites (Codex Alimentarius Commission, 1971), which were collected. Larvae viability was assessed checking for spontaneous and stimulated movements (Codex stan 244-2004). Microscopical identification (Nikon Eclipse E200) to genus level was performed according to Sakanari & McKerrow (1989) and Berland (1989). The larvae where then counted and stored (4°C in 70° alcohol).

The samples were digested (Trichineasy®, CTSV srl, Brescia) following Guardone et al. (2017). Viscera and muscle (including the skin and the belly flaps) of whole herrings were separately digested. The full content of filleted products, including marinating liquid or sauces, was digested.

Also the nematode larvae detected after digestion were observed for assessing viability, collected, identified to genus level, counted and stored in 70% alcohol. The number was registered and summed to the number of those found after the visual inspection. The larvae found at visceral and muscle level of each product where stored in separate tubes.

2.3 Molecular identification

2.3.1. DNA extraction, quantification and quality assessment. Total DNA extraction and quali-quantitative assessment from a subset of larvae (from 1 to 4 larvae per product) was performed

according to Guardone et al. (2016b). In addition, DNA integrity was assessed according to Giusti et al., (2019).

2.3.2. Amplification and sequencing of a long fragment of the mitochondrial cytochrome c oxidase subunit II (cox2) gene. Amplification and sequencing of a 629 bp fragment (long fragment) of the mitochondrial cytochrome *c* oxidase subunit II (*cox2*) gene was performed as described in Guardone et al., (2018).

2.3.3. Amplification and sequencing of a short fragment of the mitochondrial cytochrome c oxidase subunit II (cox2) gene. In case of failure of the amplification of the long fragment (section 2.3.2), a short fragment of the same gene was targeted. In order to do this, at least 10 reference sequences of the *cox2* gene, when available, for each 9 currently accepted *Anisakis* species were retrieved from GenBank and aligned using Clustal W in Bio Edit version 7.0.9 (Hall, 1999). The primer pair was designed by searching inter-species polymorphic regions throughout the sequence, being careful to avoid mismatches in critical positions (Table 1SM). Then, the ability of the selected region to discriminate to species level was verified by BLAST. Finally, the following primers, targeting a 246-bp fragment, were designed: An-F2 (5′-GTTATGAGTTTAGKGATATTCCBGG-3′) and AnR2 (5′- AGTWGGAAAACTGTAAGAAAVAG -3′). Amplification was performed with the same PCR protocol described in subsection 2.3.2 using the following cycling program: initial denaturation at 95 °C for 3 min; 45 cycles at 95 °C for 20 s, 52°C for 20 s, 72 °C for 25 s; final extension at 72 °C for 10 min., Sequences were obtained and analyzed as described in section 2.3.2.

2.4 Statistical analysis

The following parameters were calculated for both whole and filleted products and sub-categories: number of larvae per product, prevalence/positivity rate (number of products contaminated with at least one larva/number of total products), range and larval density per gram (n of larvae/gram of examined tissue). In addition, the mean abundance (MA) and the mean intensity

(MI) were calculated only for whole herrings. When possible (depending on the product type), all parameters were calculated both at visceral and muscle level.

As regards larvae found at visceral level (thus only for whole specimens), the prevalence values of golden and silver smoked herrings was compared using a chi squared test, while MA, MI, number of larvae per product and the larval density between the two subcategories were compared using a Mann-Whiney test. As regards larvae found in the muscle, the comparisons made and the tests used are shown in Table 2. All results were considered significant when $p<0.05$.

The *rho* coefficient was used for investigating the correlation between number of larvae in viscera and in muscle (for whole samples) as well as the correlation between the number of larvae and the weight of the products (whole and filleted were combined together). The non-parametric test was used because of the violation of the Pearson's *r* assumptions. The software SPSS ® vs 15 for Windows was used for all statistical analyses.

3. Results

3.1 Occurrence, distribution and viability of the larvae

In fifty-six (41.5%, 95% CI: 33.2-49.8%) out of the 135 products analysed at least one visible larva was found. Totally, 1715 visible larvae were collected, all of which microscopically identified as *Anisakis* sp. The large majority of the larvae (1559, 90.9%) was found in the viscera of 49 out of the 50 whole smoked herrings examined (prevalence: 98%, 95% CI: 94.1-100%). No differences were observed between the two subcategories of whole herrings (golden and silver) in respect to the different epidemiological parameters compared (Table 3). The weight of the specimens of the two categories was also similar (average weight 239.47 ± 71.04 g and 257.90 ± 49.06 g for silver and golden herrings, respectively).

The remaining 156 larvae (9.1% of the total larvae collected) were found at muscle level. Interestingly, a highly significant difference ($p <0.001$) emerged between the occurrence and larval density observed for the muscle of whole herrings and for filleted products. In fact, while 149 larvae

were found in the fillets of 31 whole herrings (positivity rate 62%, 3 larvae/product on average, 0.022 larval density/gram), only 7 larvae were found in 6 of the 70 filleted products (positivity rate 8.6%, 0.1 larvae/product on average, 0.001 larval density/gram) (Table 4).

No significant differences were observed comparing muscle contamination of golden and silver whole herrings and comparing smoked, marinated and canned fillets (Table 4).

Only dead parasites were found, as spontaneous and stimulated movements were never observed.

A significant correlation (*rho*=0.31 *p*=0.02) was observed between the number of the larvae in the viscera and those in the muscles: i.e. samples with a high number of larvae in the viscera are likely to have also high numbers of larvae in the muscles. Another significant correlation was found between the number of larvae and the weight of fillets (*rho*=0.56 *p*<0.001), indicating that the higher is the weight of the samples, the higher is the number of detected larvae.

As concerns data on the geographical origin, this was indicated only for 67 samples out of the 75 products falling in the scope of Regulation EU No 1379/2017 (smoked whole and fillets). Most of the products (n=48, 71.6%) reported FAO area 27 (Atlantic, Northeast), only in few cases (n=13, 19.4%) with specification of the sub area 27.2 or 27.5. The remaining 19 products reported FAO area 21(Northwest Atlantic).

3.2. *Molecular identification*

A good quality sequence was obtained from each sample belonging to the subset of larvae (n= 143) submitted to molecular analysis. For most of the specimens (n=95, 66.4%) a long fragment (629 bp) of the *cox2* gene was amplified. For the remaining 48 larvae (33.6%) only the short fragment (246 bp) was obtained using primers designed in this study. The retrieved sequences were compared to those deposited in GenBank obtaining for both fragments identity values between 99 and 100% only with sequences of *A. simplex*.

4. Discussion

4.1 Occurrence, distribution, viability and identification of the larvae

Several methods can be used to detect third-stage larvae of anisakids in fish, including classical methods such as visual inspection and candling, artificial peptic digestion, UV-press, as well as advanced imaging technologies such as X-rays, electromagnetism, spectroscopy and Magnetic Resonance Imaging (Bao et al., 2017). In this study, processed herring products sold on the Italian market were firstly analysed to detect the presence of visible endoparasites (as defined in Codex Alimentarius Commission, 1971) by visual examination (Commission Regulation (EC) no. 2074/2005), also considering this procedure could theoretically be performed by consumers. In fact, as stated by Codex Stan 244-2004, it simply consists in an observation of the fish, without magnification, in an adequately lighted room (where a newspaper may be read easily). Interestingly, 1136 larvae were easily detected by visual inspection at visceral level (accounting for 73% of the total visceral larvae found). The use of this type of examination on the muscle, which in general is known to have a lower sensitivity than the inspection of the viscera (Bao et al., 2017), was found particularly difficult for herring products given the dark colouring of the fillets (Levsen, Lunestad, & Berland, 2005), which is further intensified by treatments such as smoking. A higher sensitivity of the digestion method even when compared to candling over a fluorescent light was observed by McGladdery (1986) for herring fillets. Therefore, after the first examination, all products were submitted to a digestion protocol based on the one described by Guardone et al., (2017) and used also in Guardone et al., (2018) using the Trichineasy® system. For whole products, viscera and muscles were separately digested, as data on the location of larvae of *Anisakis* spp. in fish are of fundamental importance (Cipriani et al., 2016).

The prevalence value of 98% found in whole herrings at the visceral level corresponds to the value found by Levsen & Lunestad (2010) and it agrees with the prevalence values commonly found in the literature (Table 1: Bao et al., 2017; Levsen, Lunestad, & Berland, 2005; Tolonen & Karlsbakk, 2003). In fact, in most of the studies the prevalence values are higher than 80%, and always above 65%. However, the comparison on the basis of the geographical origin between our

results and those of the aforementioned studies is not feasible because this study relied on products sold on the market, often lacking specific geographic details on the label. In fact, only the information regarding the Major Fishing Areas were generally available (see section 3.1).

It is known that most of the larvae of *Anisakis* spp. are located in the viscera, typically encapsulated in a spiral on the surface of the organs (Mattiucci, Cipriani, Paoletti, Levsen, & Nascetti, 2017). The localization seems to be influenced by the visceral topography and the physiological stage of the individual (Smith & Hemmingsen, 2003). In particular, it has been observed that the main site of accumulation of larvae in herrings is the region occupied by the caudal part of the stomach and the pneumatic duct (Bao et al., 2017; Sluiters, 1974; Tolonen & Karlsbakk, 2003). It is likely that *A. simplex* accumulates here in clupeids due to the "Y" shape of their stomachs. Gonads can act as a physical barrier or a trap for *A. simplex* larvae (Bao et al., 2017), preventing their migration to muscle level during the breeding period (Bao et al., 2017; McPherson, Slotte, Kvamme, Meier, & Marshall, 2011). This data is relevant in light of the fact that some preparations such as whole smoked herrings, as those analysed in this study, as well as *surströmming* or bloaters do not always include fish gutting (http://www.fao.org/3/x5933e/x5933e01.htm). In addition, in some countries, egg-based dishes, often still inside the ovaries, are also consumed, such as *kazunoko*, a typical Japanese dish where the eggs are marinated with a soy sauce (https://www.japantimes.co.jp/news/2016/11/22/national/new-years-salted-herring-roe-sells-record-%C2%A5100000/). Thus, in these cases also the viscera represent “edible parts” and may be a source of human infection in case the larvae inactivation treatments were ineffective.

The prevalence of *Anisakis* spp. in the muscle results to be very variable in literature studies (values from 0 to 95% are reported, Table 1), although it is generally lower than the visceral values (Levsen, Lunestad, & Berland, 2005; Levsen et al., 2018; Smith & Wotten, 1975). Also in this study the prevalence value of larvae in the muscle (62%) of whole smoked herrings, although high,

was lower than the visceral prevalence. A correlation between the number of larvae in the viscera and in the muscle was found, in agreement with McGladdery (1986) who found the prevalence and mean intensity of visceral larvae to be higher in specimens with larvae in the muscle. A positive association between the number of larvae in the flesh and the number of larvae in the viscera was also found by Smith & Wotten (1975). In the same work an evident larval migration from the viscera to the muscle was observed when comparing herrings gutted immediately after capture or gutted 14 h or 37 h after capture and stored on ice (Smith & Wotten, 1975). This fact is important since, in general, the larvae in the muscle represent the main source of human infection (Cipriani et al., 2016).

Interestingly, a statistically significant difference in the prevalence found at muscle level in whole herrings (62%) and in industrially filleted products (7%) was found. A similar difference was also observed by Levsen & Lunestad (2010), who examined manually and industrially produced (automatically trimmed and skinned) fillets of herring at the processing plant, finding a prevalence varying from 42-70% and 8-10% in the manually and in the industrially produced fillets, respectively. The difference observed in the present study and by Levsen & Lunestad (2010) may be explained by the fact that during the industrial preparation of the fillets the belly flaps are generally discarded (Karl, 2008; Levsen & Lunestad, 2010). This ventral muscle part is known to be the most contaminated fillet region, probably due to its proximity to the visceral cavity (Bao et al., 2017; Cipriani et al., 2016; Levsen et al., 2018). In fact, as reported in Levsen et al., (2018), 87-91% of the muscular larvae are located in the belly flaps, without significant differences between the right and the left portion.

Some similarities can be found also between the present study and the work conducted by Szostakowska, Myjak, Wyszynski, Pietkiewicz, & Rokicki, (2005), in which both whole herrings (salted or smoked) and marinated, salted, spiced (ready to eat) products were examined for larvae of *Anisakis* spp. Overall, 6 types of products out of 39 (15.4%) were positive; most of the larvae were

collected from salted or smoked ungutted products, although some were found in a packet of salted fillets and of ready-to-eat fillets in spiced pickle. To be noted that two live larvae of *A. simplex* were found in the ready-to-eat spice pickle. Similarly, in a study on salted and smoked whole herrings and smoked fillets conducted in Italy in 1987 one larva still alive but not infectious. (Panebianco & Lo Schiavo, 1987). Particularly interesting are the results of the work of Hauk (1977) that investigated the presence of live larvae in fresh and processed products and found a higher prevalence of flesh larvae but a lower viability rate in processed products compared to fresh ones. On the contrary, in the present study, despite the high prevalence find both at muscular and visceral level, in particular for whole herrings, all the larvae were devitalized. In fact, the cured and smoked whole herrings analysed, according to the information provided by the producer, had undergone a salting procedure for 36 days, in accordance whit the suggestion of the Centre d'Expérimentation & Valorisation des Produits, stating that 21 days are required to inactivate *A. simplex* larvae in salted herring fillets without freezing treatment (CEVPM, 2005). Nevertheless, the companies producing whole herrings also declared a preventive treatment at -20 °C for 24 h. The presence of dead larvae is in agreement with a previous study of Panebianco & Lo Schiavo (1985), which was conducted on whole salted and smoked herrings sampled on the Italian market. It also indicates a correct management of the parasitological risk by the manufacturing companies, as required by the legislation in force (Reg. (EC) No 853/2004 and further modifications).

Larvae devitalization is very important considering the presence in the analysed products, confirmed by the molecular analysis, of *A. simplex*, both at visceral and muscle level. *A. simplex*, together with *A. pegreffii*, is the main etiological cause of human anisakiasis worldwide (Mattiucci & Nascetti, 2008). As regards the molecular identification, the amplified short fragment showed an excellent discriminatory ability, retrieving 100% of identity only with sequences of *A. simplex*, proving to be useful for the identification of *Anisakis* spp. larvae with degraded DNA in processed products. The exclusive presence of *A. simplex* is in agreement with other studies according to

which only this species is present in the Atlantic herring (Bao et al., 2017; Levsen & Lunestad, 2010; Mattiucci & Nascetti, 2006; Mattiucci, D'Amelio, & Rokicki, 1989; Tolonen & Karlsbakk, 2003). The presence of *A. simplex* further explains the high muscle prevalence, as this species is able to penetrate the fish muscle at a much higher rate than *A. pegreffii* (Cipriani et al., 2015; Suzuki, Murata, Hosaka, & Araki, 2010).

4.2 Risks related to the presence of live and dead Anisakis simplex larvae in herring products

As already mentioned, herring is one of the species most frequently associated to the presence of *Anisakis* spp. among the many fish acting as intermediate/paratenic hosts of these parasites. After the first record describing "*a nematode parasitic to herring, causing acute abdominal syndromes in man*" (Van Thiel, Kuipers, & Roskam, 1960), the Japanese authorities recognized *Anisakis* spp. as the causative agent of an already known disease, which was acknowledged as a public health problem (Berland, 2003).

Since then, other human cases related to the consumption of herrings have been reported. The first five cases of anisakiasis in Germany were described in 1982 due to ingestion of raw or undercooked herring containing larvae of *Anisakis* spp. (Lorenz, 1982). In 2001 a severe case of gastric anisakiasis with symptoms appearing four hours after the consumption of homemade herring pickled in vinegar occurred again in Germany (Plath et al., 2001). More recently a patient developed abdominal pain one day after the consumption of raw herrings from the North Sea (Ozcan, Avcu, Pauwels, Mortelé, & De Backer, 2012). As regards processed herring products, an interesting case is reported in the study of Kapral et al. (2009), describing the first Austrian case of anisakiasis. The patient regularly ate rolled fillets in brine produced by a company from Vienna (Kapral et al., 2009). Such products, known as "rollmops", are very popular in Central Europe, as well as other products in brine (Gall, Reddy, & Regenstein, 2000).

In this light, it appears clear that herring represents a potential source of zoonotic infection, following both the consumption of fresh and processed products that have not been correctly

treated. The importance of characterizing the *Anisakis* risk also for the category of processed products should be reiterated, considering that the presence of these products on the Italian market is growing, following the change in eating habits related to the need to have more practical and quick ways to prepare products (http://www.usl3.toscana.it/Sezione.jsp?idSezione=713).

Prevention of the risk of *Anisakis* infection relies on technological treatments able to devitalize larvae present in the fish (EFSA, 2010). Even though physical and chemical treatments have been proposed (Anastasio et al., 2016; D'Amico et al., 2014) freezing is at present the only treatment approved by the regulation in force (Reg. (EC) No 853/2004 and further amendments) for fish intended to be consumed raw or undercooked. Observance of this regulation is compulsory for FBOs, and food safety authorities also give general freezing recommendations for households to avoid infection (D'Amico et al., 2014).

However, it must be remembered that, despite the fact that the main parasitological risk is linked to the presence of vital larvae of nematodes, there is the possibility that even those devitalized by heat treatments are responsible for allergic reactions in sensitized patients (Bao et al., 2017; Bilska-Zając et al., 2016; EFSA, 2010; Mattiucci, Cipriani, Paoletti, Levsen, & Nascetti, 2017; Nieuwenhuizen et al., 2013), thanks to the thermostability of some antigens (Caraballo & Coronado, 2018; Gonzales-Munoz Rodriguez- Mahillo, & Moneo, 2010; Rodríguez-Mahillo, Gonzalez-Munoz, de las Heras, Tejada, & Moneo, 2010). It is believed that this occurs in subjects previously sensitized by contact with a vital larva, which, however, could be subclinical or undiagnosed (Audicana & Kennedy, 2008). To avoid the appearance of symptoms, sensitized patients are advised to consume frozen or heat-treated fishery products (Carballeda-Sangiao et al., 2016). However, in a study investigating changes over time in IgE sensitization to allergens of *Anisakis* spp. an increase of allergic symptoms and/or specific IgE titres in three allergic patients were observed during the follow-up period, in coincidence with the consumption of previously frozen fish. These findings indicate that *Anisakis* spp. allergens induce long-lived IgE responses

(Carballeda-Sangiao et al., 2016). Similarly, among allergic patients suffering from anaphylaxis in Spain at least four reactions were specifically linked to the consumption of cooked or canned pilchards (Audicana & Kennedy, 2008).

This aspect appears particularly relevant given the high prevalence of larvae in the viscera and in the muscle detected in this study. In particular, the content of larvae is more than 20 times higher in fillets obtained from whole herrings than in industrial fillets, thus exposing consumers to a higher risk of unknowingly ingest larvae, and thus allergens, of *Anisakis* spp., in accordance with what has already been observed by Levsen & Lunestad (2010).

In addition, as observed in the whole herrings examined, dead larvae may be evident, especially in the viscera (Fig. 2), potentially inducing disgust in consumers. As a fact, anisakids are important not only for public health, but also from an economic and social point of view, as they can reduce the marketability of fishery products (Bao et al., 2017).

5. Conclusions

Herring is the third commercial species traded among member States; in 2016 its trade reached 457.369 tons increasing its total value of 26 million euro. Even though consumers expect parasite-free fishery products, commercialized wild fish is naturally at risk of carrying parasites. Therefore, processing industries should pay attention to the control of larvae, as hazard and defect represented by the presence of larvae. The results of the present study are reassuring in relation to the possibility of developing anisakiasis due to the absence of live larvae in different kind of products made of herring sold on the Italian market. On the contrary, the high level of contamination, in particular of whole smoked herrings both at visceral and muscle level, still poses some issues related to the presence of obviously infected specimens on the market which may create disgust in consumers, as well as the possibility of ingesting dead larvae. Therefore, accurate gutting of whole herrings may be suggested to consumers (Panebianco & Lo Schiavo, 1987). Finally, a better management in the

herring processing industry is required to improve the whole quality and protect consumers' health and rights.

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Figures captions

Figure 1. Presentation of the products analysed in the present study: whole smoked golden herrings (A); whole smoked silver herrings (B); smoked fillets (C); smoked and marinated fillets (D); canned fillets (E).

Figure 2. Examples of obviously contaminated samples (whole smoked herrings) found in the present~~is~~ study.

References

Anastasio, A., Smaldone, G., Cacace, D., Marrone, R., Voi, A. L., Santoro, M., Cringoli, G., & Pozio, E. (2016). Inactivation of *Anisakis pegreffii* larvae in anchovies (*Engraulis encrasicolus*) by salting and quality assessment of finished product. *Food Control*, 64, 115-119.

Audicana, M. T., & Kennedy, M. W. (2008). *Anisakis simplex*: from obscure infectious worm to inducer of immune hypersensitivity. *Clinical Microbiology Reviews*, 21(2), 360-379.

Bao, M., Strachan, N. J., Hastie, L. C., MacKenzie, K., Seton, H. C., & Pierce, G. J. (2017). Employing visual inspection and Magnetic Resonance Imaging to investigate *Anisakis simplex* sl infection in herring viscera. *Food control*, 75, 40-47.

Berland, B., (1989). Identification of larval nematodes from fish. In: Möller, H. (Ed.), *Nematode Problems in North Atlantic Fish*. Report from a Workshop in Kiel 3–4 April 1989. International Councilfor the Exploration of the Sea CM/F, 6.

Berland, B. (2003). *Anisakis* spp. In: Akuffo, H., Ljungstrom, I., Linder, E., Wahlgren, M. (Ed.) *Parasites of the colder climates*, 161. CRC Press, Taylor & Francis group.

Bilska-Zając, E., Lalle, M., Różycki, M., Chmurzyńska, E., Kochanowski, M., Karamon, J., Sroka, J., Pozio, E., Cencek, T., 2016. High prevalence of Anisakidae larvae in marketed frozen fillets of pink salmons (*Oncorhynchus gorbuscha*). *Food Control* 68, 216–219.

Campbell, N., Cross, M. A., Chubb, J. C., Cunningham, C. O., Hatfield, E. M., & MacKenzie, K. (2007). Spatial and temporal variations in parasite prevalence and infracommunity structure in herring (*Clupea harengus* L.) caught to the west of the British Isles and in the North and Baltic Seas: implications for fisheries science. *Journal of Helminthology*, 81(2), 137-146.

Caraballo, L., & Coronado, S. (2018). Parasite allergens. *Molecular Immunology*, 100, 113-119.

Carballeda-Sangiao, N., Rodriguez-Mahillo, A. I., Careche, M., Navas, A., Moneo, I., & González-Muñoz, M. (2016). Changes over Time in IgE Sensitization to Allergens of the Fish Parasite *Anisakis* spp. *PLoS neglected tropical diseases*, 10(7), e0004864.

CEVPM (2005). Etude des conditions de destruction des larves d'*Anisakis simplex* dans le hareng salé au sel sec destiné à la fabrication de harengs saurs traditionnels. http://bibliomer.ifremer.fr/consult.php?ID=2007-4062 Accessed 10/01/2019.

Cipriani, P., Acerra, V., Bellisario, B., Sbaraglia, G.L., Cheleschi, R., Nascetti, G., & Mattiucci, S. (2016). Larval migration of the zoonotic parasite *Anisakis pegreffii* (Nematoda: Anisakidae) in European anchovy, *Engraulis encrasicolus*: implications to seafood safety. *Food Control*, 59, 148–157.

Cipriani, P., Smaldone, G., Acerra, V., D'Angelo, L., Anastasio, A., Bellisario, B., Palma, G., Nascetti, G., & Mattiucci, S. (2015). Genetic identification and distribution of the parasitic larvae of *Anisakis pegreffii* and *Anisakis simplex* (ss) in European hake *Merluccius merluccius* from the Tyrrhenian Sea and Spanish Atlantic coast: implications for food safety. *International Journal of Food Microbiology*, 198, 1-8.

Codex Alimentarius (2012). Code of Practice for Fish and Fishery Products. World Health Organization and Food and Agriculture Organization of the United Nations, Rome. http://www.fao.org/docrep/pdf/011/a1553e/a1553e00.pdf Accessed 10/01/2019.

Codex Alimentarius Commission (1971). Report of the eighth session of the joint FAO/WHO Codex Alimentarius Commission: recommended international standard for quick frozen filet of cod and haddock. CAC/RS-50-1971. http://www.fao.org/docrep/meeting/005/c0531e/C0531E09.htm Accessed 10/03/2018.

Codex stan 244-2004. Standard for salted atlantic herring and salted sprat. http://www.fao.org/fao-who-codexalimentarius/sh-proxy/ru/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCODEX%2BSTAN%2B244-2004%252FCXS_244e.pdf Accessed 05/12/2018

Commission Regulation (EC) No 2074/2005 of 5 December 2005 laying down implementing measures for certain products under Regulation (EC) No 853/2004 of the European Parliament and of the Council and for the organisation of official controls under Regulation (EC) No 854/2004 of the European Parliament and

of the Council and Regulation (EC) No 882/2004 of the European Parliament and of the Council, derogating from Regulation (EC) No 852/2004 of the European Parliament and of the Council and amending Regulations (EC) No 853/2004 and (EC) No 854/2004. *Official Journal of the European Union*, L 338/27.

D'Amico, P., Malandra, R., Costanzo, F., Castigliego, L., Guidi, A., Gianfaldoni, D., & Armani, A. (2014). Evolution of the *Anisakis* risk management in the European and Italian context. *Food Research International*, 64, 348-362.

Daschner, A., Cuéllar, C., & Rodero, M. (2012). The *Anisakis* allergy debate: does an evolutionary approach help? *Trends in Parasitology*, 28(1), 9-15.

EFSA (2010). Scientific opinion on risk assessment of parasites in fishery products. EFSA J. 8, 1543. http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2010.1543/epdf, Accessed 21/05/2018.

EUMOFA (2017). EU consumer habits regarding fishery and aquaculture products. Final report. https://www.eumofa.eu/documents/20178/84590/EU+consumer+habits_final+report+.pdf/5c61348d-a69c-449e-a606-f5615a3a7e4c Accessed 11/03/2018.

Gall, K., Reddy, K. P., & Regenstein, J. M. (2000). Specialty seafood products. *Marine and Freshwater Products Handbook*, 403-416.

Giusti, A., Tinacci, L., Sotelo, C. G., Acutis, P. L., Ielasi, N., & Armani, A. (2019). Authentication of ready-to-eat anchovy products sold on the Italian market by BLAST analysis of a highly informative cytochrome b gene fragment. *Food Control*, 97, 50-57.

Gonzalez- Munoz, M., Rodriguez- Mahillo, A. I., & Moneo, I. (2010). Different Th1/Th2 responses to *Anisakis simplex* are related to distinct clinical manifestations in sensitized patients. *Parasite Immunology*, 32(1), 67-73.

Grabda J. (1974). [Technological processes in salted and spiced herring production in case of nematode invasion, from the standpoint of public health. Information on studies completed in 1972.] *Cz. II, X. Rybactwo*, 221, (In Polish).

Grabda, J. (1983). Studies on viability and infectivity of *Anisakis simplex* stage III larvae in fresh salted and spiced Baltic herring. *Acta Ichthyologica et Piscatoria*, 13(2).

Greenpeace-Ixe Institute (2016). Le abitudini di consumo di prodotti ittici in Italia https://www.greenpeace.org/archive-italy/Global/italy/report/2016/mare/Greenpeace-sondaggio-consumo_pesce_IT.pdf Accessed 05/12/2018.

Guardone, L., Lodola, L.B., Guidi, A., Armani, A., 2016a. Anisakis spp. in ready-to-eat fish products. In: XXIX SOIPA National Congress, Bari 21–24 June 2016, Available at: https://www.soipa.it/2015/12/23/xxix-congresso-nazionale-soipa/ Accessed date: 24 October 2017.

Guardone, L., Malandra, R., Costanzo, F., Castigliego, L., Tinacci, L., Gianfaldoni, D., Guidi, A., & Armani, A. (2016b). Assessment of a sampling plan based on visual inspection for the detection of anisakid larvae in fresh anchovies (*Engraulis encrasicolus*). A first step towards official validation? *Food Analytical Methods*, 1-10.

Guardone, L., Nucera, D., Pergola, V., Costanzo, F., Costa, E., Guidi, A., Gianfaldoni, D., & Armani, A. (2017). A rapid digestion method for the detection of anisakid larvae in European anchovy (*Engraulis encrasicolus*): visceral larvae as a predictive index of the overall level of fish batch infestation and marketability. *International Journal of Food Microbiology*, 250, 12-18.

Guardone, L., Nucera, D., Lodola, L. B., Tinacci, L., Acutis, P. L., Guidi, A., & Armani, A. (2018). *Anisakis* spp. larvae in different kinds of ready to eat products made of anchovies (*Engraulis encrasicolus*) sold in Italian supermarkets. *International Journal of Food Microbiology*, 268, 10-18.

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In: Nucleic acids symposium series, London, vol. 41, pp. 95-98.

Hauck, A. K. (1977). Occurrence and survival of the larval nematode *Anisakis* sp. in the flesh of fresh, frozen, brined, and smoked Pacific herring, *Clupea harengus pallasi*. *Journal of Parasitology*, 515-519.

Huang, W.Y. (1988). [Anisakids and human anisakiasis. 2. Investigation of the anisakids of commercial fish in the district of Paris]. *Annales de parasitologie humaine et comparée*, 63, 197-208 (In French).

Khalil, L.F. (1969). Larval nematodes in the herring (*Clupea harengus*) from British coastal waters and adjacent territories. *Journal of the Marine Biological Association of the United Kingdom*, 49(3), 641-659.

Kapral, C., Haditsch, M., Wewalka. F., Schatzlmayr, W., Lenz, K., & Auer, H. (2009). [The first case of anisakiasis acquired in Austria]. *Zeitschrift fur Gastroenterologie*, 47, 1059-1061 (In German).

Karl, H. (2008). Nematode larvae in fish on the German market: 20 years of consumer related research. *Archiv für lebensmittelhygiene*, 59, 107–116.

Lagoin, Y. (1980). Infection of humans by the nematode *Anisakis simplex* in herrings. *Bulletin de l'Académie Vétérinaire de France*, 53, 139-146.

Levsen, A., Lunestad, B. T., & Berland, B. (2005). Low detection efficiency of candling as a commonly recommended inspection method for nematode larvae in the flesh of pelagic fish. *Journal of Food Protection*, 68(4), 828-832.

Levsen, A., & Lunestad, B. T. (2010). *Anisakis simplex* third stage larvae in Norwegian spring spawning herring (*Clupea harengus* L.), with emphasis on larval distribution in the flesh. *Veterinary Parasitology*, 171(3-4), 247-253.

Levsen, A., Svanevik, C. S., Cipriani, P., Mattiucci, S., Gay, M., Hastie, L. C., Bušelić, I., Mladineo, I., Karl, H., Ostermeyer, U., Buchmann, K., Højgaard, D. P., González, A. F., Pascual, S., & Pierce, G. J. (2018). A survey of zoonotic nematodes of commercial key fish species from major European fishing grounds—Introducing the FP7 PARASITE exposure assessment study. *Fisheries Research*, 202, 4-21.

Lorenz, G. (1982). [Morphological changes of anisakiasis]. *Zentralblatt fur allgemeine Pathologie u. pathologische Anatomie*, 126, 477-482 (In German).

Mattiucci, S., D'Amelio, S., & Rokicki, J. (1989). Electrophoretic identification of *Anisakis* sp. larvae (Ascaridida: Anisakidae) from Clupea harengus L. in Baltic Sea. *Parassitologia*, 31(1), 45-49.

Mattiucci, S., & Nascetti, G. (2006). Molecular systematics, phylogeny and ecology of anisakid nematodes of the genus *Anisakis* Dujardin, 1845: an update. *Parasite*, 13, 99-113.

Mattiucci, S., & Nascetti, G. (2008). Advances and trends in the molecular systematics of anisakid nematodes, with implications for their evolutionary ecology and host—parasite co-evolutionary processes. *Advances in Parasitology*, 66, 47-148.

Mattiucci, S., Cipriani, P., Paoletti, M., Levsen, A., & Nascetti, G. (2017). Reviewing biodiversity and epidemiological aspects of anisakid nematodes from the north-East Atlantic Ocean. *Journal of Helminthology*, 91, 422-439.

Mattiucci, S., Giulietti, L., Paoletti, M., Cipriani, P., Gay, M., Levsen, A., Klapper, R., Karl, H., Bao, M., Pierce, G.J., & Nascetti, G. (2018). Population genetic structure of the parasite *Anisakis simplex* (ss) collected in *Clupea harengus* L. from North East Atlantic fishing grounds. *Fisheries Research*, 202, 103-111.

McGladdery, S. E. (1986). *Anisakis simplex* (Nematoda: Anisakidae) infection of the musculature and body cavity of Atlantic herring (*Clupea harengus harengus*). *Canadian Journal of Fisheries and Aquatic Sciences*, 43(7), 1312-1317.

McPherson, L. R., Slotte, A., Kvamme, C., Meier, S., & Marshall, C. T. (2011). Inconsistencies in measurement of fish condition: A comparison of four indices of fat reserves for Atlantic herring (*Clupea harengus*). *ICES Journal of Marine Science*, 68(1), 52-60.

Nieuwenhuizen, N., Lopata, A. L., Jeebhay, M. F., De'Broski, R. H., Robins, T. G., & Brombacher, F. (2006). Exposure to the fish parasite *Anisakis* causes allergic airway hyperreactivity and dermatitis. *Journal of Allergy and Clinical Immunology*, 117(5), 1098-1105.

Ozcan, H.N., Avcu, S., Pauwels, W., Mortelé, K.J., & De Backer, A.I. (2012). Acute intestinal anisakiasis: CT findings. *Acta Gastroenterologica Belgica*, 75, 364-365.

Panebianco, A., & Lo Schiavo, A. (1985). [Indagine sulla presenza di larve Anisakidi in aringhe salate e affumicate del commercio. Considerazioni d'ordine inspettivo.] *Clinica Veterinaria*, 108(3), 180-184 (In Italian).

Panebianco A., & Lo Schiavo A. (1987). [Ulteriori indagini sulla presenza di larve Anisakis in aringhe salate e affumicate. Riscontro di una larva viva.] *Industrie Alimentari*, 26, 778-780 (In Italian).

Plath, F., Holle, A., Zendeh, D., Möller, F.W., Barten, M., Reisinger, E.C., & Liebe, S. (2001). [Anisakiasis of the stomach-a case report from Germany]. *Zeitschrift fur Gastroenterologie*, 39, 177-180 (In German).

Priebe, K., Jendrusch, H., & Haustedt, U. (1973). [Problems and experimental investigations on the destruction of the penetration ability of *Anisakis* larvae in herring during preparation by cold marinading]. *Archiv für lebensmittelhygiene*, 24, 21 (In German).

Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. *Official Journal of the European Union*, L 31/1-24.

Regulation (EC) (2004) No 853 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for on the hygiene of foodstuffs. *Official Journal of the European Union*, L 139/55.

Regulation (EU) No 1379/2013 of the European parliament and of the council of 11 december 2013 on the common organisation of the markets in fishery and aquaculture products, amending council regulations (EC) No 1184/2006 and (EC) No 1224/2009 and repealing council regulation (EC) No 104/2000. *Official Journal of the European Union* L354, 1-21.

Rodríguez-Mahillo, A. I., Gonzalez-Munoz, M., de las Heras, C., Tejada, M., & Moneo, I. (2010). Quantification of *Anisakis simplex* allergens in fresh, long-term frozen, and cooked fish muscle. *Foodborne Pathogens and Diseases*, 7(8), 967-973.

Sakanari, J.A., & McKerrow, J.H. (1989). Anisakiasis. *Clinical Microbiology Reviews*, 2 (3), 278–284.

Smith, J. W., & Hemmingsen, W. (2003). Atlantic cod *Gadus morhua* L.: Visceral organ topography and the asymmetrical distribution of larval ascaridoid nematodes in the musculature. *Ophelia*, 57(3), 137-144.

Smith, J. W., & Wootten, R. (1975). Experimental studies on the migration of *Anisakis* sp. larvae (Nematoda: Ascaridida) into the flesh of herring, *Clupea harengus* L. *International Journal of Parasitology*, 5, 133-136.

Sluiters, J. (1974). *Anisakis* sp. larvae in the stomachs of herring (*Clupea harengus* L.). *Zeitschrift für parasitenkunde*, 44, 279-288.

Suzuki, J., Murata, R., Hosaka, M., & Araki, J. (2010). Risk factors for human *Anisakis* infection and association between the geographic origins of *Scomber japonicus* and anisakids nematodes. *International Journal of Food Microbiology*, 137, 88–93.

Szostakowska, B., Myjak, P., Wyszynski, M., Pietkiewicz, H., & Rokicki, J. (2005). Prevalence of anisakin nematodes in fish from Southern Baltic Sea. *Polish journal of microbiology*, 54, 41.

Tolonen, A., & Karlsbakk, E. (2003). The parasite fauna of the Norwegian spring spawning herring (*Clupea harengus* L.). *ICES Journal of Marine Science*, 60, 77-84.

Unger, P., Klimpel, S., Lang, T., & Palm, H.W. (2014). Metazoan parasites from herring (*Clupea harengus* L.) as biological indicators in the Baltic Sea. *Acta Parasitologica*, 59(3), 518-528.

Van Thiel, P. H., Kuipers, F. C., & Roskam, R. T. (1960). A nematode parasitic to herring, causing acute abdominal syndromes in man. *Tropical and Geographical Medicine*, 2, 97-113.

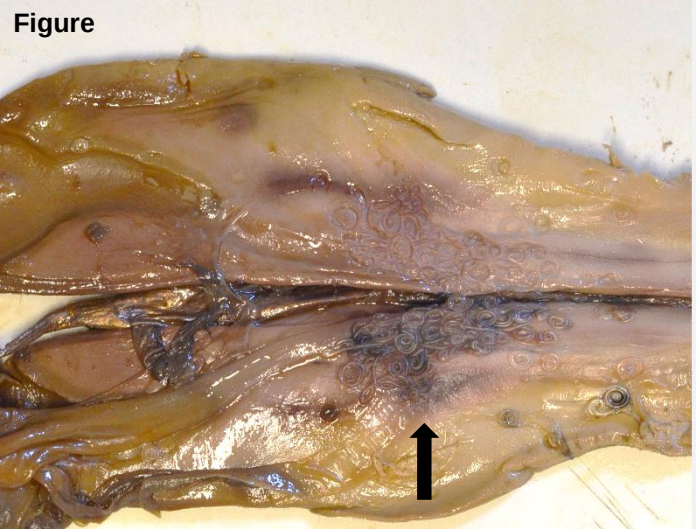
Van Thiel, P. H. (1962). Anisakiasis. *Parasitology*, 52, 16-17.

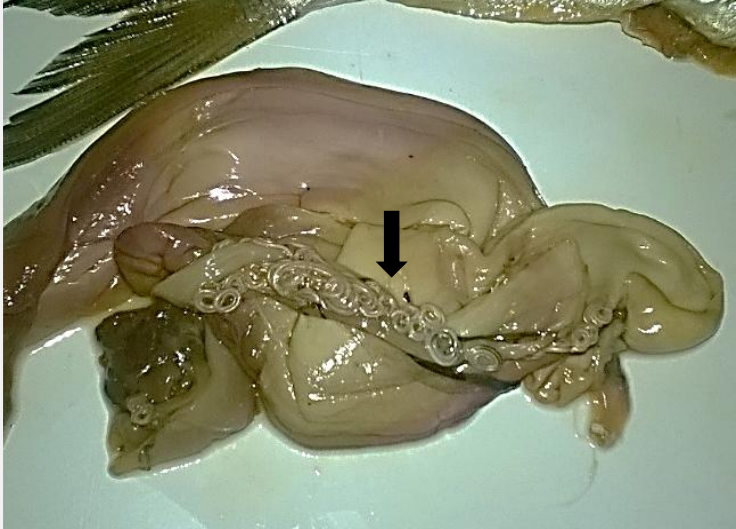
***Highlights (for review)**

- Different kinds of herring ready to eat products were analysed by digestion
- 41.5% of the products were positive for at least one visible *Anisakis* spp. larva
- A total of 1715 dead larvae were collected
- Whole smoked products were heavily contaminated at visceral and muscle level
- The product category influenced the positivity rate and larval density

Figure

Figure





Table

Table 1 Data on the occurrence and distribution of *Anisakis* spp. larvae in fresh and processed herrings. n.i.: not investigated

Reference	Origine	Number of samples	Type of product	Analytical method	Visceral/body cavity P (%)	Muscle P (%)
Levsen et al., 2018	North Sea Norw. spring spawn. Baltic Sea	1252 726 695	Whole (thawed)	UV-press	81.2 92.6 65.5	17.4 37.1 14.8
Bao et al., 2017	North Sea (ICES fishing area IVa)	209	Whole (thawed)	Press + candling	76[a]	n.i.
Ungerer et al., 2014	Baltic Sea	210	Whole (thawed)	Visual examination	2.9	n.i.
Levsen & Lunestad, 2010	Norwegian Sea	2004: 100	Viscera (whole) “manual” fillets (whole) “industrial” fillets	Visual examination Digestion	100 -- --	-- 62% 8%
		2007:350	Viscera (whole) “manual” fillets (whole) “industrial” fillets	UV-press	99 -- --	-- 51% 10%
Campbell et al., 2007	Baltic Sea, North Sea, Norwegian Sea, UK coasts	4073	Whole fresh (viscera store in ethanol)	Visual examination under microscope	60.2-100	n.i.
Levsen et al., 2005	Norw. spring spawn.	78	Whole (thawed)	Visceral examination under microscope + Candling + Digestion	94.9%	43.6%
Szostakowska et al., 2005	Poland (market)	140 74	Marinated, salted, spiced fillets and RTE dishes (30 types) Whole salted and smoked (9 types)	Digestion	--	15.4 %(6 types of products out of 39)[b]
	Southern baltic sea	31091	Whole fresh	n.i.	0-86%	n.i.

Tolonen & Karlsbakk 2003	Norw. spring spawn.	220	Whole (thawed)	Dissection under microscope	100	rare
Huang, 1988	Market (paris)	682	Whole herring	Visual inspection + digestion	82.5	0.7
Panebianco & Lo Schiavo, 1987	Italian market, imported from The Netherlands	20	Whole salted	Visual inspection and digestion	83.7[c]	9.3
		23	Whole smoked			
	Italian market, various origin	175	Smoked fillets		--	0
McGladdery, 1986	east coast of Canada	305	Whole (Thawed)	Visual examination(viscera)+ candling over a fluorescent light + digestion (fillets)	100-<5[d]	7.9
Panebianco & Lo Schiavo, 1985	Italian market, imported from The Netherlands	40	Whole salted	Visual inspection and digestion	85.7	2.9
		30	Whole smoked			
Lagoin, 1980	French supermarket		Whole smoked		78-97	
Hauck et al., 1977	Pacific Ocean[e]	39	Whole fresh	Digestion	n.i.	38.5
		120	Whole frozen		n.i.	42.5
		20	Whole salted		n.i.	50
		21	Whole cold smoked		n.i.	57.1
		20	Whole eviscerated cold smoked		n.i.	95
Smith & Wotten, 1975	North Sea	38	Whole fresh gutted	Digestion	-	39.5-73.7

			immediately (Exp.1)[f]			
		38	Whole fresh gutted immediately (Exp.2)[f]	Digestion	-	39.5-73.7
Khalil, 1969	English coast	5646	Whole eviscerated smoked	Visual inspection (viscera) + slicing and candling (muscle)	34[g]	

[a]Prevalence given for ascaridoid nematodes (mainly *A. simplex* s.l. and infrequently *Hysterothylacium* spp.); [b]there were found mainly in salted and smoked not gutted herring but they were also found in one wrapping of salted fillet and in one container of ready-to-eat fillets in spiced pickle. Almost all larvae were dead, but two *A. simplex* larvae found in a packet of ready to-eat fillets in spiced pickle were alive; [c]one live larva was found in one of the whole smoked herrings; [d]depending on the area of origin, 6 different areas of the Canadian coast were sampled; [e]The survey involved speciemens of *Clupea pallasi*; [f]whole fresh gutted herrings left on ice and examined after 14 h and 37h, which were also investigated in the study, were not included in the table as epidemiological values are influenced by the storage; [g]overall viscera and muscle;

Table

Table 2 Variables compared, and tests used for statistical comparisons for the larvae found in the muscle. MA: Mean Abundance, MI: Mean Intensity, MW: Mann-Whitney test, KW: Kruskal-Wallis, N.A.: non applied. Numbers 1 and 2 refer to the two different type of products, whole and filleted. 1a and 1b refer to the two subcategories of type 1, while 2a, 2b and 2c refer to the three subcategories of type 2.

	Variables to be compared				
Comparisons	**Positivity rate**	**MA**	**MI**	**N larvae/product**	**Larval density/gram**
whole (1) / **fillets (2)**	$\chi 2$	n.a.	n.a.	MW	MW
whole golden (1a) / **whole silver (1b)**	$\chi 2$	MW	MW	MW	MW
smoked fillets (2a) / **marinated fillets (2b)** / **canned fillets (2c)**	$\chi 2$	n.a.	n.a.	KW[a]	KW[a]

[a] if overal significance was achieved, MW was used for further comparisons using alfa=0.01 in order to reduce type I error.

Table

Table 3 Results of the analysis of the 50 whole smoked herrings at visceral level. Results are given overall and according to the subcategories (golden and silver). N= number of products, N pos= number of positive samples, P % = prevalence expressed in percentage, MA = Mean abundance, MI = Mean Intensity, CI: Confidence Interval

Type of product	N	N pos.	P % (95% CI)	n larvae	MA	MI	range	Mean density
whole total	50	49	98 (3.9%)	1559	31.2	31.8	0-172	0.63
whole golden	25	24	96 (7.7%)	738	29.5	30.8	0-172	0.72
whole silver	25	25	100	821	32.8	32.8	5-76	0.55

Table

Table 4 Results of the analysis of the muscle of the 50 whole smoked herrings and of the 70 filleted products. Results are given overall for the two types of products and according to the subcategories. N = number of products, N pos = number of positive samples, % pos = percentage of positive products, CI = Confidence Interval

Type of products	N	N pos	% pos (95% CI)	n larvae	larvae/prod tot	larvae/prod pos	range	Mean density
Whole	50	31	62.0 (13.5%)	149	3.0	4.8	0-17	0.022
Filleted products	70	6	8.6 (6.6%)	7	0.1	1.2	0-2	0.001
whole golden	25	16	64.0 (18.8%)	78	3.1	4.9	0-15	0.021
whole silver	25	15	60.0 (19.2%)	71	2.8	4.7	0-17	0.023
smoked fillets	25	3	12.0 (12.7%)	4	0.2	1.3	0-2	0
marinated fillets	30	3	10.0 (10.7%)	3	0.1	1.0	0-1	0.001
canned fillets	15	0	0.0	0	0.0	0.0	0	0

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