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Seafood safety and food-borne zoonoses from fish

Examining the risk of *Anisakis* in the Portuguese Population and Consumer Risk Perceptions of Fish Consumption

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Abstract

Parasitic nematodes of the genus *Anisakis* are the causative agent of anisakiosis, an important fish-borne zoonosis. Humans are infected through consumption of raw or undercooked fish, contaminated with the parasite. Infection can result in both gastrointestinal and allergic symptoms. There are few reports of anisakiosis in Portugal, but evidence of *Anisakis* allergy exists, indicating that exposure is occurring in the population. The European Food Risk Assessment Fellowship Programme (EU-FORA) work programme, entitled: 'Food safety of fish and zoonoses: fish consumption and microbiological risk assessment and perception, from fisherman to final consumers in Portugal' was hosted by the Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), in Porto, Portugal. It aimed to gather information on risk perception and attitudes in the Portuguese population to contamination of fish with *Anisakis* spp. and on their knowledge of methods to prevent infection. In addition, it aimed to examine the risk of anisakiosis in the Portuguese population.

Keywords: *Anisakis*, fish parasite, food safety, Zoonoses

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Table of contents

1. Introduction

1.1. European Food Risk Assessment Fellowship Programme (EU-FORA)

The European Food Risk Assessment Fellowship Programme (EU-FORA) is an initiative of the European Food Safety Authority (EFSA). It aims to build the European Union's (EU) scientific assessment capacity and knowledge community, in line with the key objectives outlined in EFSA's strategy 2020. It provides scientists working in food safety organisations across Europe with the opportunity to increase their knowledge and gain experience in food risk assessment, taking a 'learning by doing' approach to training. (Bronzwaer et al., 2016). The fellow was hosted by the Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), in Porto, Portugal. The work programme was entitled: Food safety of fish and zoonoses: fish consumption and microbiological risk assessment and perception, from fisherman to final consumers in Portugal. The programme included a 3-week induction training course and four 1-week modules, each focused on different aspects of risk assessment and risk communication. These modules would usually be held in different food safety institutes across the EU; however due to the Covid-19 pandemic, they were conducted on an online platform.

1.2. Background to Risk Assessment

Each year, almost one in 10 people fall ill from eating contaminated food. The World Health Organization has estimated that food-borne disease resulted in 420,000 deaths in 2010, with a global burden of 33 million Disability Adjusted Life Years (DALYs) (WHO, 2015). Food-borne zoonoses from fish and shellfish products are of significant public health concern, with high numbers of people at risk worldwide in both developed and developing countries (Lima dos Santos and Howgate, 2011). Anisakiosis is a fish-borne parasitic zoonosis caused by nematodes of the genus *Anisakis,* Dujardin 1845. These parasites have an indirect life cycle, using marine mammals, usually cetaceans as their definitive host. Invertebrates such as fish or squid are intermediate or paratenic hosts and crustaceans act as first intermediate hosts (Klimpel et al., 2004; EFSA BIOHAZ Panel, 2010; Smith and Wootten, 1978). Humans are infected with *Anisakis* spp. through consumption of the third-stage larval (L_3) form of the parasite in raw, smoked, marinated, salted or undercooked fish or squid (Audicana et al., 2002; Caldeira et al., 2021). They are accidental hosts for the parasite, so it does not mature, but on reaching the gastrointestinal tract, the larvae can cause disease (anisakiosis) (Mattiucci et al., 2018).

Van Thiel first described gastric anisakiosis in the 1960s (Van Thiel, 1962; Van Thiel and Van Houten, 1966; Buchmann and Mehrdana, 2016), the symptoms include nausea, vomiting and abdominal pain, and are caused by the larvae penetrating the gastrointestinal tract tissues (Caramello et al., 2003; Buchmann and Mehrdana, 2016). Patients can experience both abdominal and hypersensitivity symptoms and this condition is described as gastroallergic anisakiosis (Daschner et al., 2000). The other condition associated with *Anisakis* infection is an allergic response to fish products that contain parasite allergens. In these cases, live parasites may not be necessary to induce an allergic reaction, although it is generally believed that an initial *Anisakis* infection must occur to sensitise individuals to parasite antigens. However, it has not been possible to definitively rule out the occurrence of sensitisation through exposure to antigen alone (Audicana et al., 2002; EFSA BIOHAZ Panel, 2010).

There has been an increase in notifications of anisakiosis over the past few decades. This is probably due to increased inspection measures for fishery products, higher detection rates of contaminated seafood and of human infections due to improved detection and diagnostic techniques, along with the increasing popularity of raw or lightly cooked fish products (McCarthy and Moore, 2000; Chai et al., 2005; Audicana and Kennedy, 2008; Caldeira et al., 2021). The economic consequences of this parasite can be substantial, caused by increased rejection rates of contaminated products and decreased consumer confidence due to the negative aesthetic effects of visible nematode larvae in seafood products (D'amico et al., 2014; Llarena-Reino et al., 2015). *Anisakis* was ranked fourth of 14 food-borne parasites within the category of international trade importance in a report from the FAO/WHO (2014).

There are very few reported cases of anisakiosis in Portugal (Santos et al., 2022). The first case was reported in 2017 by Carmo et al. (2017). A case–control study by Falcão et al. (2008) found that 7% of the control group in the study population were sensitised to *Anisakis simplex,* while 22.5% of the patients in the case group, suffering from acute, relapsing urticaria, were sensitised. An earlier study by Nunes and Ladeira (2003) demonstrated that 8% of a population tested in a coastal region of

Portugal were allergic to *Anisakis* spp. indicating that exposure to the parasite is occurring in the population. Portugal does not have a tradition of eating raw fish, but this may change with the global trend towards consumption of raw or lightly processed foods (McCarthy and Moore, 2000; Käferstein, 2003). Also, grilling is a common method of cooking fish and this does not always result in sufficiently high temperatures throughout the fish to inactivate larvae. Furthermore, dead *Anisakis* larvae may be allergenic to previously sensitised individuals. Hake gonads are a popular Portuguese dish and this area of the fish is frequently parasitised by *Anisakis* (Santos et al., 2022). The factors outlined above indicate that the Portuguese population is at risk of exposure to this parasite.

2. Description of work programme

2.1. Aims

The work programme had three main aims:

i) Use survey data to characterise the situation in Portugal with regard to the zoonotic fish parasite *Anisakis*. The survey gathered information on general fish consumption, raw fish consumption and on consumer risk perception and attitudes to contamination of fish with *Anisakis* spp. Also, on consumer knowledge of methods of preventing infection.
ii) Examine the risk of anisakiosis in the Portuguese population from consumption of raw or undercooked fish.
iii) Determine the prevalence of *Anisakis* spp. in European hake (*Merluccius merluccius*), captured in North-East Atlantic Portuguese waters.

2.2. Activities/Methods

2.2.1. Assessing raw fish consumption trends and sociodemographic and health characteristics of raw fish consumers

A survey had been carried out prior to the start of the work programme consisting of 33 questions that gathered information on demographics, general health, fish consumption habits for both cooked and raw/undercooked products and knowledge of *Anisakis*. Data from questions regarding raw fish consumption were analysed in conjunction with data from sociodemographic and health-based questions, including those relating to allergy. This allowed analysis of the subset of respondents that consumed raw fish in relation to the type and volume of raw fish consumed, their sociodemographic profile, perceived health status and frequency of allergies.

2.2.2. Assessing consumer risk perception, awareness of *Anisakis* and 'willingness to pay' for *Anisakis*-free fish

Survey respondents were asked to describe what hazards they associated with fish consumption, to indicate if they had any prior knowledge of *Anisakis* and if this had affected their purchasing habits in the past. They were also asked if they were aware of methods to prevent infection with this parasite. In the final section of the survey, respondents were provided with information on *Anisakis* and also presented with a hypothetical scenario regarding a treatment that could remove all parasites and parasite antigens from the fish without affecting the quality. They were then asked if they would be willing to pay extra for such a product and given options of price ranges to choose from, for those that were willing to pay extra. This scenario and question were included to facilitate a contingent valuation study as an additional analysis of the survey data. Contingent valuation (CV) studies are used to assess the monetary trade-offs that consumers would be willing to make for a good or service (Carson, 2012). This approach was used by Bao et al. (2018) to ascertain the value that Spanish consumers would place on *Anisakis*-free fish.

2.2.3. Examine the risk of anisakiosis in the Portuguese population from consumption of raw or undercooked fish

The initial plan for this part of the project had been to carry out a quantitative risk assessment regarding the risk of anisakiosis posed to the Portuguese population from consumption of a given species of fish, raw or undercooked. One of the objectives of assessing raw fish consumption trends (see Section 2.2.1) was to identify a suitable hazard vehicle for this analysis. However, following

analysis of these data, no suitable hazard vehicle was identified. It was decided to focus on the five species most frequently eaten raw or undercooked in Portugal, identified by our survey, and examine the risks that they might pose to the population.

2.2.4. Determining the prevalence and distribution of *Anisakis* spp. larvae in a sample of European hake

2.2.4.1. UV-Press analysis

Forty-five European hake were examined for *Anisakis* L_3. The UV-Press method was used to estimate the number of parasites in the muscle and viscera of the fish. This method was first described by Karl and Leinemann (1993). *Anisakis* larvae fluoresce under UV light after freezing, allowing them to be identified and their location marked on the plastic bag. The larvae were removed from the samples, washed in 0.9% saline solution and examined using a stereoscope to distinguish between Type I and Type II *Anisakis* L_3.

This was the first time that the UV-Press method had been used in the laboratory, so some work was required to optimise the method such as establishing the most suitable light source to obtain the best visualisation of the larvae.

2.2.4.2. Statistical analysis of UV-Press results

From the findings of the UV-Press analysis, the intensity and abundance of infection per fish were calculated, as defined by Bush et al. (1997), as well as the intensity and abundance of infection in the muscle and viscera of each fish. The density of infection in the muscle and viscera was also calculated (Bush et al., 1997). The Mann–Whitney U test was used to establish if measurements such as infection abundance differed significantly between heavier and lighter fish, and Spearman's rank-order correlation was used to evaluate some host–parasite correlations, such as fish weight and length with intensity and density of infection in the muscle and viscera. SPSS version 27 was used for this analysis.

2.2.5. Molecular analysis of *Anisakis* L_3 obtained from a range of fish species

Anisakis L_3 isolated from a sample of 16 fish of the subclass Elasmobranchii and 14 gurnards were selected for identification using conventional PCR. DNA was extracted and amplified by PCR, using primers for Internal Transcribed Spacer rDNA (ITS) (Gasser et al., 1993; Gasser and Hoste, 1995), Mitochondrial-encoded cytochrome c oxidase subunit 2 (COX-2) (Nadler and Hudspeth, 2000) and elongation factor 1 alpha 1 (Mattiucci et al., 2016). PCR products were analysed by electrophoresis on a 1% agarose gel and submitted for forward and reverse Sanger sequencing with an external company. The sequences obtained were analysed using MEGA software and compared with sequences deposited on GenBank.

3. Conclusions

The survey analysis provided the fellow with hands-on training in data analysis and an understanding of how contingent valuation studies are conducted. It resulted in a data set with useful insights into the fish consumption habits of the Portuguese population, their knowledge and perception of risks associated with the parasite *Anisakis* and their awareness of methods of preventing infection.

Through working on the UV press method, the fellow also gained experience in introducing a new method to the host institute laboratory and this allowed collection of data regarding prevalence and distribution of *Anisakis* larvae in European hake from Portuguese waters. In addition, the fellow received training in molecular techniques to allow speciation of *Anisakis* larvae.

Preparation for the quantitative risk assessment that was planned, allowed the fellow to go through the process of gathering and organising data for such an assessment. When it became apparent from analysis of survey data, that no suitable hazard vehicles could be identified, the fellow worked on examining the risks of anisakiosis posed to the Portuguese population from the species of fish most commonly consumed raw in Portugal. This consolidated the knowledge of the topic already gained over the course of the programme, and provided an opportunity to acquire a broader understanding of this food safety issue, through a comprehensive review of the relevant literature.

The fellow also had the opportunity to attend regular on-line seminars at the host institute, learning about a wide variety of research projects conducted there. The EU-FORA modules provided training in a broad range of topics related to risk assessment, along with the opportunity to network with other scientists in this field.

Results of the fellow's project were presented in two posters at the international conference of the European Association of Fish Pathologists 2021 (Annex A and B), and will be published in four articles in peer-reviewed journals.

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Abbreviations

CIIMAR	Interdisciplinary Centre of Marine and Environmental Research
CV	Contingent Valuation
DALY	Disability Adjusted Life Years
FAO	Food and Agriculture Organization
L3	Stage-three parasite larva
PCR	Polymerase Chain Reaction
QMRA	Quantitative Microbiological Risk Assessment
UV	Ultraviolet
WHO	World Health Organisation

Annex A – Poster on UV-Press method results presented at the European Association of Fish Pathologists 20th international conference (virtual) on diseases of fish and shellfish 20–23 September 2021

Anisakis spp. in European hake (*Merluccius merluccius*) – a risk to Portuguese consumers?

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INTRODUCTION

Anisakiosis is a fish-borne zoonosis, caused by parasitic nematodes of the genus *Anisakis.* These marine parasites have an indirect life cycle, using marine mammals, usually cetaceans, as their definitive host. Humans are accidental hosts for *Anisakis* spp; the larvae do not mature, but on reaching the gastrointestinal tract can cause disease.

Portugal has one of the highest levels of fish consumption in the world and the European hake is one of the most popular fish in Portugal.

AIMS

Establish the level of contamination of European hake with *Anisakis* spp., in a sample caught in Portuguese waters. And characterise the distribution of infection between the viscera and muscle of infected fish.

MATERIAL & METHODS

Forty-five European hake of mixed age were measured, weighed and the viscera and muscle were examined for *Anisakis* spp. larvae using the UV-Press Method.

RESULTS

The fish analysed had a mean±SD length of 31.6±3.7 cm and weight of 212.6±85.7 g.

A total of 473 Anisakis stage 3 larvae were found, with a prevalence of 95.6% (95% CI 89.5-100%).

Three fish were fully eviscerated and these were excluded when calculating the intensity, abundance and density per gram of viscera.

A significantly higher number of larvae were found in the viscera and the muscle of the larger fish (Mann-Whitney test, Z = -2.21, and p<0.03). However, the muscle density values were not significantly different between large and small fish (Mann-Whitney test, Z =-0.07, and p=0.95).

Fish length was significantly, positively correlated with the total larval abundance per fish (Spearman correlation coefficient: ρ =0.64, p<0.01), the visceral abundance (ρ = 0.68, p<0.01) and the muscle abundance (ρ =0.393, p<0.01). Also with the density of parasites in the viscera (ρ = 0.49, p<0.01).
Fish weight was significantly, positively correlated with the total larval abundance per fish (ρ = 0.63, p<0.01), the visceral abundance (ρ = 0.67 , p<0.01) and the muscle abundance (ρ =0.38, p<0.01). And with the density of parasites in the viscera (ρ = 0.45, p<0.01).
The larval abundance in the viscera was significantly correlated with the larval abundance in the muscle (ρ = 0.44, p<0.01).

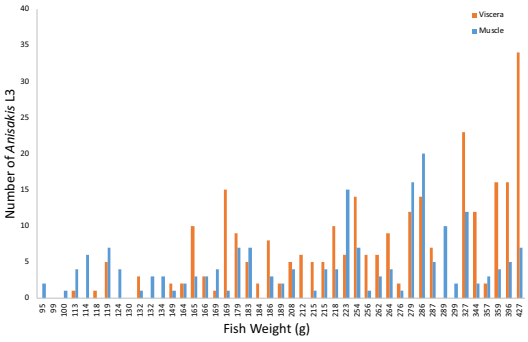

Figure 1. *Anisakis* spp. Larval Stage 3 Abundance in Muscle and Viscera

	Mean	Standard Deviation	Range
Intensity	11.3	9.7	1-41
Total Abundance	11	9.8	0-41
Visceral Abundance	6.6	7.1	0-34
Muscle Abundance	4.4	4.4	0-20
Density per gram of viscera	0.54	0.5	0-2.53
Density per gram of muscle (n=45)	0.05	0.04	0-0.16

Table 1. Summary of results for forty-two European hake caught in Portuguese waters in April 2021. Fish were examined for stage 3 *Anisakis* spp. larvae using the UV-press method.

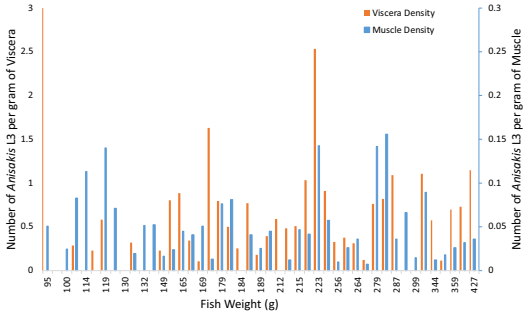

Figure 2. *Anisakis* spp. Larval Stage 3 Density per Gram of Viscera and Muscle

CONCLUSIONS

There is not a tradition of consuming raw or undercooked fish in Portugal, however these products are becoming increasingly popular, and common cooking methods such as grilling do not always reach sufficient temperatures to ensure death of parasite larvae. Although there are few reports of anisakiosis in Portugal, studies in other, neighbouring, countries have highlighted that this is a highly under-reported disease due to the non-specific symptoms and lack of awareness of the condition. Our findings form a basis from which we can establish a more accurate estimate of the risk posed to Portuguese consumers from the consumption of hake. The high prevalence of *Anisakis* larvae in our sample highlights a need for consumer education about this parasite.

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Annex B – Poster on survey results presented at the European Association of Fish Pathologists 20th international conference (virtual) on diseases of fish and shellfish 20–23 September 2021

Anisakis spp. and the potential risks to human health; an assessment among consumers in Portugal

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INTRODUCTION

Anisakiosis is a zoonosis resulting from the accidental ingestion of viable *Anisakis* spp. larvae in raw or undercooked fish products. Portugal has one of the highest levels of fish consumption in the world and although raw fish is not part of the traditional Portuguese diet, the growth of international trade and increasing popularity of lightly cooked or raw food means that consumers may be at increased risk of exposure to this parasite.

AIMS

To assess the level of knowledge within the Portuguese population of *Anisakis* spp. and their potential risks; also, to assess awareness of methods to prevent infection.

MATERIALS & METHODS

A questionnaire consisting of 33 questions was circulated online. There were 746 respondents. Information was collated on socio-demographic factors and fish-consumption habits. Data on consumers' attitudes to contamination of fish with *Anisakis* spp., risk perception and knowledge of prevention methods were also analysed.

RESULTS

The majority of respondents were aged between 30 and 49 (55%) and were female (72%). About 67% had post-graduate degrees, 39% of respondents worked in the field of Biological and Health Sciences.

Most respondents (86%) cited "transmission of parasites" as a risk associated with the consumption of raw fish. Most respondents had not heard of *Anisakis* spp. (66%) or prevention methods (79%). Of those that stated they were aware of prevention methods, the majority cited "cooking thoroughly" and "freezing" as the most important.
Only 7% of people have avoided buying or eating fish due to the presence of worms. In these cases, the fish most commonly avoided were cod (29%), anchovies (26%), salmon or trout (22%).

Thirty-five per cent of respondents would be willing to pay between € 1 and € 2.5 extra for a fish product that was treated to remove *Anisakis* larvae and allergens. Thirteen percent of respondents stated that they would not buy this *Anisakis*-free product and 25% would not pay any extra for it. In these two cases, the main reason given was that they should not have to pay extra to have access to safe food.

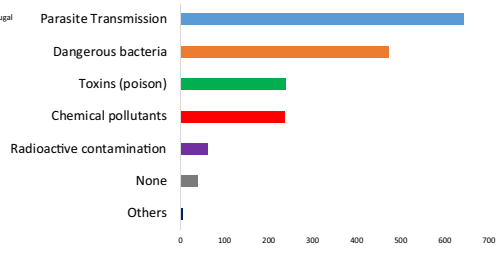

Figure 1. Survey responses regarding hazards associated with fish consumption

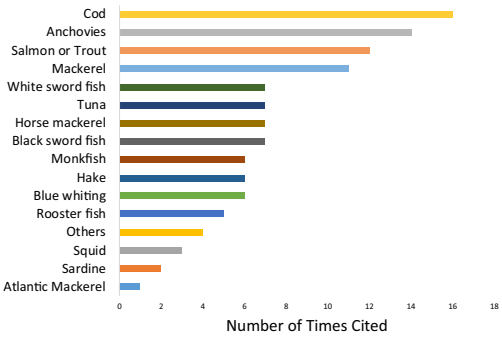

Figure 2. Types of fish avoided by the **55** respondents that have avoided buying or eating fish due to concerns about *Anisakis* spp.

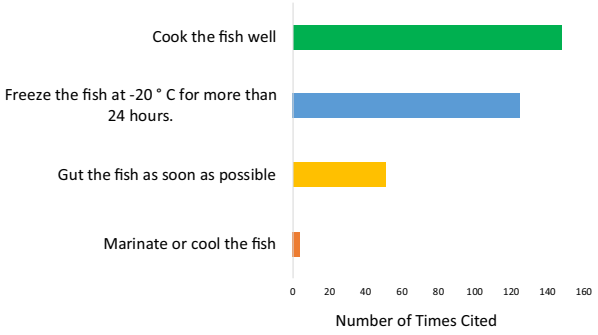

Figure 3. Prevention methods cited by the **156** respondents that stated they had both heard of *Anisakis* spp. and were aware of prevention methods.

CONCLUSIONS

A significant number of participants had little or no knowledge of *Anisakis* spp., the risks associated with them and prevention methods. The majority of respondents had a post-graduate degree, mostfrequently in th e area of Biological or Health Sciences. Itis clear thatthere is a need to raise awareness ofthis parasite, so that consumers know the risks assoc iated with eating raw or lightly processed fish, and are familiar with effective methods to preventinfection. This information should be inclu ded in consumer awareness programs regarding safe food.

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