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A survey of zoonotic nematodes of commercial key fish species from major European fishing grounds—Introducing the FP7 PARASITE exposure assessment study

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ABSTRACT

Harvesting and exploiting limited fisheries resources in a sustainable manner also implies achieving maximum added value from the raw material. However, the presence of parasites in the products may adversely affect consumer perception and/or pose a direct health hazard. As a major stepping-stone of the PARASITE project, an epidemiological survey was carried out to provide the basis for analysis and prediction of consumer exposure risk due to the presence of anisakid nematodes in fish from European wild-catch fisheries. The project consisted of nine separate workpackages (WP) where the exposure risk assessment survey was organized within WP2. The surveillance task also provided the data or samples needed for data management and sample storage (WP3, Biobank), molecular and genetic parasite species identification (WP4), and statistical modelling and inference (WP8). In total 17,760 fish belonging to 16 teleost species were examined for anisakids, with special emphasis on economically and ecologically important species such as Atlantic mackerel, herring, European hake, Atlantic cod and anchovy. The target fish species were sampled at four major European fishing areas including the Barents Sea, North Sea, Baltic Sea, Grand Sole Bank, waters off NW Spain and Portugal, central and western parts of the Mediterranean Sea, and the Adriatic Sea. Thus, the survey represents the largest and most comprehensive epidemiological data compilation of anisakids ever generated in terms of geographical range as well as number of fish host species and sample size. An important requirement of the survey was the use of commonly accepted nematode detection methods, i.e. the UV-press method or artificial digestion, to quantify infection level and spatial distribution of anisakid larvae in the target fish species. The basic layout, set-up and principles of the method, along with a discussion of possible source of errors are described. Additionally, the molecular and genetic markers which were used to identify and characterize different species and populations of anisakids from the targeted fish host species and geographical areas, are reviewed as well. Some basic parasite infection characteristics of each fish host species, and any relationships with the presumably most important infection predictors, i.e. fish host body size and fishing locality, are presented and discussed. Emphasis is put on anisakid occurrence in the flesh of the fish. Based on the findings, a graphical exposure risk profile is introduced, including fish species or products thereof, which due to common processing or preparation practices, are at highest risk to act as source of anisakiasis in Europe.

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1. Introduction

The EU fisheries industry is the fifth largest in the world. In the European Union, close to 5 million tons of wild fish catches are processed every year. Fishing and fish processing provide jobs for at least 275,000 people. Moreover, the EU is among the leading fish markets in the world with imports accounting for approximately €21 billion in 2014, more than 40% of world fish imports in value, with increasing trend. It should be noted, however, that Norway as an EU third country accounts for 23% of the EU seafood imports alone. The average consumption of fisheries products in the EU-28 countries was 24.9 kg/person in 2011. The annual per capita consumption rate varies greatly, however, from 5.3 kg in Hungary to 56.8 kg in Portugal (European Commission, 2016).

Nevertheless, consumers expect safe and healthy fish and fishery products. However, some of the most important fish species caught by the European fishing industries are at risk of carrying parasites when put on the market. In Europe, anisakid nematodes are the most relevant group of parasites in terms of consumer health risk and product quality, with *Anisakis* and *Pseudoterranova* as the genera of greatest concern because several species are considered a human health hazard (Mattiucci et al., 2017a). The term anisakiasis refers to the zoonotic disease provoked through accidental ingestion of viable larvae of certain *Anisakis* species which infect the edible parts of fish or squid. Among the nine nominal species belonging to the genus *Anisakis* (Mattiucci and Nascetti, 2008; Mattiucci et al., 2014), *A. simplex (sensu stricto)* and *A. pegreffii* have been confirmed to cause disease in humans (D'Amelio et al., 1999; Umehara et al., 2007; Mattiucci et al., 2011; , 2013; Lim et al., 2015; Mladineo et al., 2016; Bao et al., 2017). It was further demonstrated that *A. pegreffii* may provoke gastric (GA), intestinal (IA) and gastro-allergic anisakiasis (GAA) (Mattiucci et al., 2011, 2013; Lim et al., 2015; Mladineo et al., 2016), while both *A. simplex* (*s. s.*) and *A. pegreffii* larvae may cause allergic reactions in humans (Daschner et al., 2000). Although international regulations, e.g. EU No. 1276/2011, demand deep-freezing for at least 24 h of any fishery product to be consumed raw or semi-raw, this so-called freezing requirement is not necessarily practiced by private households or local guesthouses and restaurants. Thus, consumption of local or privately prepared dishes based on fresh, only lightly processed fish such as boquerones in Spain and marinated anchovies in Spain and Italy, probably represents a major source of anisakiasis in Europe.

Self-control programs such as HACCP (hazard analysis and critical control points) procedures in the fish industry are hampered by the fact that the epidemiology of anisakid parasites in fish caught and marketed in Europe is not well understood. The collection of data on the complete life cycle, geographical and seasonal distribution, prevalence, intensity, and infection site of parasites of public health importance in wild fish stocks and fishery products has so far been based mainly on non-systematic and opportunistic sampling, lacking appropriate monitoring programs coordinated on a pan-European scale. Therefore, a systematic epidemiological survey of the economically most important fish species and stocks from European fishing grounds could provide the basis for analyzing and modelling parasite prevalence and abundance.

In fish, the majority of *Anisakis* larvae are typically seen as whitish to greyish, flat and tight coils, measuring a few mm across. Larvae that reside in the fish flesh are very hard to detect by the naked eye since they are often transparent and may have penetrated deeply into the fillets. Moreover, the larval occurrence in terms of their abundance and spatial distribution seems largely to depend on fish host species and their respective feeding behavior. Thus, piscivorous species such as adult hake and cod are usually more heavily infected with anisakid larvae compared to strict plankton feeders such as sardine, anchovy and capelin (for reviews of the literature, see Mladineo and Poljak, 2014; Šimat et al., 2015; Cipriani et al., 2016; Levsen et al., 2016; Zorica et al., 2016). However, we know only little about the spatial distribution of anisakid larvae in various economically important fish species, i.e. where in the fish the larvae primarily reside. This is especially important whenever anisakid larvae occur in the flesh (fillets and belly flaps) of fish.

The main objective of the anisakid exposure assessment workpackage (WP2) of the PARASITE project was to provide comprehensive and comparable epidemiological data with respect to zoonotic parasites in the economically most important fish species or stocks originating from major European fishing grounds. The study focused on *Anisakis* species (mainly *A. simplex* and *A. pegreffii*), extending to other species such as *Pseudoterranova decipiens* (*s. l.*), *Contraceacum osculatum* (*s. l.*) and *Hysterothylacium aduncum* (non-zoonotic species but may have aesthetical quality reducing effect if present abundantly), where adequate data were available. Thus, the current report provides a basic overview of the methods which were commonly applied to detect anisakid nematodes in the actual fish samples, and to identify them molecularly to species level. The report further summarizes some basic epidemiological results with emphasis on larval occurrence in the fish flesh, and analyses through GAM modelling the relationships between larval occurrence and fish host body size which is already known to act as important driver of anisakid infection patterns in many fish species and fishing areas. Finally, we introduce a graphical exposure risk profile based on prevalence data of *Anisakis* spp. in the flesh of several fish species which are commonly prepared and consumed in a raw or only lightly processed state.

2. Material and methods

2.1. Target fish species

The primary decision criteria for the target fish species of the survey concerned their importance in terms of: 1) annual consumption volume/sales value, 2) significance for the fresh fish market, 3) basis for raw or semi-raw products such as sushi and sashimi, and 4) parasite history (e.g., former RASFF –Rapid Alert System for Food and Feed – notifications). Thus, the pelagic fish species included in the survey were herring (*Clupea harengus*), sardine (*Sardinus pilchardus*), anchovy (*Engraulis encrasicolus*), Atlantic mackerel (*Scomber scombrus*), chub mackerel (*S. colias*) and blue whiting (*Micromesistius poutassou*). European hake (*Merluccius merluccius*), haddock (*Melanogrammus aeglefinus*), Atlantic cod (*Gadus morhua*) and monkfish (*Lophius piscatorius* and *L. budegassa*), in addition of two flatfish species – plaice (*Pleuronectes platessa*) and four-spotted megrim (*Lepidorhombus boscii*) – represented species preferring demersal habitats. On a smaller scale, or whenever available, we also investigated whiting (*Merlangius merlangus*), European sea bass (*Dicentrarchus labrax*) and silver scabbardfish (*Lepidopus caudatus*) since these, too, are commercially utilized on an industrial scale and are of importance in a number of major European seafood markets including Spain, UK, Italy and France. Some of the fish species to be included in the survey, e.g. Atlantic mackerel, cod and hake, occur and are commercially utilized in several of the present NE Atlantic fishing areas. Thus, the epidemiological data obtained from these species and areas have been particularly analyzed for the effect of specific habitat characteristics, geographical location and fish host migration patterns on the diversity and distribution of anisakid species (see also Levsen et al., 2017; Gay et al., 2017; Pascual et al., 2017).

2.2. Sample size and fish host biometric data

Fish host sample size varied among host species, sampling localities and sampling date/year. In general, smaller or medium sized species, e.g. anchovy, herring, mackerel or blue whiting were sampled and examined in greater quantities compared to larger species such as Atlantic cod, haddock or monkfish (Table 1). This was partly due to the fact that processing and UV-inspection of smaller fish is less labor- and time-intensive than examining larger fish. Additionally, samples of some other fish species, e.g. European sea bass, were more costly to obtain