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Anisakid infection levels in fresh and canned cod liver: Significant reduction through liver surface layer removal

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ABSTRACT

Canned liver is a highly valuable and healthy product from the important commercial fish species cod *Gadus morhua*. Due to high levels of contaminants such as dioxins and dioxin-like PCBs (polychlorinated biphenyls) in cod liver products from the Baltic Sea, the grounds for supply of cod liver were relocated to areas around Iceland in the Irminger Sea with lower levels of contaminants. In the present study, 62 canned samples, as well as 132 fresh cod livers from Greenland and Iceland, were analysed in order to assess the infection levels with anisakid nematode species. High prevalence and abundance of the potentially zoonotic nematode species *Anisakis simplex s.s.* and *Pseudoterranova decipiens s.s.* were found in the fresh livers. Lower infection levels in canned livers compared to fresh livers might indicate successful industrial processing measures in order to reduce nematode larvae in the final products. However, two heat stable allergens, Ani s 1 and Ani s 4, were verified from isolated nematode larvae from canned products. The effectiveness of methods to further reduce the number of nematode larvae during industrial processing of cans with cod liver is discussed.

1. Introduction

Atlantic cod *Gadus morhua* (Linnaeus, 1758) is one of the most important commercial fish species in the North Atlantic, inhabiting the continental shelves of cold Arctic waters throughout the North Atlantic and more temperate waters in the North and Baltic Sea, as well as waters along the North American coast (Froese & Pauly, 2017; Pörtner et al., 2001). The global annual catch is estimated to be above 1.1 million t (FAO, 2011). In 2016, a total catch of 251,134 t of cod in Iceland (ICES Division 5a) was reported, whereas in East Greenland (ICES subarea 14 and NAFO Division 1.F) the total catch was 14,818 t (ICES, 2017a, 2017b).

Cod liver is a highly valuable by-product used in many traditional culinary dishes around Europe, e.g. in Germany, Iceland, Denmark, Poland, Latvia, and Lithuania. Some famous dishes are, for example, the North Norwegian dish *mølje,* the Spanish dish 'Foie de Bacalao' and the French dish 'Foie gras de mer'. The liver was and is still mainly used for the production of cod liver oil, which has been administered as a food supplement to children for centuries (Bennett, 1841). Cod liver contains approximately 20% protein and 50–60% lipids. Additionally, it is rich in vitamins A and D, iodine and has a high content of n-3 polyunsaturated fatty acids (Birgisdottir et al., 2012; Fooddata, 2017; Kolakowska et al., 2002).

Until 2008 considerable amounts of canned cod liver were produced in countries around the Baltic Sea. The production stopped, however, after dioxin concentrations were found to be above allowable EU limits (maximum levels of the sum of dioxins and dioxin-like PCBs 20 pg/g wet weight (European Commission, 2011; Godliauskienė, Petraitis, Jarmalaitė, & Naujalis, 2012)). Several studies showed that contaminant levels in cod liver from northern fishing areas, such as Greenland, Iceland or Barents Sea, were relatively low (Julshamn et al., 2013; Jörundsdóttir, Löfstrand, Svavarsson, Bignert, & Bergman, 2010; Karl & Lahrssen-Wiederholt, 2009), and thus were suitable for human consumption. Today 70%

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of canned cod liver for the West European market is produced in Iceland (Jónsson & Viðarsson, 2016). The Icelandic production of canned liver has reached approximately 3800 t in 2015, corresponding to 19 million cans (Jónsson & Viðarsson, 2016).

Due to its economic value, Atlantic cod is probably one of the most extensively studied marine fish species, not only on the various aspects of the biology of this species, but also with special regards to its parasite fauna. An impressive number of parasitological studies suggest that as a top predator in North Atlantic waters, cod acts as a host for an exceptionally rich and diverse parasite fauna (Hemmingsen & MacKenzie, 2001; Hemmingsen, Lile, & Halvorsen, 1992; Münster, Klimpel, Fock, MacKenzie, & Kuhn, 2015; Perdiguero-Alonso, Montero, Raga, & Kostadinova, 2008; Sobecka, Łuczak, Więcaszek, & Antoszek, 2011). Furthermore, a substantial number of studies have been conducted on the spatio-temporal dynamics and abundances of anisakid nematodes, i.e. *Anisakis*, *Contracaecum*, and *Pseudoterranova*, due to their economic and medical significance (Boily & Marcogliese, 1995; Buchmann & Mehrdana, 2016; Hemmingsen & MacKenzie, 2001; Mehrdana et al., 2014; Mouritsen et al., 2010; Strømnes & Andersen, 1998, 2000). Larvae of these zoonotic endohelminths are known to occur in vast numbers as third-stage larvae in the musculature, body cavity and internal organs (including the liver) of cod, which poses a potential health risk for the consumer when ingested raw or slightly cooked. Whereas the consumption of live worms with fish products is associated with severe clinical symptoms such as fever, epigastric pain, diarrhoea, stenosis as well as chronic, ulcer-like symptoms known as anisakidosis (Hochberg & Hamer, 2010), the exposure to inactivated nematodes in processed fish products has been associated with a range of allergic manifestations in sensitive patients. Both types of symptoms, disease or allergic reactions, highlight the need for preventive consumer health protection (Audicana, Ansotegui, Fernández de Corres, & Kennedy, 2002; Nieuwenhuizen & Lopata, 2013). With the presence of visible parasites causing consumer rejection (EFSA, 2010), and EU regulations not allowing parasite-infected fishery products to be sold (Regulation (EC) 853/2004, 2004 European Hygiene Package, section VIII), methods to reduce the infection levels are needed.

The aim of the present study was to examine and compare the abundance of larval nematodes in canned cod liver products from Iceland with those of fresh cod livers from Greenland and Iceland. Results from nematode species identification, as well as the allergenic potential of canned cod liver and isolated nematode larvae are discussed in the context of consumer health protection. As a number of *Anisakis* antigens are thermo resistant, e.g. Ani s 1 and Ani s 4 (Carballeda-Sangiao et al., 2014), the presence of allergens following industrial processing was assessed. Furthermore, the effectiveness of technology to reduce the number of nematode larvae in cod liver products before canning was tested.

2. Material and methods

2.1. Sample collection

2.1.1. Fresh cod livers

129 specimens of cod (*Gadus morhua*) were sampled in 2013 and 2014 during research cruises 369 and 379 of the FRV Walther Herwig III from different fishing grounds along the east coast of Greenland in the fishing areas of Dohrn Bank, Angmagsalik, Kleine Bank, Fylkir Bank (catching positions 65°30′N - 59°39′N; 030°01′W - 044°14′E). Total length, weight, and sex of the fish were determined and the cod liver was removed. In 2013, 100 cod livers were visually inspected directly on board the research vessel, while the 29 cod livers collected in 2014 were deep frozen for further analysis.

Additionally, three large fresh cod livers were obtained from an Icelandic processor. Livers were weighed, analysed for anisakid nematode intensity, and tested for the effectiveness of removal of the liver surface sheath during industrial processing.

2.1.2. Canned cod livers

Fifty cans containing cod liver in liquid and oil produced in Iceland by three different companies (A: Ingredients: Cod liver (*Gadus morhua* 99%), B: Cod liver (*Gadus morhua* 98%), salt; C: Cod liver, salt, smoke) were collected from various German retail shops and super markets. Twelve cans of cod liver in oil for the Danish market were supplied by an Icelandic processor.

2.2. Visual inspection

Cod livers were visually inspected to investigate the predictability of nematode intensity of the whole liver by estimation of the surface infection level at first glance. During the research cruise in 2013 cod livers of freshly caught cod specimens from Greenland ($n = 100$) were visually inspected directly on board. After opening the abdominal cavity, visual inspections were done within 5 s and followed four evaluation categories: 1) Apparently uninfected: no nematodes visible (= 0 nematode larvae (NL)), 2) Slightly infected: very few nematodes detected (= 1–10 NL), 3) Clearly infected: several nematodes instantly visible (= 11–20 NL), 4) Heavily infected: liver surface covered by nematodes (≥20 NL). Livers were removed, weighed, and all nematodes on the liver surface were counted. Cod livers obtained in 2014 cod caught in Greenland ($n = 29$) were visually inspected at land and all nematodes on the surface layer were counted.

2.3. Nematode larvae analysis

2.3.1. Nematode larvae detection

After the visual inspection and counting of nematode larvae on the surface of liver samples from Greenland, livers were placed into plastic bags and pressed by means of a hydraulic press to a thickness of 2 mm. Total number of nematodes were determined on a “candling” dish (3000 Lux in 30 cm distance) (Karl & Leinemann, 1993). “Candling” means a brief visual inspection on a light table. The fishing industry uses it as a common approach for nematode larvae detection (Levsen, Lunestad, & Berland, 2005).

Fresh Icelandic cod livers were divided into subsamples which were compressed (thickness 2 mm) between two glass plates (150 × 150 × 10 mm). The plate was placed under a dissection microscope and examined for nematode larvae at 5–50× magnification (Buchmann, 2007).

For detection of anisakid nematode larvae in canned liver products, cans were opened, the liquid was poured through a sieve (mesh size 500 µm) and livers were weighted. Canned cod livers bought at German markets were placed into plastic bags, pressed and carefully examined on a candling dish (3000 Lux in 30 cm distance); products from the Danish market were pressed between two glass plates as described above. Nematode larvae were recovered and further identified.

2.3.2. Accuracy of the candling method

To assure the accuracy of the visual inspection by candling, a subsample of 40 pressed livers from Greenland samples was thawed, transferred to a beaker, and artificially digested applying the pepsin/HCL method according the CODEX standard for salted Atlantic herring and salted sprat (CODEX STAN, 2004). Comparison of results showed excellent agreement between the candling and digestion method (Spearman correlation $S = 58.25$, $p < 0.01$,