



Occurrence of *Anisakis pegreffii* (Nematoda: Anisakidae) Larvae in Imported John Dory (*Zeus faber*) from Senegalese Coast Sold in Turkish Supermarkets

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Abstract

Background West African goatfish *Pseudupeneus prayensis*, bluespotted seabream *Pagrus caeruleostictus* and John Dory *Zeus faber* are commercially marketed as fresh and frequently imported from Senegalese coast (FAO area 34.3.12) in Turkish supermarkets.

Purpose The aim of the current study was to collect data of occurrence and molecular identification of *Anisakis* species in imported *P. prayensis*, *P. caeruleostictus* and *Z. faber* caught in the Senegalese coast and to support epidemiological report for a risk evaluation of *Anisakis* species in Turkish supermarkets.

Methods Forty imported fish from each species at a total of 120 samples were investigated for the presence of *Anisakis* larvae. Based on ITS region of RFLP analysis *Anisakis* larvae were identified and randomly selected five larvae were also sequenced for further confirmation for *cox2* gene.

Results No *Anisakis* larvae were isolated from *P. prayensis*, *P. caeruleostictus* whereas *Anisakis* larvae were only found in *Z. faber*. A total of 156 *Anisakis* larvae were collected from *Z. faber*. All larvae were molecularly identified as *Anisakis pegreffii*. The prevalence (%), intensity and abundance of *Anisakis* infection in *Z. faber* were detected to be 82.5%, 8.3 and 6.8, respectively.

Conclusion This is the first assessment of the occurrence of *A. pegreffii* in imported *Z. faber* from the Senegalese coast in Turkish supermarkets. Moreover, consuming imported *P. prayensis* and *P. caeruleostictus* present low to non-existent risk for anisakiasis in Turkish consumers. Furthermore, the presence of *A. pegreffii* larvae in imported *Z. faber* from the Senegal waters could have public health implications in Turkish consumers.

Keywords *Anisakis pegreffii* · *Pseudupeneus prayensis* · *Pagrus caeruleostictus* · *Zeus faber* · Senegal · Turkish supermarkets

Introduction

The West African goatfish *P. prayensis*, the bluespotted seabream *P. caeruleostictus* and the John Dory *Z. faber* are known from the ECA (FAO area 34). These fish species are commercially most important demersal species in Senegal. Large quantities are exported to Europe and other countries as fresh, frozen, dried-salted and smoked [8, 15,

32]. In Turkey, these fish species are marketed as fresh and frequently imported from Senegal (FAO area 34.3.12) especially during legal fishing ban period (April–October) and are commonly consumed by Turkish population.

Anisakis species belonging to the family Anisakidae are important fish-borne nematode larvae with zoonotic significance. *Anisakis pegreffii* especially causes oesophageal anisakiasis, gastric anisakiasis, intestinal anisakiasis, ectopic (extra-gastrointestinal) anisakiasis and gastro-allergic anisakiasis in consumers [13, 14, 20–23, 25, 37]. Infection and/or allergies in consumers could be seen by ingestion of raw or undercooked fish, cephalopods and/or seafood products [4, 21, 35]. Therefore, occurrence and accurate identification of *Anisakis* species larvae in different fish species used for

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human consumption is very important in terms of seafood safety and public health.

To date there is no information on *Anisakis* infection and/or allergies in Turkish consumers. However, zoonotic *A. pegreffii* was genetically identified from different marine fish from Turkish waters [28]. Additionally, *A. simplex* (s.s.) were detected in imported *Scomber scombrus* from Norway (FAO area 27) sold in Turkish supermarkets [27]. To date, the occurrence of *Anisakis* larvae in imported West African goatfish *P. prayensis*, bluespotted seabream *P. caeruleostictus* and John Dory *Z. faber* from Senegal (FAO area 34.3.12) sold in Turkish supermarkets have not been investigated by researchers.

The aims of the current study were to collect data on prevalence and molecular identification of *Anisakis* species in the imported West African goatfish *P. prayensis*, bluespotted seabream *P. caeruleostictus* and John Dory *Z. faber*, which are commonly consumed in Turkey, caught in the ECA waters (FAO area 34.3.12) and to support epidemiological report for a risk evaluation of *Anisakis* species in Turkish supermarkets.

Materials and Methods

Fish Sampling and Parasitological Analysis

A sample size of 40 imported whole fresh fish samples from each investigated fish species, at a total of 120 fish samples, 100–200 g West African goatfish *P. prayensis*, 300–400 g bluespotted seabream *P. caeruleostictus* and 200–300 g John Dory *Z. faber* were randomly purchased from supermarkets

that import these fish species Senegal to Turkey from May to August of 2016. All fish species were captured in the ECA's FAO area 34.3.12 (Fig. 1). A routine parasitological examination for *Anisakis* larvae in the viscera was visually performed under a stereomicroscope. Then, eviscerated fish were cut into two dorsal and ventral fillets and each fillet was only digested with pepsin solution [6, 18]. The *Anisakis* larvae were counted and placed in 70% ethanol. *Anisakis* larvae were cut into three segments for morphological and molecular analysis. A small middle part was excised for molecular identification. Anterior and posterior ends were cleared with Amann's lactophenol and examined for their morphological characteristics. The larvae were identified based on distinct morphological criteria described [5]. Subsequently, all detected larvae were identified as *Anisakis* type I were subjected to molecular analysis to identify the species.

DNA Amplification, PCR and RFLP Analysis and DNA Sequencing

Total DNA was extracted from the middle part of larvae using the DNA extraction kits. The ITS region (ITS1-5.8-ITS2) was amplified using the specific primer pairs NC5/NC2 [38]. PCR amplicons were electrophoresed on 1.5% agarose gels and then visualized by UV illumination. RFLP profiles of ITS rDNA of *Anisakis* larvae were digested with *HhaI* and *HinfI* [9, 29]. The PCR and RFLP analyses were followed according to Pekmezci et al. [28]. One hundred and fifty-six larvae were identified based on the ITS RFLP profiles and randomly selected five larvae among them were sequenced for further confirmation for mtDNA *cox2* gene. PCR was used to amplify the mtDNA *cox2* gene using the



Fig. 1 Geographical area of subdivision 34.3.12 of FAO Major Area 34 [11]

specific primer pairs 210/211 [26]. The mtDNA *cox2* gene was also subjected to sequencing using the same primers. The quality of the sequences were checked using Geneious R11 (Biomatters Ltd) and Vector NTI Advance 11.5 (Invitrogen). Later, sequences were verified by forward and reverse comparisons, assembled and edited with Contig Express in Vector NTI Advance 11.5 (Invitrogen) and Geneious R11 (Biomatters Ltd). Sequences of the specimens found in the current study were compared with other sequences available in GenBank by using the BLASTn [2]. A sequence of the mtDNA *cox2* of *A. pegreffii* (isolate SN-10) was submitted to Genbank under accession number: MK599139.

Statistical Methods

Infection parameters were estimated following Rózsa et al. [31]. Prevalence (*P*), mean intensity (*mI*) and abundance (*mA*) calculations were performed using Quantitative Parasitology (QP) program [30]. *P* was calculated with Sterne's exact 95% confidence limits and a bootstrap procedure with 2000 replications to set 95% confidence intervals of *mI* and *mA* were used [30]. Indeed, *mI* and *mA* are affected with a small sample size (< 40), while *P* is comparatively not significantly affected by sample size [19] and more than 15 specimens is enough to obtain a good estimate of *P* [16]. In the present study, for better estimation of parasitological parameters and sample costs was selected samples size equal to 40 fish for each species.

Results

Parasitological Results and *Anisakis* Prevalence

All *Anisakis* larvae visually detected in body cavities and viscera of imported *Z. faber* were identified as *Anisakis* type I via light microscopy. No *Anisakis* larvae were isolated from *P. prayensis* and *P. caeruleostictus* whereas *Anisakis* larvae were only found in *Z. faber*. The prevalence of *Anisakis* larvae in *Z. faber* was 82.5% (33/40) (95% confidence limits 67.6–91.6%). Moreover, mean intensity and abundance of *Anisakis* larvae were 8.3 (bootstrap 95% confidence limits

7.2–9.3) and 6.8 (bootstrap 95% confidence limits 5.5–8.1), respectively (Table 1). A total of 156 *Anisakis* larvae were removed from the viscera and body cavities, while no *Anisakis* larvae were detected in the muscle, were isolated from *Z. faber* in the ECA's FAO area 34.3.12. Moreover, live *Anisakis* larvae were not detected in the viscera and body cavities of *Z. faber*.

RFLP Analysis and DNA Sequencing

One hundred and fifty-six larvae were identified based on ITS region of RFLP profiles. All RFLP profiles were the same patterns and these RFLP profiles perfectly matched to *A. pegreffii* reported previously [9, 29]. The RFLP profiles of *A. pegreffii* were confirmed by mtDNA *cox2* sequence analysis. The mtDNA *cox2* gene of *A. pegreffii* revealed a 624-bp sequence. The mtDNA *cox2* sequences of *A. pegreffii* (MK599139) from *Z. faber* in the ECA's FAO area 34.3.12 showed 100% identity with *A. pegreffii* (DQ116428) from short-beaked common dolphins (*Delphinus delphis*) in the Northeast Atlantic (Major Fishing Area 27) [34].

Discussion

Fish markets are increasing worldwide with the accessibility of new fresh and marine fish species from tropical and sub-tropical seas. Nowadays people can reach these varieties of fish even in the markets in distant countries [10]. Imported Atlantic mackerel *Scomber scombrus*, West African goatfish *P. prayensis*, bluespotted seabream *P. caeruleostictus* and John Dory *Z. faber* are among the most frequently consumed marine fish in Turkey during the legal fishing ban period (April–October) in Turkish waters. Atlantic mackerel are marketed as whole deep-frozen and typically imported from Norway (FAO area 27). Moreover, whole fresh *P. prayensis*, *P. caeruleostictus* and *Z. faber* are frequently imported from Senegal (FAO area 34.3.12).

Anisakiasis is an emerging parasitic zoonosis throughout the world [20]. Especially, *A. pegreffii* induces infection and allergic symptoms in consumers [3, 9, 12, 13, 17, 20–25, 33, 37]. Within the present study crucial evidence with regard

Table 1 Fish species from the Senegalese coast (FAO 37.4.2) examined for *Anisakis* larvae and their level of infection

Fish species	Mean length (range, cm)	Infected//tested	<i>P</i> (%) (CI)	<i>mI</i> (CI)	<i>mA</i> (CI)	Number of collected larvae
<i>Pseudupeneus prayensis</i>	14.8 (13.1–16.8)	0/40	–	–	–	–
<i>Pagrus caeruleostictus</i>	21.6 (20.4–24.2)	0/40	–	–	–	–
<i>Zeus faber</i>	35.5 (30.7–38.9)	33/40	82.5 (67.6–91.6)	8.3 (7.2–9.3)	6.8 (5.5–8.1)	156

P prevalence, *mI* mean intensity, *mA* mean abundance, *CI* confidence intervals

to sanitary risk is the overall P (%) of *Anisakis* larvae in *Z. faber* of 82.5%. This is the first assessment of the occurrence of *A. pegreffii* in imported *Z. faber* from the ECA's FAO area 34.3.12 in Turkish supermarkets. Furthermore, the presence of *A. pegreffii* in imported *Z. faber* could represent a sanitary risk for Turkish consumers. Similarly, in our previous studies, *A. simplex* (s.s) was found in imported Atlantic mackerel *S. scombrus* from FAO area 27 [27]. Moreover, *A. pegreffii* was genetically identified in different marine fish in Turkish waters during the legal fishing period [28].

Another important finding of this study, there are no *Anisakis* larvae in both *P. prayensis* and *P. caeruleostictus* imported into Turkish supermarkets. Therefore, consuming fresh *P. prayensis* and *P. caeruleostictus* imported from Senegal during the legal fishing ban period in Turkish waters have low to non-existent risk for anisakiasis in Turkish consumers.

In Turkey, thoroughly cooking meals, a native culinary practice, can prevent infections, but not allergies. Moreover, cooking or freezing kill parasites but its allergenic potency still remains. Although well-cooked fish meals are a tradition in Turkey could represent a risk to human health. Moreover, well-cooked fish parasitized by the previously mentioned *Anisakis* larvae can cause allergic processes because these parasites have antigens that act as allergens [4].

Anisakis pegreffii is a dominant species in the Mediterranean Sea [20] and its prevalence of *A. pegreffii* in *Z. faber* from Mediterranean Sea has been reported ranging from 82 to 100% [7, 28]. The current study represents high prevalence values as 82.5% for *A. pegreffii* in *Z. faber* captured in the ECA's FAO area 34.3.12. However, in the present study, no *Anisakis* larvae were detected in the samples of *P. prayensis* and *P. caeruleostictus* from the ECA waters. Since *Z. faber* is a large predatory fish with a high mean trophic level, a high prevalence of *A. pegreffii* was found in *Z. faber* in the present study.

Similarly, *Anisakis* larvae in *P. prayensis* and *P. caeruleostictus* from the ECA (off Senegal) have not been reported before [36]. Moreover, *A. pegreffii*, *A. typica* and *A. physeteris* have been recently reported in *Trichiurus lepturus* and *Nealotus tripes* from Senegalese coast [1].

Consequently, outcomes of the present study demonstrate that there is low to non-existent risk of anisakiasis from the consumption of fresh West African goatfish *P. prayensis* and bluespotted seabream *P. caeruleostictus* from the ECA's FAO area 34.3.12 for Turkish consumers. On the contrary, the presence of *A. pegreffii* larvae in John Dory *Z. faber* from the ECA's FAO area 34.3.12 could have public health implications in Turkey, Europe and other countries. These results are also consistent with our previous study about fish consumers [27, 28]. Therefore, precautions in the Hazard Analysis Critical Control Point (HACCP) program should be revised to decrease the risk of *Anisakis*-related allergies. As

hazards from fish-borne parasitic zoonoses are tremendously important for public health, it is crucial to take appropriate precautions, such as including RASFF (Rapid Alert System for Food and Feed) in the legal system for fish that are imported in Turkey.

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