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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Ongoing Data from Presence of Zoonotic *Anisakis* Larvae in Imported Fish in Turkish Supermarkets: Frozen Atlantic Mackerel (*Scomber Scombrus*) and Smoked Atlantic Salmon (*Salmo Salar*)

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Abstract: *Anisakis pegreffii* and *A. simplex* sensu stricto (s.s.) are the main etiological agents causing human anisakiasis. Here, we aimed to investigate based on the pepsin digestion method of the occurrence of *Anisakis* larvae in imported deep-frozen whole Atlantic mackerel (*Scomber scombrus*) from FAO 27 to Turkey and smoked Norwegian farmed Atlantic salmon fillets (*Salmo salar*) between 2018 and 2019. A total of 100 whole Atlantic mackerel and 180 Atlantic salmon fillets were randomly sampled from local Turkish supermarkets. No *Anisakis* larvae were detected in smoked Atlantic salmon fillets. In total, 827 *Anisakis* larvae were found in mackerel, and the prevalence was 68% (68/100). The mI and mA of *Anisakis* larvae in mackerel were 13.1 and 8.2, respectively. Whereas the 95.28% (788/827) of the *Anisakis* larvae were found in abdominal cavity/viscera, the 4.72 % (39/827) of the larvae in the muscle. The prevalence and mI of *Anisakis* larvae in the abdominal cavity/viscera and muscle of mackerel was 63.0% (63/100) and 42.0% (42/100), and 12.5 and 0.9, respectively. The subsample of 100 larvae was identified by molecular methods. The 99 (99.0%) larvae were identified as *A. simplex* (s.s.), and 1 (1.0%) larva was *A. pegreffii*. Consequently, there is low or no risk of anisakiasis in smoked farmed Atlantic salmon for Turkish consumers. The 42.0% prevalence of zoonotic *Anisakis* species larvae in imported Atlantic mackerel fillets could have public health risk in Turkish consumers for anisakiasis or allergy

Keywords: FAO 27, ITS region, parasite, PCR–RFLP, zoonoses.

Türk Süpermarketlerindeki İthal Balıklarda Zoonotik *Anisakis* Larvalarının Varlığına İlişkin Devam Eden Veriler: Dondurulmuş Atlantik Uskumru (*Scomber Scombrus*) ve Füme Atlantik Somonu (*Salmo Salar*)

Öz: *Anisakis pegreffii* ve *A. simplex* sensu stricto (s.s.) türleri insan anisakiasisine neden olan ana etiyolojik ajanlardır. Bu çalışmada *Anisakis* larvalarının varlığı 2018-2019 yılları arasında FAO 27 avcılık sahasında avlanıp ve sonrasında Türkiye'ye ithal edilen derin dondurulmuş bütün Atlantik uskumru (*Scomber scombrus*) ve tütsülenmiş Norveç çiftlik Atlantik somon filetolarında (*Salmo salar*) pepsin sindirim yöntemine göre araştırılması amaçlanmıştır. Toplam 100 bütün Atlantik uskumru ve 180 Atlantik somon filetosu Türk süpermarketlerinden örneklendi. Füme Atlantik somon filetolarında *Anisakis* larvası tespit edilmedi. Uskumruda toplam 827 *Anisakis* larvası bulunmuş olup, larvaların enfeksiyon oranı %68 (68/100) olarak saptanmıştır. Uskumruda *Anisakis* larvalarının ortalama yoğunluk (mI) ve ortalama bolluk (mA) değerleri sırasıyla 13,1 ve 8,2 idi. *Anisakis* larvalarının %95,28'i (788/827) karın boşluğu/iç organlarında bulunurken, larvaların %4,72'si (39/827) kaslarda bulunmuştur. *Anisakis* larvalarının uskumrunun karın

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boşluğu/iç organları ve kasındaki enfeksiyon oranı ve mI değerleri sırasıyla %63,0 (63/100) ve %42,0 (42/100) ile 12.5 ve 0.9 idi. Çalışmada örneklenen 100 larva moleküler metotlara göre identifiye edildi. Araştırmada 99 (%99,0) larva *A. simplex* (s.s.) ve 1 (%1,0) larva *A. pegreffii* olarak teşhis edildi. Sonuç olarak Türk tüketiciler için tütsülenmiş çiftlik Atlantik somonunda anisakiasis riski düşüktür veya hiç yoktur. İthal Atlantik uskumru filetolarındaki zoonotik *Anisakis* türü larvalarının %42,0 oranındaki yaygınlığı Türk tüketicilerde anisakiasis veya alerji açısından halk sağlığı riski oluşturabilir.

Anahtar kelimeler: FAO 27, ITS gen bölgesi, parazit, PCR–RFLP, zoonoz .

INTRODUCTION

Anisakiasis is a significant fish-borne zoonotic disease. Anisakid nematodes of the genus *Anisakis* Dujardin, 1845 are ascaridoid nematodes colonizing the digestive system of marine vertebrates. Marine mammals (mainly cetaceans) serve as definitive hosts, fish and squids are intermediate or paratenic hosts, while planktonic or semiplanktonic crustaceans act as first intermediate hosts. Consumers may be infected with consuming raw, processed fish products or undercooked fish and squids containing *Anisakis* larvae (Mattiucci et al., 2018). Gastric, gastroallergic, intestinal, and ectopic anisakiasis have been reported in consumers (Audicana & Kennedy, 2008; EFSA, 2010; Mattiucci et al., 2018). *Anisakis pegreffii* and *A. simplex* (s.s.) species are major etiological agent of anisakiasis worldwide (Umehara et al., 2007; Mattiucci et al., 2011, 2013; Bao et al., 2017; Mattiucci et al., 2017a, 2017b, 2018). Several nuclear markers including internal transcribed spacer, ITS1-5.8-ITS2 (ITS) (the region used in RFLP analysis), allozymes, elongation factor 1 alpha1 nDNA (EF1 α-1 nDNA region), and Beta-tubulin (β-TUB) have been successfully used for the identification and genetic analysis of *Anisakis* species (D'Amelio et al., 2000; Pekmezci, 2014; Pekmezci et al., 2014; Mattiucci et al., 2016; Gómez-Mateos et al., 2020; Simsek et al., 2020; Aydın & Pekmezci, 2023).

We aimed to investigate the occurrence of zoonotic *Anisakis* larvae in whole frozen Atlantic mackerel and smoked Atlantic salmon fillets imported from FAO 27 geographical areas to Turkey, and determine their epidemiological data.

MATERIAL AND METHOD

***Fish sampling and parasitological examination*:** One hundred, frozen, 200-400 g, eviscerated Atlantic mackerel (*Scomber scombrus*) imported from FAO 27 and 180 smoked Norwegian farmed Atlantic salmon fillets (*Salmo salar*) packed in 100 g packages were randomly sampled from local supermarkets in Samsun province of Turkey between 2018 and 2019. After thawing, Atlantic mackerel were carefully eviscerated and filleted into dorsal and ventral fillets. Viscera and fillets of each Atlantic mackerel and Atlantic salmon fillets were examined using the pepsin digestion method for inspection of *Anisakis* larvae (Llarena-Reino et al., 2013, Lunestad, 2003). *Anisakis* larvae were washed with physiological saline and then individually cut into three parts. Mid-body of larvae were used in genetic analyses. The rest parts were cleared using lactophenol solution, placed on slides, and made morphological identification based on literature (Berland, 1961; Petter & Maillard, 1988).

***Molecular identification*:** One-hundred subsample of *Anisakis* larvae were randomly selected, and their genomic DNA were individually obtained with DNA extraction kit. The entire ITS region was amplified with PCR methods (Zhu et al., 1998). All PCR protocols were applied according to Pekmezci et al. (2014). PCR products were digested with *Hinf* I and *Hha* I by restriction fragment length polymorphism (RFLP) technique, and RFLP patterns were analysed (D'Amelio et al., 2000). The ITS regions of five specimens (four *A. simplex* (s.s), one *A. pegrefii*) were also sequenced to confirm their identity. Obtained ITS raw data were assembled and edited with Contig Express in Vector NTI Advance 11.5 (Invitrogen). The consensus sequences were compared with those already obtained for the same gene in previous study (Mattiucci et al., 2014).

Epidemiological data: Prevalence (P), intensity (mI), and abundance (mA) were analysed by QP 3 (Reiczigel et al., 2019).

RESULTS

Parasitological and epidemiological findings: No *Anisakis* larvae were found in 180 smoked salmon fillets, while 68 out of the 100 (68.0%) mackerel were detected. In total 827 larvae were collected, and identified as *Anisakis* type I (Fig. 1). The mI and mA of *Anisakis* larvae in mackerel were determined as 13.1 and 8.2, respectively. Whereas 788 (95.28%) of the 827 *Anisakis* larvae were found in the abdominal cavity/viscera, 39 (4.72%) larvae were found in the fillets from mackerel. The P and mI of larvae in the abdominal cavity/viscera and muscle of mackerel was 63% (63/100) and 42% (42/100), and 12.5 and 0.9, respectively.

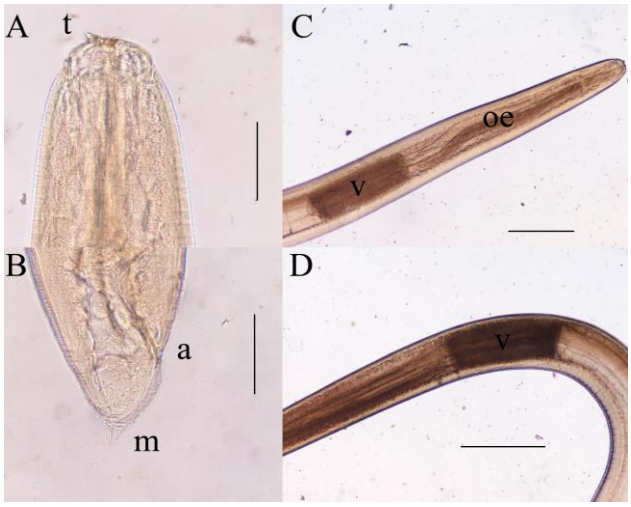

Figure 1. *Anisakis* type I larva. **A:** detail of anterior end, scale: 100 µ; **B:** Posterior end, scale: 100 µ, **C:** anterior end, scale: 400 µ, **D:** ventriculus, scale: 500 µ.

Molecular findings: PCR products of all larvae were successfully digested with *Hha*I and *Hinf* I restriction enzymes. While the 99 out of 100 (99.0%) of *Anisakis* larvae were identified as *A. simplex* sensu stricto (s.s.), (*Hha*I: ~550 and ~430 bp; *Hinf*I: ~620 bp and ~280 bp), 1 larva (1.0%) was hybrid (*Hha*I: ~550 and ~430 bp; *Hinf*I: ~620 bp, ~370 bp, ~300 bp, and ~250 bp) based on RFLP analyses (Fig. 2A–B). RFLP results were also confirmed by ITS sequencing. The ITS data of four specimens of *A. simplex* (s.s.) herein showed 100% of identical with adults *A. simplex* (s.s.) sequence (JX535521) in the Norwegian coast. Our sequence of the ITS region of one hybrid matched 100% with adult *A. pegreffii* sequence (JX535520) in the Mediterranean Sea. Although one species has hybrid patterns by RFLP method, this species was considered as *A. pegreffii* by ITS sequence analysis in the present study.

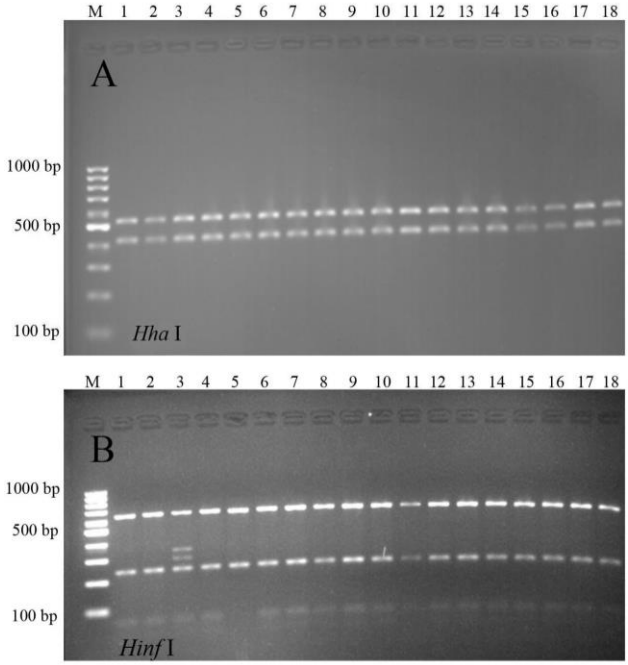

Figure 2. RFLP patterns obtained by digestion of the ITS region of the rDNA with the restriction enzymes Hha I (A) and Hinf I (B) shown by the species of the genus *Anisakis*. Lanes: 1–2: *A. simplex* (s.s), 3: Hybrid, 4–18: *A. simplex* (s.s), M: 100 bp ladder.

DISCUSSION

Herein, no *Anisakis* larvae were found in the 180 smoked Norwegian farmed Atlantic salmon fillets. Our results are consistent with previous findings of the absence of *Anisakis* larvae in Norwegian farmed Atlantic salmon (Angot & Brasseur 1993; Lunestad, 2003; Levsen & Maage, 2016; González et al., 2020; Simsek et al., 2020). However, the 42.0% (42/100) high prevalence of zoonotic *Anisakis* species have been found in the edible muscles of imported Atlantic mackerel in the present study. *Anisakis* larvae has been reported from edible parts of imported Atlantic mackerel in Turkey, and their prevalence of were 17,5% (7/40) and 11% (11/100) (Pekmezci, 2014; Simsek et al., 2020). In the current study, the prevalence of *Anisakis* larvae in the imported Atlantic mackerel was found to be higher than those previous studies in Türkiye, and zoonotic *A. pegreffii* species was also genetically identified unlike previous studies. Furthermore, current findings indicate that imported Atlantic mackerel fillets still continue to public health risk in Turkish consumers for anisakiasis or allergy.

Among all *Anisakis* specimens, *A. simplex* (s.s.) is the dominant species in the FAO 27 catching areas (Mattiucci et al., 2018). *Anisakis pegreffii* has also been detected with very low prevalence (1 %) in the FAO 27 geographical areas compared with the Mediterranean Sea (Madrid et al., 2016; Levsen et al., 2018). Herein, 99.0 % of all larvae detected in Atlantic mackerel were molecularly identified as *A. simplex* (s.s.).

Turkish people usually consume well-cooked fish meat. Because *Anisakis* allergens does not destroy by heat-cooking methods (Caballero & Moneo, 2004; Moneo et al., 2005), consumption of fillets of imported Atlantic mackerel infected with zoonotic *Anisakis* larvae could have public health risk in Turkish consumers. Therefore, we suggest that the HACCP systems should be revised to reduce the risk of *Anisakis* allergies for Turkish consumers.

CONCLUSION

Our study present ongoing data from presence of zoonotic *Anisakis* larvae in imported fish in Turkish supermarkets. Although frozen Atlantic mackerel (*Scomber scombrus*) fillets pose a risk for Anisakiasis, smoked Atlantic salmon (*Salmo salar*) does not for allergy for Turkish consumers.

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