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RESEARCH ARTICLE

Effect of *Anisakis pegreffii* (Nematoda: Anisakidae) on Biochemical and Haematological Characteristics of Chub Mackerel (*Scomber japonicus* Houttuyn, 1782) Caught in the Dardanelles at Çanakkale, Türkiye

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Abstract: Chub mackerel (*Scomber japonicus* Houttuyn, 1782) were obtained from the commercial fishermen in Çanakkale, Turkey, in July, 2017. We examined a total of 40 fish (20 non-infested and 20 infested) and assessed the biometric indices, haematological parameters and serum biochemical variables. The hepatosomatic index and gonadosomatic index of infested chub mackerel fish were lower than those of non-infested fish. Blood haematocrit ratio and haemoglobin concentration in naturally parasite-infested chub mackerel fish were significantly lower than those in non-infested fish. However, white blood cell counts of the parasite-infested chub mackerel fish were higher than those of healthy ones. Serum total protein, globulin, glucose, cholesterol, triglyceride, urea, chlorine and iron levels in naturally parasite-infested chub mackerel fish were significantly lower than those in non-infested fish. Moreover, serum lactate dehydrogenase, aspartate aminotransferase, alkaline phosphatase and alanine aminotransferase activities of the parasite-infested chub mackerel fish were higher than those in healthy ones. Therefore, observed variations in haematological parameters, serum biochemical variables and biometric indices influenced by the parasite, A. pegreffii, may potentially increase sensitivity of the chub mackerel, Scomber japonicus, to diseases and environmental conditions.

Anahtar kelimeler:

Kurt
Parazit
Anisakiasis
Sağlık
Biyometrik İndeksler
18S rRNA

Çanakkale Boğazı'ndan Yakalanan Kolyoz'un (*Scomber japonicus* Houttuyn, 1782) Biyokimyasal ve Hematolojik Karakteristikleri Üzerine *Anisakis pegreffii* (Nematoda: Anisakidae)'nin Etkisi

Öz: Kolyoz (*Scomber japonicus* Houttuyn, 1782) Temmuz 2017'de Çanakkale, Türkiye'deki ticari balıkçılardan elde edildi. Toplam 40 balığın (20 parazitsiz, 20 parazitli) biyometrik indeksleri, hematolojik parametreleri ve serum biyokimyasal değişkenleri incelendi. Parazitli kolyoz balıklarının hepatosomatik indeksi ve gonadosomatik indeksi, parazitsiz balıklardan daha düşüktü. Doğal olarak parazitle istila edilmiş kolyoz balıklarında kan hematokrit oranı ve hemoglobin konsantrasyonu, parazitsiz balıklardan önemli ölçüde daha düşüktü. Bununla birlikte, parazitli kolyoz balıklarının beyaz kan hücresi sayıları sağlıklı olanlardan daha yüksekti. Doğal olarak parazitli kolyoz balıklarında serum total protein, globulin, glikoz, kolesterol, trigliserit, üre, klor ve demir seviyeleri, parazitsiz balıklardan önemli ölçüde daha düşüktü. Ayrıca, parazitli kolyoz balıklarının serum laktat dehidrojenaz, aspartat aminotransferaz, alkalin fosfataz ve alanin aminotransferaz aktiviteleri sağlıklı olanlardan daha yüksekti. Bu bulgular ışığında, parazitin kolyoz, *Scomber japonicus*'un hematolojik parametreler, serum biyokimyasal değişkenler ve biyometrik indeksler de meydana getirdiği değişimlerin balığı farklı hastalıklara ve/veya çevresel koşullara karşı daha duyarlı hale getirebileceği söylenebilir.

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Introduction

Anisakis pegreffii, is a nematode parasite belonging to Anisakidae family (Campana-Rouget and Biocca, 1955) with a complex life cycle that affects marine organisms at different trophic levels. In the life cycle of the *A. pegreffii*, small crustaceans are the intermediate host, fish and cephalopods are the intermediate and/or paratenic hosts while marine mammals are the definitive host (Mattiucci et al., 2008). The third-stage *A. pegreffii* larvae (L3) are infective to fish and cephalopods and are commonly found in the viscera and the musculature of these host species. Consuming infected animals raw or undercooked causes a disease called Anisakiasis in humans (Macchioni et al., 2021; Mattiucci et al., 2008; Mattiucci & D'Amelio, 2014; Mladineo & Poljak, 2014; Sakanari & McKerrow, 1989).

Anisakis pegreffii larvae have been reported in various fish species in many regions so far such as Africa, Australia, Oceania, Europe, South America and also in Turkish coastal waters (Aibinu et al., 2019; Özbakış Beceriklisoy et al., 2020; Pekmezci et al., 2014) . *A. pegreffii* is the most important anisakid nematode larva in pelagic and demersal fish living in the Mediterranean waters, and life cycle of the parasite mostly includes fish species belonging to the families Salmonidae, Gadidae, Scombridae, Merlucciidae and Carangidae (Mattiucci et al., 2018; Menconi et al., 2022).

The relationship between parasites and hosts in both aquaculture and natural populations and their adverse effects on growth and survival have been demonstrated in parasite-host systems (Bichi & Dawaki, 2010). Damage on the host depends on the parasite species, the type of deterioration in the hosts tissue, the amount of parasite and the host's health condition (Tavares-Dias et al., 1999). Parasites can cause a decrease in haemoglobin concentrations, hematocrit levels and erythrocyte counts which called anemia (Martins et al., 2004). Also parasites affect the energy metabolism of their hosts. In many host-parasite relationships, parasites increase the metabolic rate of the host (Devevey et al., 2008). Therefore, changes associated with haematological and serum biochemical variables due to different parasites form a database that can be used in diagnosing diseases and preventive measures (Çelik & Aydin, 2006; Nnabuchi et al., 2015).

Chub mackerel, *Scomber japonicus* Houttuyn, (1782) is a pelagic fish species, which is distributed in the Atlantic, Black Sea, Mediterranean Sea, Indian and Pacific Oceans (Whitehead et al., 1984). Small pelagic fish such as anchovy, sardines, sprats, silversides and small invertebrates are the main food source of this species while *S. japonicus* is one of the most important element in the diet of other larger pelagic species and sea mammals (Collette & Nauen, 1983; Zardoya et al., 2004). However, there is no study on the impact of *A. pegreffii* on the biometric indices and blood variables of chub mackerel. Therefore, the present study was carried out to investigate the impact of *A. pegreffii* infestation on the biometric indices, haematological and serum biochemical parameters of the chub mackerel, *S. japonicus*.

Material and Methods

Fish and parasite sampling

Chub mackerels (*Scomber japonicus* Houttuyn, 1782) were obtained from commercial fishermen in Çanakkale, Turkey in July, 2017. We examined a total of 40 fish (20 non-infested, 20 infested) and assessed the haematological and biochemical parameters. After recording wet body mass and body length, all individuals were dissected and the liver, spleen and gonad of each fish were weighed. Then, individuals were immediately examined for the parasites. For this purpose, the abdominal cavity was visually checked for the presence of nematodes and the fish with parasites were immediately examined. Nematode larvae were collected from the muscles, body cavum and visceral organs and fixed in 70% ethanol. The collected *Anisakis* larvae were morphologically identified according to Quiazon et al. (2009).

Calculations of biometric indices

The condition factor (CF), splenosomatic index (SSI), hepatosomatic (HSI) and gonadosomatic index (GSI) were calculated according to the formulae proposed by Nikolsky (1963) and White and Fletcher (1985).

Blood sampling

1-1.2 mL blood were taken from the tail vein through a plastic syringe, and 400-500 µL blood transferred to EDTA tubes for haematological analysis. 600-700 µL blood was harvested in serum separation tubes. Then, the serum samples were separated by centrifugation at 4000 ×*g* for 5 min and kept in a −80 °C freezer for analysis.

Haematological analysis

Red blood cell (RBC, 10^6 mm^{-3}) counts, haematocrit (Hct, %) ratios and haemoglobin (Hb, g/dL) concentrations were determined according to Blaxhall and Daisley (1973). The white blood cell (WBC, 10^4 mm^{-3}) counts were estimated indirectly by the methods of Yilmaz et al. (2016).

Biochemical analysis

Albumin (g/dL), globulin (g/dL), total protein (g/dL), urea (mg/dL), uric acid (mg/dL), creatinine (mg/dL), glucose (mg/dL), cholesterol (mg/dL), triglyceride (mg/dL), alkaline phosphatase (U/L), aspartate aminotransferase (U/L), lactate dehydrogenase (U/L), alanine aminotransferase (U/L), calcium (mg/dL), iron (µg/dL) and phosphorus (mg/dL) values were measured using commercial Bioanalytic kits in a spectrophotometer, as suggested by Yilmaz (2018), Yilmaz et al., (2012) and Yilmaz and Ergün (2012).

DNA extraction, PCR amplification and DNA sequencing

Nematode larvae were first homogenized with a bead-based homogenizer (Bullet Blender Storm - Next Advance Inc.) using glass beads (1.0 mm) for 5 min and then, genomic DNA was obtained using the commercial PureLink Genomic DNA Mini Kit (Catalog number: K182001, Invitrogen) following the manufacturer's instructions. To identify the parasites, partial 18S rRNA gene was amplified using a universal eukaryotic primer set F-566 and R-1200 (Hadziavdic et al., 2014). The PCR mix containing 0.4 µM of each primer, PCR Master Mix (2X) (Thermo Scientific), template DNA (approximately 50 ng) was used for amplifications using a thermal cycler (Biometra TAdvanced - Analytik Jena AG). The cycler was programmed as follows: initial denaturation 94°C for 5 min; 35 cycles of amplification (94°C for 30 s; 60°C for 30 s; 72°C for 1 min) and final extension at 72°C for 10 min. Agarose gel electrophoresis (1.6% wt/vol) was used for visualizing the PCR products in TAE buffer and electrophoresis was performed. All PCR products were purified and sequenced by a sequencing company (Medsantek Ltd., Istanbul/Turkey). Sequence editing and analyses were performed in Bioedit v7.0.0 (Hall, 1999) using the ClustalX 2.1 (Larkin et al., 2007) and BLASTN 2.2.20 algorithm (Zhang et al., 2000).

Statistical analysis

The statistical analysis was performed using SPSS 19.0 (SPSS Statistics) and the significance level was considered to be 0.05. All parameters were analyzed by T-test (Student's t-test).

Results

According to their morphological characteristics and partial 18S rRNA gene (Fig. 1) sequencing results with 100% similarity (acc. no. MF037865), the parasite was identified as *Anisakis pegreffii*.

The hepatosomatic index and gonadosomatic index of infested chub mackerel fish were significantly ($p<0.05$) lower than those of non-infested fish (Table 1). However, the means of body weight, body length, splenosomatic index and condition factor were not significantly different ($p>0.05$).

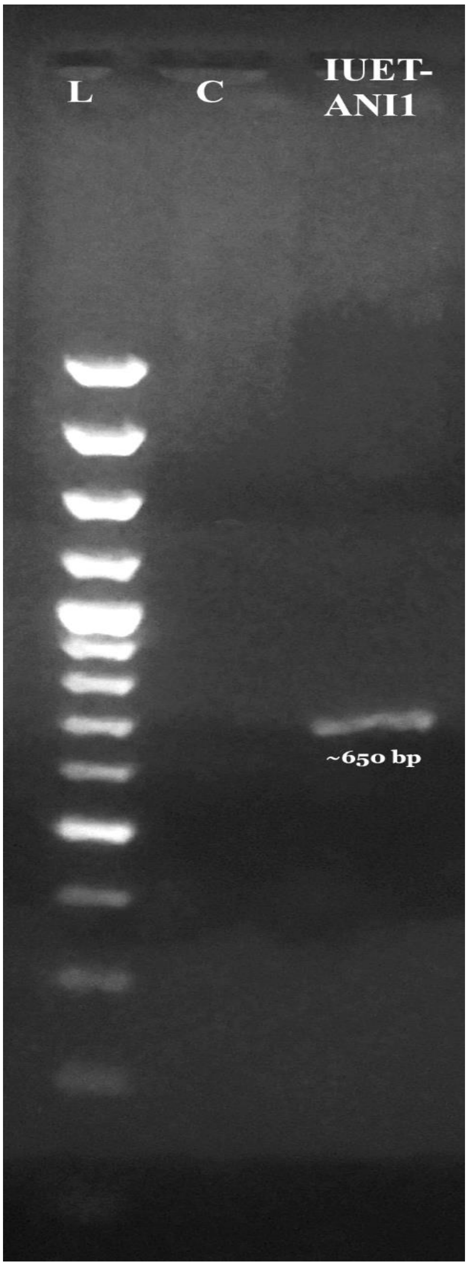

Figure 1. Agarose gel electrophoresis image of the PCR product Lane 1: (L) GeneRulerTM 100 bp DNA Ladder (Thermo Scientific), Lane 2: Negative control, Lane 3: Partially amplified 18S rRNA gene of the nematode

Table 1. The length, weight, hepatosomatic index (HSI), splenosomatic index (SSI), condition factor (CF), rate of gonadosomatic index (GSI) in the studied chub mackerel

Characteristic	Naturally parasite-infested fish mean ± SEM (n=20)	Healthy fish mean ± SEM (n=20)
Body weight (g)	126.12±2.15	138.15±3.11
Body length (cm)	24.62±0.21	24.98±0.41
HSI (%)	$0.85±0.01^{b}$	$1.12±0.05^{a}$
SSI (%)	0.15±0.02	0.16±0.02
CF (%)	0.79±0.10	0.82±0.05
GSI (%)	$3.56±0.25^{b}$	$5.98±0.24^{a}$

Values within a row denoted with different small letter is significantly different according to t-test

Blood haematocrit ratio and haemoglobin concentration in naturally parasite-infested chub mackerel fish were significantly ($p<0.05$) lower than in non-infested fish (Table 2). However, white blood cell counts of the parasite-infested chub mackerel fish were higher than healthy ones ($p<0.05$).

Serum iron, total protein, globulin, cholesterol, urea, triglyceride and glucose levels in naturally parasite-infested chub mackerel fish were significantly ($p<0.05$) lower than in non-infested fish (Table 3). Moreover, serum aspartate aminotransferase, alkaline phosphatase, lactate dehydrogenase and alanine aminotransferase activities of the parasite-infested chub mackerel fish were higher than healthy ones ($p<0.05$).

Table 2. Haematological parameters of naturally and healthy parasite-infested chub mackerel

Haematological parameters	Naturally parasite-infested fish mean ± SEM (n = 20)	Healthy fish mean ± SEM (n=20)
Haematocrit (%)	31.09±1.56 [b]	36.54±1.85 [a]
Haemoglobin (g/dL^{-1})	11.12±0.28 [b]	13.16±0.91 [a]
Red blood cell (10^6 mm^{-3})	2.98±0.47	3.02±0.56
White blood cell (10^4 mm^{-3})	2.56±0.18 [a]	1.73±0.34 [b]

Values within a row denoted with different small letter is significantly different according to t-test

Table 3. Serum biochemical characteristics of naturally parasite-infested and healthy chub mackerel

Serum Biochemical Parameters	Naturally parasite-infested fish mean ± SEM (n = 20)	Healthy fish mean ± SEM (n=20)
Total protein (g/dL)	2.56±0.01 [b]	3.84±0.21 [a]
Albumin (g/dL)	1.11±0.12	1.42±0.13
Globulin (g/dL)	1.45±0.06 [b]	2.42±0.12 [a]
Creatinine (mg/dL)	0.14±0.01	0.15±0.02
Urea (mg/dL)	1.32±0.08 [b]	2.96±0.18 [a]
Uric acid (mg/dL)	7.76±0.12	7.55±0.34
Glucose (mg/dL)	25.85±0.96 [b]	56.47±1.25 [a]
Cholesterol (mg/dL)	85.26±2.55 [b]	108.22±3.12 [a]
Triglyceride (mg/dL)	46.28±2.16 [b]	81.22±1.98 [a]
Aspartate aminotransferase (U/L)	95.21±10.08 [a]	46.22±10.17 [b]
Alanine aminotransferase (U/L)	25.16±1.13 [a]	11.21±0.96 [b]
Alkaline phosphatase (U/L)	59.14±0.67 [a]	32.21±1.18 [b]
Lactate dehydrogenase (U/L)	1268.16±21.58 [a]	854.21±62.13 [b]
Calcium (mg/dL)	14.44±1.16	15.84±1.51
Phosphorus (mg/dL)	16.31±0.76	16.09±0.85
Iron (µg/dL)	101.87±10.19 [b]	155.85±10.27 [a]

Values within a row denoted with different small letter is significantly different according to t-test

Discussion

In the present study, *Anisakis pegreffii* were recovered within the body cavity, liver, gonad and muscles of chub mackerel (*S. japonicus*) caught in the Dardanelles, Çanakkale. Similarly, *Trachurus trachurus* (Mattiucci et al., 2008; Utuk et al., 2012), *S. japonicus*, *Scomber scombrus, Micromesistius poutassou*, *Merluccius merluccius*, *Trachurus mediterraneus*, *Mullus barbatus* (Pekmezci et al., 2014) and *Zeus faber* (Yardimci et al., 2014) were the fish intermediate hosts for *A. pegreffii* in the Turkish seas.

To our knowledge, this is the first report to evaluate the biometric indices, haematological parameters and serum biochemical variables of chub mackerel naturally parasitized by *A. pegreffii*.

Biometric indices, haematological parameters and serum biochemical variables are important parameters of health status used in determining the effect of parasitic diseases in fish (Çelik & Aydin, 2006; Özdemir et al., 2016; Maqbool & Ahmed, 2016). In this study, the mean HSI and GSI levels of parasite-infested chub mackerel fish were significantly lower than that in healthy ones. Previous studies showed that high numbers of parasites caused destruction of organ structures and subsequent decrease in organ size (Ryberg et al., 2020; Ryberg et al., 2022). This result is in accordance with that of Ondračková et al. (2010) indicating that larval parasitic nematode *Raphidascaris acus* decreased GSI level in infested female *Neogobius kessleri*. Similarly, Çelik and Aydin (2006) declared that average HSI levels of the *Scorpaena porcus* fish naturally infested with *Trachelobdella lubrica* was lower than that of non-infested fish. However, *Anisakis* infections did not affect condition factor, total length, total weight, hepatosomatic index and gonadosomatic index of *Scomber colias* and *Scomber scombrus* (Santos et al., 2017). These different findings might be related with differences in season, geographic region, fish species and/or parasite species.

In this study, slight reduction of red blood cell count and a significant reduction in serum iron, Hb and Hct values were observed in *A. pegreffii*- infested fishes. Previous studies indicated that parasites like *T. lubrica* (Çelik & Aydin, 2006), *C. oestroides* (Özdemir et al., 2016), *Trypanosoma kashmirensis* (Maqbool and Ahmed, 2016), *Trypanosoma carassii (*McAllister et al., 2019), *Dactylogyrus* sp., *Trichodina* sp. and *Lernaea cyprinacea* (Panjvini et al., 2016) and *Trypanosoma* spp. (Sousa et al., 2020) often caused anemia which is characterized by decreased red blood cell count, Hb concentration and/or Hct ratio.

White blood cells, serum proteins, albumin and globulin play significant roles in the immune response in parasitic infections (Çelik & Aydin, 2006; Özdemir et al., 2016). The high number of leucocytes in infested chub mackerel can be a reaction of cellular immune system to *A. pegreffii* infection. Similar results were also observed in *Dactylogyrus* sp., *Trichodina* sp. and *L. cyprinacea* infested *Cyprinus carpio* (Panjvini et al., 2016), *Thelohanellus mrigalae* infested *Cirrhinus mrigala* (Manna & Naskar, 2021) and *Argulus* sp. infested *Carassius auratus* (Shameena et al., 2021).

The decreased serum total protein and globulin concentrations may occur during infectious diseases and nutritional imbalances (Çelik & Aydin, 2006). The same results were also obtained in *C. oestroides* infested *Boops boops* (Özdemir et al., 2016). Ryberg et al. (2020) reported that the plasma protein level decreased while plasma globulin level increased significantly with increasing *Contracaecum osculatum* density in *G. morhua*.

Previous studies demonstrated that creatinine, urea and uric acid levels could be interpreted as a biomarker for determining the damage of gill and kidney diseases (Campbell, 2004). Similar to our result, Çelik and Aydin (2006) who found that serum urea concentration in naturally infested black scorpion (*S. porcus*) were significantly lower than those in non-infested fish.

The low serum glucose, triglyceride and cholesterol levels in the infested fish group may be the result of undernourished condition, liver disorders and inflammation. Similarly, the decreased serum glucose, triglyceride and cholesterol levels were also observed in *S. porcus* infested with *T. lubrica* (Çelik & Aydın, 2006). Özdemir et al. (2016) reported that serum glucose and triglyceride levels were decreased in the *C. oestroides* infested fish.

Serum enzyme activities are accepted as important indices of tissue damages, for example aspartate aminotransferase and alanine aminotransferase are indices of liver damage, lactate dehydrogenase for liver or muscle damages, and alkaline phosphatase for biliary system damage in fish (Yilmaz et al., 2019). Therefore, the increase in these enzymes in *A. pegreffii* infested fish could be attributed to liver and muscle tissue damage. In agreement with our findings, earlier studies reported increases of lactate dehydrogenase, aspartate aminotransferase, alkaline phosphatase and/or alanine aminotransferase enzymes in response to parasitic infections in fish (Çelik & Aydın, 2006; Datta et al., 2022; Kundu et al., 2016; Nabi et al., 2022; Özdemir et al., 2016; Rastiannasab et al., 2016).

In conclusion, observed variations in haematological parameters, serum biochemical variables and biometric indices influenced by the parasite, *A. pegreffii*, may potentially increase sensitivity of the chub mackerel, *Scomber japonicus*, to diseases and environmental conditions.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contributions

E. Şanver Çelik: Study Idea; Investigation; Collect Data; Writing Original Draft, R. E. Kanat: Writing Original Draft, P. Dermanci: Writing Original Draft, D. Kahraman Yilmaz: Writing Original Draft; Review & Editing, E. Turgay: Writing Original Draft; Methodology; Review & Editing, S. Karataş: Writing Original Draft; Methodology, S. Yılmaz: Methodology; Statistical Analyses; Writing Original Draft; Review & Editing, All authors contributed to the study conception, design and writing.

Ethics Approval

This study was carried out the approval of Canakkale Onsekiz Mart University Animal Experiments Local Ethics Committee (Protocol Number: 2017/05-05).

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