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Infection of *Hysterothylacium aduncum* (Namatoda: Anisakidae) in farmed rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792)

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Farmed rainbow trout (*Oncorhynchus mykiss*) were examined for anisakid nematodes at fish farms in Elazıg city, Turkey. A total of 439 fish (246 from fish farm ponds and 193 from net-cages) were monthly investigated in the period from February 2000 to May 2003. Only the endoparasite, *hysterothylacium aduncum* (Nematoda: Anisakidae) was recorded in the digestive tract of the 91 cultured rainbow trout, *O. mykiss* fed with minced marine fish. Prevalence, mean intensity, and the abundance of *H. aduncum* on fish obtained from fish farm ponds were 36.99%, 16.00 ± 1.15, and 5.92 ± 0.15, respectively. However, this values in fish fed with freshly minced marine fish were 100%, 16.00 ± 1.15 and 16.00 ± 1.15, respectively. *H. aduncum* was found in the oesophagus, stomach, intestine, and pyloric caeca of fish. All *H. aduncum* were adult and was not found on fish fed with commercial pellets.

Key words: *Hysterothylacium aduncum*, rainbow trout, nematoda, anisakidae.

INTRODUCTION

Nematodes are usually considered the most economically important helminth parasites of fishes in the world (Dick and Choudhury, 1995). Most adult nematodes are found in the intestine of fish, but larval stages are sometimes found in the flesh and viscera which cause disease and economical loses. It is also the larval stages which are infective to humans and which have the greatest impact on consumer acceptance of fish as a source of protein (Dick and Choudhury, 1995; Moravec, 1994).

Members of the family Anisakidae parasitise are fish, mammals, birds and reptiles (Moravec, 1994; Zhu et al., 1998). Anisakids are among most common nematodes of fish. They cause patholagical symptoms and mortalities, and reduce the commercial value of fish (Dick and Choudhury, 1995). Larval and adult anisakids infect freshwater fishes (Cyprinidae, Ictaluridae, contrarchidae, percidae and salmonidae) (Hoffman, 1998; Ekingen, 1983).

The presence of larval and adult nematodes belonging to the genus *hysterothylacium* was reported in the freshwater and marine fish farms (Moravec, 1994; Hoffman, 1998; Gonzalez, 1998). Furthermore, it is known that species of marine fish can act as intermediate, paratenic or definitive host (Zhu et al., 1998). *Hysterothylacium aduncum* is mainly found in marine piscivorous fish (*Gadus morhua* Linnaeus, 1758) and its larvae occur in a variety of prey fishes including smaller cod (*G. morhua* Linnaeus, 1758) and mackerel (*Scomber scombrus* Linnaeus, 1758) (Dick and Choudhury, 1995).

Inoue et al. (2000) investigated the possibility of larval

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anisakid infection in farmed salmon, *Oncorhynchus mykiss* (Walbaum, 1792) in Tokyo, Japan. The life cycle of *H. aduncum* was shown experimentally by Gonzalez (1998) and Yoshinaga et al. (1987). The third-stage larva of *H. aduncum* was defined in flounder (*Platichthys flesus* Linnaeus, 1758; Koie, 1999). Ismen and Bingel (1999) studied *H. aduncum* infection in the whiting, (*Merlangius merlangius* euxinus Nordmann, 1840), off Turkish coast of the Black Sea. Shih and Jeng (2002) observed *H. aduncum* infecting a herbivorous fish, (*Siganus fuscescens* Houttuyn, 1782), off the Taiwanese coast of the Northwest Pacific.

In this study, the existence of anisakid nematodes was investigated in organs and tissues of rainbow trout cultured with respect to a prevalence mean intensity of infection and mean abundance in freshwater ponds and net-cages in Elazıg city, in Turkey.

MATERIALS AND METHODS

Fish

A total of 439 cultured rainbow trout (246 from freshwater ponds and 193 from net-cages) were examined for endoparasites throughout the study period. Farmed rainbow trout (*O. mykiss*) (age 0+, weight, 200 to 250 g and total length, 20 to 26 cm) for this study were monthly obtained from two different commercial net-cages farms in the Keban Dame Lake and from a commercial fish farm ponds in Elazıg, Turkey in the period from February 2000 to May 2003. These were farmed in freshwater ponds and net-cages for about 10 to 12 months after hatching. Cultured fish in net-cages were fed with commercially prepared pellets. While some of fish obtained from fish farm ponds were fed with only commercially prepared pellets, some others were fed with only freshly minced marine fishes such as anchovy (*Engraulis engrasicholus* Linnaeus, 1758), whiting (*Merlangius merlangus* Linnaeus, 1758) and scad (*Trachurus trachurus* Linnaeus, 1758). All fishes were fed three times in a day.

Examination of rainbow trout for anisakid nematodes

The organs and tissues of rainbow trout were examined for anisakid nematodes using the methodology of Chubb and Powell (1966). The muscle of fish was sliced and carefully examined for the presence of nematodes (Inoue et al., 2000). The digestive tract, liver, spleen, kidney and heart were taken into petri dish separately, with a lancet and carefully investigated. The inner surface of the abdominal cavity was also checked according to the methods described by Inoue et al. (2000) and Chubb and Powell (1966).

Identification of nematode species

Parasites found were identified by the morphological characteristics given by Moravec (1994) and Bykhovskaya-povlovskaya et al. (1964). The number of *H. aduncum* in each fish was counted. The specimens were fixed in 70% alcohol and then transferred to lactophenol for becoming transparent (Kennedy, 1990; Pritchard and Kruse, 1982). The locations of *H. aduncum* in individual fish were also recorded. The data were analysed with respect to the infection prevalence (number of fish infected with *H. aduncum*/number of fish examined), mean intensity (the mean number of *H. aduncum* per infected fish) and abundance (the mean number of *H. aduncum* per studied fish) of worms (Bush et al., 1997).

RESULTS

The anisakids and other nematodes were not found in the muscle of 469 farmed rainbow trout between 2000 and 2003. Adult *H. aduncum* was only determined in the oesophagus, stomach, intestine and piloric-caeca of 91 trout fed with minced marine fish. Neither larvae nor adult *H. aduncum* was found in 348 farmed rainbow trout and fed with commercially prepared pellets (Table 1). No nematode was determined on the surface of the liver, spleen, kidney, muscle, heart or the inner surface of the abdominal cavity of examined fish.

The infection prevalence (%), mean intensity and mean abundance of *H. aduncum* are given in Tables 1 and 2. *H. aduncum* were found only in the digestive tract of infected 91 fishes, corresponding to a prevalence of 20.73%, a mean intensity of 16.00 ± 1.15 *H. aduncum* per infected fish, and an abundance of 3.32 ± 0.12 *H. aduncum* per examined fish. On the other hand, 91 rainbow trout fed with minced marine fish were infected by *H. aduncum* with the prevalence of 100%, the mean intensity of 16.00 ± 1.15 nematodes per infected fish, and the abundance of 16.00 ± 1.15 nematodes per fish (Table 1). Infection prevalence, mean intensity, and mean abundance of *H. aduncum* in farmed rainbow trout in the freshwater ponds were 36.99%, 16.00 ± 1.15 and 5.92 ± 0.15, respectively (Table 2). A total of 1456 *H. aduncum* (551 male and 905 female) were counted through the host of investigation period.

Morphology of *H. aduncum* (Rudolphi, 1802)

The body of *H. aduncum* is cylindrical. Females (Figure 1) are larger than males (Figure 2). Lips are approximately equal in size. Oesophagus is narrow, ending with a small ventriculus and the intestine is dark and straight. The nerve ring encircled the oesophagus approximately at the border of first, second and fifths of its length. Excretory pore is situated just below the nerve ring and rectum is short. Measurements of *H. aduncum* are given in Table 3.

DISCUSSION

Marine fishes are usually intermediate of anisakid nematodes. A few cases of transmission of these nematodes to humans have been reported (Dick and Choudhury, 1995; Moravec, 1994; Post, 1987). The *in vitro* culture and an experimental infection of *H. aduncum* demonstrated that the third larval stage hatching from the eggs easily infects their first intermediate host a calanoid

Table 1. Infection prevalence (%) of nematode, mean intensity, mean abundance and locality of *H. aduncum* in the rainbow trout (*O. mykiss*) fed with freshly minced marine fish. in the freshwater ponds.

Source of food	No. of fish examined	No. of infected fish	No. of parasites (male/female)	Infection prevalence (%)	Mean intensity (±S.E.)	Mean abundance (±S.E.)	Locality of *H. aduncum*
Freshly minced marine fish	91	91	1456 (551/905)	100	16.00 ± 1.15	16.00 ± 1.15	Digestive tract*
Commercially prepared pellets	348	0	0	0	0	0	-
Total	439	91	1456 (551/905)	20.73	16.00 ± 1.15	3.32 ± 0.12	

(*) Oesophagus, stomach, intestine and piloric-caeca. No., number.

Table 2. Infection prevalence (%), mean intensity and mean abundance of H. aduncum in farmed rainbow trout (O. mykiss) in the freshwater ponds and net-cages.

Sample	No. of fish examined	No. of fish infected	No of parasites (male/female)	Infection prevalence (%)	Mean intensity	Mean abundance
Freshwater ponds	246	91	1456 (551/905)	36.99	16.00 ± 1.15	5.92 ± 0.15
Net-cages	193	0	0	0	0	0
Total	439	91	1456 (551/905)	20.73	16.00 ± 1.15	3.32 ± 0.12

or harpacticoid copepod, *Tisbe longisetosa* Gurney, 1927 (Gonzalez, 1998). In this study, *H. aduncum* infections were recorded in farmed rainbow trout in ponds fed with freshly minced marine fish such as; anchovy, whiting and scad.

This result is the same with those reported by Yoshinaga et al. (1987). They experimentally demonstrated that rainbow trout could be the final host for *H. aduncum*, and are fed by marine fish infected with the 3rd stage larva of the nematode. They defined *H. aduncum* in the net-cages of Chilean marine farms in the inner ocean (Gonzalez, 1998) and *A. simplex* in the wild salmons (Inoue et al., 2000). But in this study, infection of *H. aduncum* was not determined in rainbow trout fed with commercial pellets in the net-cages in the Keban Dame Lake in Elazıg. *H. aduncum* were not observed in any wild fish species in the previous studies conducted in the Keban Dame Lake.

The larvae and adults of *H. aduncum* were not determined in the muscle of farmed rainbow trout. This result is in accordance with the finding of Inoue et al. (2000) and Koie (1999). In this study, *H. aduncum* was recorded in the digestive tract such as; oesophagus, stomach, intestine and pilloric-caeca of rainbow trout, in contrast to be defined only in the intestine of sea trout (Byrne et al., 1999).

The infection prevalence of *H. aduncum* was recorded as 8 to 44% in the intestine of flounder *P. flesus* (Koie, 1999) and 21.8 to 54.8% in the visceral organs of whiting, *M. merlangus* euxinus (Ismen and Bingel, 1999). According to this study, the infection prevalence of *H. aduncum* in farmed rainbow trout in the freshwater ponds was 36.99%. Ninety-one rainbow trout fed with minced marine fish were infected by *H. aduncum* with the prevalence of 100%. These results are

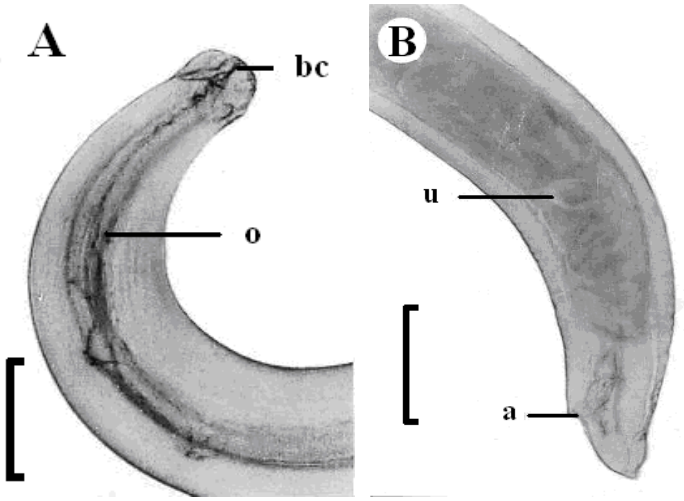

Figure 1. The anterior (A) and posterior (B) view of female *Hysterothylacium aduncum*. Scale bar = 0.5 mm. a, anus; bc, buccal capsule; o, oesophagus; u, uterus.

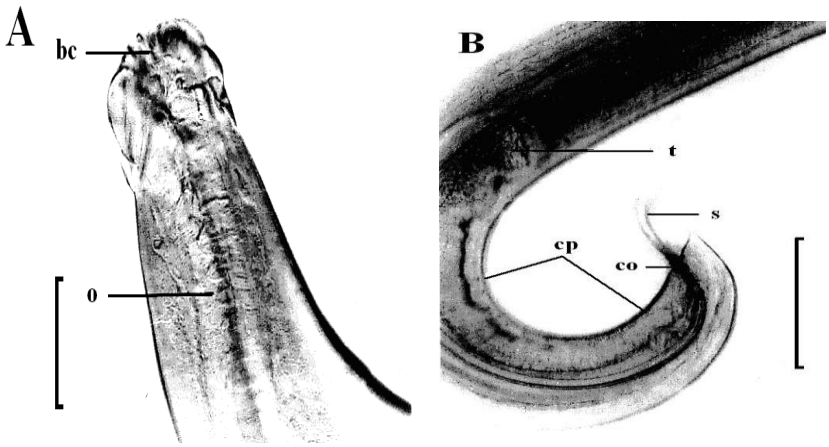

Figure 2. The anterior (A) and posterior (B) view of male *Hysterothylacium aduncum*. Scale bar = 0.5 mm. bc, buccal capsule; co, cloacal opening; cp, caudal pa -1921. pillae, o, oesophagus; s, spicules; t, testis.

Table 3. Measurements of *H. Aduncum.*

Measurement	Male (Mean ± SE) (range)	Female (Mean ± SE) (range)
Length of body	14.0 ± 2.7 mm (8.0 to 18.0)	25.7 ± 2.6 mm (15.2 to 42.0)
Width of body	0.40 ± 0.01 mm (0.20 to 0.50)	0.76 ± 0.06 mm (0.45 to 1.25)
Length of dorsal lip	0.14 ± 0.01 mm (0.13 to 0.15)	0.14 ± 0.05 mm (0.14 to 0.15)
Width of dorsal lip	0.13 ± 0.01 mm (0.12 to 0.14)	0.15 ± 0.02 mm (0.14 to 0.17)
Length of oesophagus	0.83 ± 033 mm (0.81 to 1.79)	1.83 ± 0.43 mm (1.41 to 3.54)
Ventriculus	0.12 to 0.14 x 0.09 to 0.11 mm	0.16 to 0.17 x 0.15 to 0.16 mm
Length of intestinal caecum	0.45 to 0.75 mm	0.91 to 0.99 mm
Nerve ring	0.43 to 0.44 mm	0.56 to 0.80 mm
Long of spicules 1 and 2	0.97 to .91 mm	to
Eggs	to	0.059 to 0.065 x 0.040 to 0.042 mm

SE, standard error.

similar to the infection prevalence of *H. aduncum* to that of researchers in *P. flesus* and *M. merlangus* euxinus.

The abundance of anisakids observed in the chum salmon by Inoue et al. (2000) was nearly equal to that of the present study (16.0 ± 1.15). The prevalence mean intensity and mean abundance of *H. aduncum* on the sea trout were 61.2, 9.3 and 6.3%, respectively (Byrne et al., 1999). Our results were found rather higher on the rainbow trout fed with freshly minced marine fish in the freshwater ponds.

Conclusion

In conclusion, in order to prevent farmed fish from this parasites infection. It is not advisable to use the freshly minced fish for farmed fish feeding. If it is necessary, this kind of food has to pass some processes to eliminate the parasites.

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