Presence of *Hysterothylacium gadi aduncum* (Rudolphi, 1802) (Anisakidae) in cultured Rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) in fresh water farms from Turkey and its mortality

Pekmezci G.Z.[1*]; Umur S.[2]

Abstract

The presence of the anisakid nematode *Hysterothylacium gadi aduncum* (Rudolphi, 1802) is reported for the first time in cultured rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) as a new host in freshwater farms from Turkey. This marine parasitic nematode is thought to complete the life cycle in fresh water and rainbow trout could act as the definitive host for this parasite under fresh water culture conditions. Mortalities in the rainbow trout infected with *H. gadi aduncum* (Rudolphi, 1802)*,* were seen after three to four months period of feeding program with marine fish offal (*Sprattus sprattus, Engraulis encrasicolus*) and pellet fish food. In the present study, the main cause of mortalities was detected as stomach obstruction formed by aggregation of the adult nematodes which developed from infective third stage larvae. Original measurements and figures are presented.

Keywords: *Hysterothylacium gadi aduncum*, Rainbow trout, Fresh water environment, Turkey

1-Department of aquatic animal diseases, Faculty of Veterinary Medicine, University of Ondokuz Mayis, 55139, Kurupelit, Samsun, Turkey

2-Department of parasitology, Faculty of Veterinary Medicine, University of Ondokuz Mayis, 55139, Kurupelit, Samsun, Turkey

*Corresponding author's email: zpekmezci@omu.edu.tr

Introduction

Adult anisakid nematodes of the genus *Hysterothylacium* Ward et Magath, 1917 are common parasites in the digestive tract of fishes in marine, brackish and fresh water environments (Moravec, 2004). *Hysterothylacium aduncum* (Rudolphi, 1802) Deardorff and Overstreet, 1981, has a circumpolar distribution in the Northern Hemisphere, and is found mainly in marine teleost in temperate and cold waters (Berland, 1961, 1991) although Moravec *et al.* (1985) and Yoshinaga *et al.* (1987) also recorded it in fresh water hosts, suggesting that *H. aduncum* (Rudolphi, 1802) can complete its life cycle even in fresh water environment. Salmonids reared in farm conditions could be infected, where they are fed with raw marine fish offal infected with marine nematode larvae (e.g. *Anisakis* sp., *Hysterothylacium* sp.). Heavy infections can be seen due to intestinal impactions and mortalities can occur in these infections (Wootten and Smith, 1975; Berland and Egidius, 1980; Berland, 1987; 1991). The presence of *H. aduncum* (Rudolphi, 1802) in different marine fish species previously have been reported in Turkey by other researchers (Doganay, 1994; Ismen and Bingel, 1999; Keser *et al.*, 2007; Kalay *et al.*, 2009). However, notifications of this species and subspecies in rainbow trout cultured in fresh water environment is new in Turkey. Also, *H. gadi aduncum* (Rudolphi, 1802) as a subspecies is detected for the first time in rainbow trout in Turkey and rainbow trout featured as a new host for this parasite.

Materials and methods

A total of 516 rainbow trouts (*Oncorhynchus mykiss* Walbaum 1792) were obtained from fresh water farms in the Derbent Dam Lake in Northern Turkey (41˚25′06″ North latitude, 35˚49′52″ East longitude) started in April 2008 and lasted to March 2009. All of the fresh water trout farms which are raising market size fish are located in aquaculture sites in the Northern part of Turkey. Rainbow trouts are reared in floating net cages in fresh water systems. Rainbow trouts were caught by hand-netting and placed in plastic bags within farm water. The trouts were transferred to the laboratory and examined within 24-48 hours. Standard investigation for fish parasites was conducted according to Moravec (1994, 2004) and Buchmann (2007). The recovered nematodes were washed in physiological saline and then fixed in hot 70% ethanol and then were cleared with lactophenol for light microscopical examination. The nematodes were measured with a microscope (Eclipse 80i, Nikon Corporation) connected to a digital camera with a liquid crystal display and a measurement specific software (Nikon Digital Sight1 DS-L1). Drawings were made with the aid of a Nikon drawing attachment. After examination, the specimens were stored in vials with 70 % ethanol. All measurements were made in millimeters. Parasitological parameters (prevalence and mean intensity) were calculated according to Bush *et al.* (1997) and by using Quantitative Parasitology 3.0 Statistical Software (Reiczigel and Rózsa, 2005).

Results

H. gadi aduncum (Rudolphi, 1802) (Fig. 1)

Syn.: *Ascaris adunca* (Rudolphi, 1802), *Contracaecum benimasu* (Fujita, 1932), *C. hyppoglossi* (Fujita, 1932), *C. hypomesi* (Fujita, 1932), *C. ochotense* (Fujita, 1932), *C. crassicaudatum* (Fujita, 1939), *C. elongatum* (Fujita, 1939), *C. longispiculum* (Fujita, 1940), *C. mesopi* (Fujita, 1940), *C. okadai* (Fujita, 1940), *C. oshoroensis* (Fujita, 1940), *C. okadai* (Fujita, 1940), *C. salvelini* (Fujita, 1940).

Type host: Rainbow trout (*O. mykiss*) (Walbaum 1792, Salmonidae).

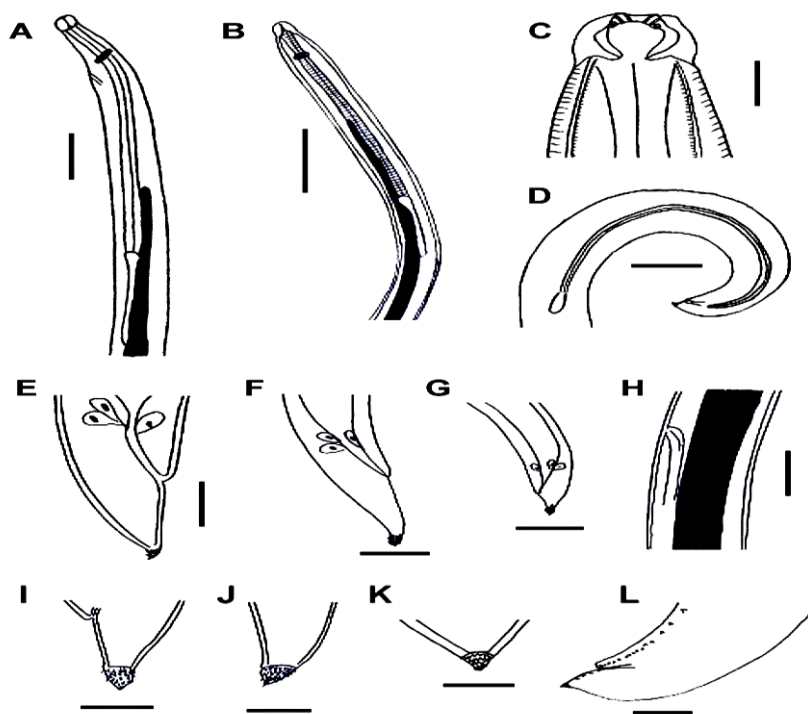Site of infection: Pyloric caeca and intestine.

Figure 1: *H. gadi aduncum* (Rudolphi, 1802): A- cephalic end of female (scale 0.5 mm); B- head end of fourth-stage larva (scale 0.5 mm); C- cephalic end of male (scale 0.1 mm); D- posterior end of male (scale 0.4 mm); E- tail of female (scale 0.2 mm); F, G- tail of male and female fourth-stage larva (F scale 0.1 mm; G scale 0.2 mm); H- vaginal opening of female fourth-stage larva, covered with cuticle (scale 0.2 mm); I- tail tip of male fourth-stage larva (scale 0.1 mm); J- tail tip of female fourth-stage larva (scale 0.1 mm); K- tail tip of male (0.05 mm); L- posterior end of male (scale 0.2 mm.). (Salmonidae, *O. mykiss* (Walbaum, 1792), stomach and pyloric caeca), (Original).

Type locality: Derbent Dam Lake (fresh water environment), Samsun (41°25'6″ North latitude, 35°49'52″ East longitude), Black Sea Region, Turkey.

Prevalence and intensity: 3.2 % (in 17 out of 516 fish examined); 12-396 (mean 201) nematodes per fish.

Deposition of specimens: Department of Parasitology, Faculty of Veterinary Medicine, Samsun, Turkey, Helminth Coll. No. 5–22.

Female (based on 10 gravid specimens) Length of body 41.16 (37.87-50.86) mm and maximum width 0.628 (0.380-0.750) mm. Maximum cervical alae width 0.044 (0.038-0.055) mm. Cervical alae wider than lateral alae; ratio of oesophagus/maximum width of cervical alae: 82.7 and ratio of ventricular appendage/maximum width of cervical alae: 19.4. Nerve ring and excretory pore 0.655 (0.567-0.754) mm and 0.721 (0.626-0.837) mm respectively, from anterior extremity. Length of esophagus 3.639 (3.205-3.982) mm, ventricles 0.211 (0.165-0.265) x 0.150 (0.110-0.194) mm in size and ventricular appendix 0.856 (0.722-0.974) mm long. Length of intestinal caecum 1.35 (1.11-1.57) mm, forming 34-39 % of esophagus length. Ratio of intestinal caecum to ventricular appendix 1:0.63 (1:0.61-0.64). Vulva pre-equatorial, 14.45 (12.48-19.96) mm from anterior end of body. Vagina muscular, directed posteriorly from vulva. Tail conical, 0.371 (0.292-0.429) mm long, ending in small spinose process and tail tip 0.027 (0.021-0.037) mm. Eggs oval to almost spherical, thin-walled, smooth, non-embryonated; mature eggs (n=10) measuring 0.048 x 0.043 mm.

Male (based on 10 mature specimens) Length of body 30.25 (22.30-33.07) mm, maximum width 0.482 (0.330-0.685) mm. Maximum cervical alae width 0.040 (0.029-0.048) mm. Cervical alae wider than lateral alae; ratio of esophagus/maximum width of cervical alae: 67 and ratio of ventricular appendage/maximum width of cervical alae: 16.9. Nerve ring and excretory pore 0.494 (0.292-0.554) and 0.555 (0.349-0.642) mm respectively, from anterior extremity. Length of esophagus 2.682 (2.299-3.136) mm. Ventricles 0.167 (0.139-0.196) x 0.108 (0.098-0.127) mm in size and ventricular appendix 0.677 (0.484-0.785) mm long. Length of intestinal caecum 0.971 (0.776-1.186) mm, forming 33-38 % of esophagus length. Ratio of intestinal caecum to ventricular appendix 1:0.69 (1:0.67-0.71). Spicules slender, alate, equal, 2.034 (1.690-2.552) mm long, representing 7.5-7.7 % of body length. Gubernaculum absent. Caudal papillae: 20-22 pairs of small subventral preanal papillae, one to two pair of minute adanal papillae and three to four minute postanal papillae. Tail conical, 0.131 (0.109-0.155) mm long, tail tip 0.015 (0.009-0.023) mm and ending in small spinose process.

Female fourth-stage larvae (based in 10 specimens)
Body length 11.86 (10.69-12.88) mm; maximum width 0.202 (0.192-0.280) mm. Maximum cervical alae width 0.022 (0.014-0.028) mm. Cervical alae wider than lateral alae; ratio of esophagus/maximum width of cervical alae: 67.8 and ratio of ventricular appendage/maximum width of cervical alae: 21.6. Esophagus 1.492 (1.424-1.592) mm long. Ventricles 0.095 (0.080-0.116) x 0.067 (0.057-0.089) mm and ventricular appendix 0.095 (0.080-0.116) mm long.

Intestinal caecum 0.636 (0.527-0.760) mm long, forming 37-47 % of esophagus length. Ratio of intestinal caecum to ventricular appendix 1:0.75 (1:0.70-0.79). Excretory pore slightly posterior to nerve ring. Nerve ring and excretory pore 0.298 (0.254-0.321) mm and 0.331 (0.286-0.365) mm from anterior end. Vulva covered by cuticle. Length of tail 0.199 (0.154-0.223) and tail tip 0.027 (0.020-0.036) mm with rudimentary spines and characteristic cactus-tail present.

Male fourth-stage larvae (based in 10 specimens)

Body length 10.32 (8.72-11.83) mm; maximum width 0.207 (0.180-0.240) mm. Maximum cervical alae width 0.021 (0.015-0.029) mm. Cervical alae wider than lateral alae; ratio of esophagus/maximum width of cervical alae: 61.7 and ratio of ventricular appendage/maximum width of cervical alae: 20.5. Esophagus 1.297 (1.047-1.423) mm long. Ventricles 0.083 (0.071-0.098) x 0.059 (0.043-0.080) mm and ventricular appendix 0.432 (0.403-0.521) mm long. Intestinal caecum 0.514 (0.416-0.585) mm long, forming 39-41 % of esophagus length. Ratio of intestinal caecum to ventricular appendix 1:0.84 (1:0.89-0.96). Nerve ring and excretory pore 0.305 (0.277-0.340) mm and 0.341 (0.302-0.372) mm from anterior end. Length of tail 0.103 (0.092-0.125) and tail tip 0.023 (0.020-0.030) mm with rudimentary spines and and characteristic cactus-tail present.

Discussion

Moravec (2004) indicated the subspecies *H. gadi aduncum* (Rudolphi, 1802) and *H. gadi gadi* (Müller, 1776) based on shape and width of lateral alae. Furthermore, Petter and Cabaret (1995) delineated the subspecies *H. aduncum aduncum* (Rudolphi, 1802) and *H. aduncum gadi* (Müller, 1776) based on cluster analysis of morphological parameters. Presence of cervical alae hardly wider than lateral alae; the ratio (esophagus length: maximum width of cervical alae) differentiates the subspecies: in *H. aduncum gadi* (Müller, 1776) it is <54 whereas in *H. aduncum aduncum* (Rudolphi, 1802) it is >54. Moreover, the ratio (ventricular appendage: maximum width of cervical alae) differs among specimens of these subspecies, i.e., <15 and >15 in *H. aduncum gadi* (Müller, 1776) and *H. aduncum aduncum* (Rudolphi, 1802), respectively (Petter and Cabaret, 1995). The morphology of nematodes (based on the shape and width of lateral alae) of the present material is, more or less, in agreement with the description of *H. gadi aduncum* (Rudolphi, 1802) provided by Moravec (2004). Besides, based on the cluster analysis of morphological parameters of the present material described above can also be identified as the subspecies *H. aduncum aduncum* (Rudolphi, 1802) by the following combination of characters: presence of cervical alae hardly wider than lateral alae; ratio of esophagus/maximum width of cervical alae >54 and ratio of ventricular appendage/maximum width of cervical alae >15 (Petter and Cabaret, 1995).

The general morphology of these specimens has shown that they are very similar (both in morphology and measurements) to the nematodes described by Moravec *et al*. (1985) as *H. aduncum* (Rudolphi, 1802) from salmonid and some other fishes from fresh waters in Hokkaido, Japan, which evidently also belonged to the subspecies *H. gadi aduncum* (=*H. aduncum aduncum*) (Rudolphi, 1802). Moreover, metrical differences are probably associated with the state of development of the parasites (Moravec *et al*., 1985), and it could be a consequence of the state of development of the previous larval stages in intermediate hosts (Navone *et al*., 1998). *H. aduncum* (Rudolphi, 1802) is found mainly in different marine teleost in temperate and cold waters (Berland, 1961, 1991; Carvajal *et al*., 1995; González and Carvajal, 1995; Moravec and Nagasawa, 2000). On the other hand, (Moravec *et al*., 1985) suggested that its life cycle could be completed in fresh water environment; this was demonstrated experimentally by Yoshinaga *et al*. (1987). Within the present study, this marine parasitic nematode is thought to complete the life cycle in fresh water and rainbow trout could act the definitive host for this parasite under fresh water culture conditions. Salmonids reared in farm conditions could be infected, whether they are fed with raw marine fish offal infected with marine nematode larvae (e.g. *Anisakis* sp., *Hysterothylacium* sp.). Heavy infections could be seen due to intestinal impactions and mortalities could occur in these infections (Wootten and Smith, 1975; Berland and Egidius, 1980;

Berland, 1987, 1991). Mortalities in the rainbow trout infected with *H. gadi aduncum* (Rudolphi, 1802), in the present study were seen after a three or four months period of feeding program with raw marine fish offal (*S. sprattus, E. encrasicolus*) and pellet fish food. At the same time, it is recognized that the parasites in the rainbow trout were getting matured because of the feeding program of the rainbow trout, which consists of raw marine fish offal infected with the infective third stage marine nematode larvae. The main cause of mortalities was detected as stomach obstruction formed by the matured aggregated infective third stage marine nematode larvae in the present study. In such cases, the rainbow trout have to be fed with pellet dry feed instead of feeding with raw marine fish offal in trout farming to eliminate the Anisakidae. Similarly, Skov *et al.* (2009) did not find nematode larvae in rainbow trout, fed with the pellet food.

The infection rate and the mean intensity of *H. gadi aduncum* (Rudolphi, 1802) were detected as 3.2 %, and 201 respectively, during one year of research period in the present study. Moravec and Nagasawa (2000) reported the infection rate as 100 % and the mean intensity as 12 in the rainbow trout fished from the North Pacific Ocean. However, *H. aduncum* (Rudolphi, 1802) was also detected in the rearing farms of rainbow trout in Japan and southern Chile, the information about the infection rate and the mean intensity was not reported (Moravec *et al*., 1985; Carvajal *et al*., 1995).

Nematodes in fresh water rainbow trout reared in net cages are firstly

recorded within this research. Furthermore, *H. gadi aduncum* which is a marine nematode was detected and caused mortalities in fresh water rainbow trout reared in net cages. Indeed, existence of *H. gadi aduncum* in rainbow trout could be explained by feeding of the raw marine fish offal containing the infective third stage nematode larvae. Consequently, mortalities due to marine nematodes in cultured salmonid reared in net cages could be prevented by feeding heat-treated pellet dry feed instead of raw marine fish offal.

Acknowledgments

References

Berland, B., 1961. Nematodes from some Norwegian marine fishes. *Sarsia*, 2, 1–50.

Berland, B. and Egidius, E., 1980. Rund mark i oppdrettsfisk - større problem enn antatt?. *Norsk Fiskeoppdret*, 5(3), 16.

Berland, B., 1987. Helminth problems in sea-water aquaculture. in: Stenmark, A., Malmberg, G. (Eds.), Parasites and diseases in natural waters and aquaculture in Nordic countries. Zoo-Tax, Naturhistoriska Riksmuseet, Stockholm. pp. 56-62.

Berland, B., 1991. *Hysterothylacium aduncum* (Nematoda) in fish. Ices identification leaflets for diseases and parasites of fish and shellfish. *International Council for the Exploration of the Sea*, 44, 1–4.

Buchmann, K., 2007. An introduction to fish parasitological methods-classical and molecular techniques. Copenhagen: Biofolia Press. 130P.

Bush, A.O., Lafferty, K.D., Lotz, J.M. and Shostak, A.W., 1997. Parasitology meets ecology on its own terms: Margolis et al. revised. *Journal of Parasitology*, 83, 575-583.

Carvajal, J., Gonzáles, L. and Toledo, G., 1995. New record of *Hysterothylacium aduncum* (Rudolphi, 1802) (Nematoda: Anisakidae) in salmonid cultured in sea farms from southern Chile. *Research and Reviews in Parasitology*, 55, 195–197.

Deardorff, T.L. and Overstreet, M., 1981. Review of Hysterothylncium and Iheringascaris (both previously - Thynnascaris) (Nematoda: Anisakidae) from the northern Gulf of Mexico. *Proceedings of the Biological Society of Washington*, 93, 1035-1079.

Doganay, A., 1994. A record of *Hysterothylacium aduncum* (Rudolphi, 1802) in cod-fish (*Gadus* sp.) from Black Sea. *Veterinary Journal of Ankara University*, 41, 208-217.

González, L. and Carvajal, J., 1995. New host records of larval *Hysterothylacium aduncum* (Nematoda: Anisakidae) in fauna associated with salmonid sea farms

in Chile. *Research and Reviews in Parasitology*, 55, 247–253.

Ismen, A. and Bingel, F., 1999. Nematode infection in the whiting *Merlangius merlangus euxinus* off Turkish Coast of the Black Sea. *Fisheries Research*, 42, 183–189.

Kalay, M., Donmez, A.E., Koyuncu, C.E., Genc, E. and Sahin, G., 2009. Seasonal variation of *Hysterothylacium aduncum* (Nematoda: Raphidascarididae) infestation in sparid fishes in the Northern Mediterranean Sea. *Turkish Journal of Veterinary and Animal Science*, 33, 517-523.

Keser, R., Bray, R.A., Oguz, M.C., Celen, S., Erdogan, S., Doguturk, S., Aklanoglu, G. and Martı, B., 2007. Helminth parasites of digestive tract of some teleost fish caught in the Dardanelles at Çanakkale, Turkey. *Helminthologia*, 44(**4**), 217-221.

Moravec, F., Nagasawa, K. and Urawa, S., 1985. Some fish nematodes from fresh water in Hokkaido, Japan. *Folia Parasitologica*, 32, 305–316.

Moravec, F., 1994. Parasitic nematodes of freshwater fishes of Europe. Dodtrecht-Netherlands.: Academia and Kluwer Academic Publishers. 477P.

Moravec, F. and Nagasawa, K., 2000. Some anisakid nematodes from marine fishes of Japan and the North Pacific Ocean. *Journal of Natural History*, 34, 1555–1574.

Moravec, F., 2004. Metazoan parasites of salmonid fishes of Europe. Praha.: Academia. 512P.

Navone, G.T., Sardella, N.H. and Timi, T., 1998. Larvae and adults of *Hysterothylacium aduncum* (Rudolphi, 1802) (Nematoda: Anisakidae) in fishes and crustaceans in the South West Atlantic. *Parasite*, 5, 127-136.

Reiczigel, J. and Rózsa, L., 2005. Quantitative parasitology 3.0. Statistical Software, Budapest.

Petter, A.J. and Cabaret, J., 1995. Ascaridoid nematodes of teleostean fishes from the Eastern North Atlantic and seas of the North of Europe. *Parasite*, 2, 217-230.

Skov, J., Kania, P.W., Olsen, M.M., Lauridsen, J.H. and Buchmann, K., 2009. Nematode infections of maricultured and wild fishes in Danish waters: A comparative study. *Aquaculture*, 298, 24-28.

Wootten, R. and Smith, J.W., 1975. Observational and experimental studies on the acquisition of *Anisakis* sp. larvae (Nematoda: Ascaridida) by trout in fresh water. *International Journal for Parasitology*, 5, 373–378.

Yoshinaga, T.M., Ogawa, K. and Wakabayashi, H., 1987. Experimental life cycle of *Hysterothylacium aduncum* (Nematoda: Anisakidae) in fresh water. Fish Pathology, 22(**4**), 243–251.