

Original Paper

Possibility of anisakid larvae infection in farmed salmon

KOJI INOUE,*^a SYUN-ICHIRO OSHIMA, TATSUYOSHI HIRATA AND IKUO KIMURA

Central Research Laboratory, Nippon Suisan Kaisha Ltd., Hachioji, Tokyo 192-0906, Japan

SUMMARY: Nematodes belonging to the family Anisakidae including *Anisakis simplex* and *Pseudoterranova decipiens* are known to cause anisakiasis when their live larvae are ingested by humans. We estimated the possibility of anisakid infection to salmonids, farmed in sea net-pens at Onagawa Bay, Miyagi, Japan, in 1992, 1998 and 1999, by direct examination of the edible muscle and examination of the contents of the alimentary canal. From direct examination of the muscle, no nematode was found in the 249 farmed coho salmon *Oncorhynchus kisutch* and 40 farmed rainbow trout *Oncorhynchus mykiss*. In contrast, third-stage larvae of *Anisakis simplex* were found in seven of 14 wild coho salmon caught in Russia and all the 40 wild chum salmon *Oncorhynchus keta* caught at Nemuro and Kesennuma in 1998. The stomach and intestines of 521 farmed coho salmon and 40 farmed rainbow trout were examined carefully for the existence of possible carrier organisms such as crustaceans, fish or squid. Such carrier organisms were not found in the stomach and intestines of farmed fish. Thus, we conclude that the possibility of anisakid infection is very low in farmed salmonids.

KEY WORDS: *Anisakis*, chum salmon, coho salmon, nematode, rainbow trout.

INTRODUCTION

Nematodes in the family Anisakidae including *Anisakis simplex* and *Pseudoterranova decipiens* are known to cause anisakiasis when their third-stage larvae are ingested with raw or undercooked host fish or squids.¹ Larvae ingested sometimes penetrate into gastrointestinal mucosa and cause gastric or intestinal anisakiasis.¹ In some cases, the parasite may perforate the gastrointestinal wall and cause extragastrointestinal anisakiasis.² In Japan, raw fish are often served as 'sashimi' and thus anisakiasis is a very important problem. It is interesting, however, that serving salmonids as sashimi had traditionally been avoided, although salmonids are a very popular food in Japan. It is reasonable to have avoided eating raw salmonids because *Anisakis* larvae are often present in the muscle of salmonids while they infect viscera in most other fish species.³

In recent years, salmon farming has become a major industry in Japan as well as in other countries. With the development of salmon farming, sashimi and sushi made from salmon is becoming more and more popular because cultured fish are supposed to be free from anisakid infec-

tion. This hypothesis is based on the fact that the major diet of farmed fish is commercially prepared foods such as dry pellet and expanded pellet. However, scientific evidence regarding the safety of farmed salmon for anisakids infection is insufficient. One available report is by Deardorff and Kent⁴ in which coho salmon, chinook salmon and Atlantic salmon farmed in Washington State, USA were shown to have no anisakid larvae. Niizuma *et al.*⁵ also inspected farmed salmon imported to Japan and showed that they were negative for anisakid infection. Salmonids farmed in Japan, however, have never been examined for the existence of anisakid larvae. In this study, we examined the existence of anisakid nematodes in the muscle of coho salmon and rainbow trout cultured in seawater net-pens in Onagawa Bay, Miyagi Prefecture, Japan. In addition, we investigated the food eaten by the farmed salmon and trout by inspecting stomach and intestine contents and presumed the possibility of becoming infected with anisakid nematodes.

MATERIALS AND METHODS

Fish

Cultured coho salmon (age, 1+; average weight, 1.81 kg) and rainbow trout (age, 1+; average weight 1.68 kg) were sampled at net-pens at Oura, Onagawa Town, Miyagi

*Corresponding author: Tel: 81-3-5351-6465. Fax: 81-3-5351-6463. Email: inouek@ori.u-tokyo.ac.jp

^aPresent address: Ocean Research Institute, University of Tokyo, Nakano, Tokyo 164-8639, Japan.

Received 28 December 1999. Accepted 19 June 2000.

Prefecture, Japan, in June 1992, May to July 1998, and June 1999. These fish were farmed in freshwater ponds for about 9 months after hatch-out and introduced to sea net-pens in autumn or winter. They were fed with commercially prepared pellets throughout freshwater and seawater stages and fasted for 3–4 days before catch.

To compare infection levels between farmed and wild salmon, wild chum salmon (average weight, 3.00 kg) were obtained at Nemuro, Hokkaido ($n=10$) and Kesennuma, Miyagi ($n=30$), Japan just after arriving at port. Frozen wild coho salmon ($n=14$) caught from along Russian coast (average weight, 1.79 kg) were purchased from a commercial source.

Examination of the muscle

Salmon and trout were processed into fillet and then all the edible muscle was sliced at a thickness of 5 mm. The sliced muscle was placed on a light box, stretched with a transparent glass board and carefully examined for the existence of nematodes.

Examination of the stomach and intestine

The stomach and intestine of 521 coho salmon and 40 rainbow trout were examined carefully to know whether they had eaten material other than pellets, including possible carrier organisms such as crustaceans, fish or squids. The stomach and intestines were taken out onto a stainless steel dish, torn with scissors and the contents

were investigated carefully. In addition, after removal of the stomach and intestine, the surface of the liver and the inner surface of the abdominal cavity were checked for the existence of nematodes.

Species identification

Nematodes found were identified by the morphological characteristics according to Urawa.³

RESULTS

Anisakid in the muscle

Neither anisakids nor other nematodes were found in the muscle of 249 coho salmon and 40 rainbow trout farmed in 1992, 1998 and 1999 (Table 1). All of 10 and 30 wild chum salmon caught in 1998 at Kushiro and Kesennuma, respectively, were infected with *Anisakis simplex*, whose mean intensity was 18 larvae on average (Table 2). *Anisakis simplex* larvae were also found in seven of 14 wild coho salmon caught from along the Russian coast.

Contents in the stomach and intestine

It was found that the stomach and intestines of most fish were almost empty (Table 3). The only materials found in a few coho salmon were a small piece of seaweed, a small piece of nylon net and a piece of pine leaf

Table 1 Results of direct examination of the edible muscle of coho salmon and rainbow trout farmed in Onagawa, Miyagi, Japan for the existence of anisakid larvae

Species	Sampling date	No. of fish examined	No. of infected fish	Prevalence (%)	Total no. of <i>Anisakis</i> larvae	Abundance of <i>Anisakis</i> per fish
Coho salmon	24 Jul. 1992	22	0	0	0	0
	12 May 1998	35	0	0	0	0
	16 Jun. 1998	25	0	0	0	0
	1 Jul. 1998	6	0	0	0	0
	5 Jul. 1998	6	0	0	0	0
	14 Jul. 1998	3	0	0	0	0
	24 Jul. 1998	25	0	0	0	0
	28 Jul. 1998	17	0	0	0	0
	30 May 1999	5	0	0	0	0
	6 Jun. 1999	35	0	0	0	0
	22 Jun. 1999	34	0	0	0	0
	30 Jun. 1999	36	0	0	0	0
	Total	249	0	0	0	0
Rainbow trout	12 May 1998	10	0	0	0	0
	16 Jun. 1998	10	0	0	0	0
	24 Jul. 1998	10	0	0	0	0
	6 Jun. 1999	5	0	0	0	0
	30 Jun. 1999	5	0	0	0	0
	Total	40	0	0	0	0

Table 2 Results of direct examination of the edible muscle of wild coho salmon and chum salmon for the existence of anisakid nematodes

Species	Locality	Sampling date	No. of fish examined	No. of infected fish	Prevalence (%)	Total no. of <i>Anisakis simplex</i> larvae	Abundance of <i>Anisakis</i> larvae
Chum salmon	Nemuro	Sep. 12 1998	10	10	100	150	15.0
	Kesennuma	Oct. 12 1998	30	30	100	559	18.6
	Total		40	40	100	709	17.7
Coho salmon	Russia	Aug 1998*	14	7	50	10	0.71

* Exact date is unknown.

Table 3 Results of examination of contents in the stomach and intestine of farmed coho salmon and rainbow trout

Species	Date	No. of fish examined	Contents in the stomach and intestine (No. of fish)
Coho salmon	9 Jul. 1992	50	NF
	10 Jul. 1992	50	NF
	18 May 1998	35	A small piece of seaweed (2)
	16 Jun. 1998	145	NF
	5 Jul. 1998	6	NF
	27 Jul. 1998	25	NF
	28 Jul. 1998	90	A small piece of nylon net (1)
	6 Jun. 1999	35	NF
	22 Jun. 1999	45	Pine leaf (1)
	30 Jun. 1999	40	A small piece of seaweed (1)
	Total	521	
Rainbow trout	12 May 1998	10	NF
	16 Jun. 1998	10	NF
	24 Jul. 1998	10	NF
	6 Jun. 1999	5	NF
	30 Jun. 1999	5	NF
	Total	40	

NF, Nothing found.

(Table 3). Organisms such as fish, crustaceans and molluscs and the parts of them such as bone and shell were not found at all in the stomach and intestines. In addition, no nematode was found on the surface of the liver and the inner surface of the abdominal cavity of farmed salmon and trout.

DISCUSSION

The definitive hosts of anisakid nematodes are marine mammals such as seals, dolphins and whales. Anisakids mature and spawn eggs in the marine mammals and the eggs are released to the sea. Although it has been believed that they develop to second larval stages in the sea and to the third stage in crustaceans including krill, a recent paper showed that eggs develop to the third stage before hatching.^{3,6} The third-stage larvae are ingested by fish and squid via infected crustaceans. Salmon become infected by eating crustaceans, fish or squid containing anisakid larvae.³ Therefore, farmed salmonids are believed to be free from anisakid infection because they are fed with artificially prepared feed such

as dry pellets and expanded pellets. In this study, we estimated the possibility of anisakid infection in farmed salmon. One approach was direct observations of the edible muscle to show the absence of anisakids. Similar attempts were made by Deardorff and Kent⁴ in which they showed farmed salmonids are free from anisakid infection. Another approach was observation of the contents in the alimentary canal. Farmed salmon and trout examined in this study had been fed until satiation almost every day and fasted for 3–4 days before harvest. Fish suddenly fasted must be hungry and would be likely to actively seek other food. We found, however, no evidence showing the ingestion of organisms such as small fish and invertebrates in their stomach and intestine. These results indicate a low possibility of ingestion of organisms carrying anisakids.

From the result of these two different types of examinations, we conclude that the possibility of anisakid infection is very low in farmed salmonids. It is impossible, however, to examine all the fish by direct observation of the muscle. It is also impossible to monitor all the food during the entire culturing period. Therefore, we cannot conclude that farmed salmonids are absolutely

free from anisakid infection. For a more complete conclusion, immunological detection of anti-anisakid antibody in salmon serum, for example, may be useful, although it is still unknown whether salmon produce an antibody against anisakids. So far, an immune response to *Diphyllbothrium dendriticum* has been reported in rainbow trout.^{7,8} In addition, serodiagnosis of nematodes infection has shown to be possible in eel.^{9,10} Such a diagnostic system should be examined in the future.

ACKNOWLEDGMENT

The authors are grateful to Dr Shigehiko Urawa of the National Salmon Resources Center for critical reading of the manuscript and valuable discussion.

REFERENCES

1. Ishikura H. General survey of *Anisakis* and Anisakiasis in Japan. In: Ishikura H, Namiki M (eds). *Gastric Anisakiasis in Japan: Epidemiology, Diagnosis, Treatment*. Springer-Verlag, Tokyo. 1989; 3–11.
2. Yoshimura H. Clinical patho-parasitology of extra-gastrointestinal anisakiasis. In: Ishikura H, Kikuchi K (eds) *Intestinal Anisakiasis in Japan*. Springer-Verlag, Tokyo. 1990; 145–154.
3. Urawa S. The parasites of salmonid fishes—II. The biology of anisakid nematodes and the prevention of their human infections. *Fish Eggs* 1986; **156**: 52–70 (in Japanese).
4. Deardorff TL, Kent ML. Prevalence of larval *Anisakis simplex* in pen-reared and wild-caught salmon (Salmonidae) from Puget Sound, Washington. *J. Wildlife Dis.* 1989; **25**: 416–419.
5. Niizuma J, Higuchi S, Osada N, Ishibashi O, Tanaka K, Suzuki T, Higashijima H, Ootaka M. Studies on parasitic infection of imported fresh salmon for raw fishes. *Food Saint. Res.* 1996; **46**: 51–59 (in Japanese).
6. Köie M, Berland B, Burt MDB. Development to third-stage larvae occurs in the eggs of *Anisakis simplex* and *Pseudoterranova decipiens* (Nematoda, Ascaridoidea, Anisakidae). *Can. J. Fish. Aquat. Sci.* 1995; **52**: 134–139.
7. Sharp GJE, Pike AW, Secombes CJ. The immune response of wild rainbow trout, *Salmo gairdneri* Richardson, to naturally acquired plerocercoid infections of *Diphyllbothrium dendriticum* (Nitzsch, 1824) and *D. ditremum* (Creplin, 1825). *J. Fish Biol.* 1989; **35**: 781–794.
8. Sharp JGE, Pike AW, Secombes CJ. Sequential development of the immune response in rainbow trout [*Oncorhynchus mykiss* (Walbaum, 1972)] to experimental plerocercoid infections of *Diphyllbothrium dendriticum* (Nitzsch, 1824). *Parasitology* 1992; **104**: 169–178.
9. Inui T, Ushikoshi R, Mano N, Hirose H. A competitive-ELISA for the serodiagnosis of anguillicolosis in Japanese eel, *Anguilla japonica*. *Fish Pathol.* 1999; **34**: 25–31 (in Japanese).
10. Ushikoshi R, Inui T, Nogami S, Hirose H. A method for specific antibody detection from Japanese eel by indirect ELISA using cuticular antigen of *Anguillicola crassus*. *Fish Pathol.* 1999; **34**: 81–82.