



Anisakid larva in the viscera of a farmed Atlantic salmon (*Salmo salar*)

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ABSTRACT

Parasitic nematodes of the family Anisakidae occur in the visceral cavity and surrounding tissues of many marine fish species at a prevalence as high as 100% in wild salmon samples. Human consumption of fish products containing these parasites can result in the zoonotic disease anisakiasis, and *Anisakis simplex* is most commonly associated with human disease. Previous studies of farmed salmon have found no anisakids in viscera or muscle, presumably because formulated feed production renders parasite larvae nonviable. However, among farmed Atlantic salmon (*Salmo salar*) examined by histopathology as part of a provincial government auditing program in British Columbia, Canada, 1 of 894 (0.11%) had an anisakid larva partly embedded in the wall of an intestinal cecum. Skeletal muscle is not examined as part of the government's program, but other studies have correlated anisakids in the viscera and muscle. Using anisakid prevalence in viscera as an estimate of its prevalence in muscle, the risk ratio of anisakid parasites in commercial product is 570 times less in farmed than in wild Atlantic salmon.

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1. Introduction

Parasitic nematodes of the family Anisakidae are common in many marine fish species, and consumption of fish products containing these parasites can result in two forms of zoonotic disease. First, the disease traditionally recognized as anisakiasis results from ingestion of live parasites in fresh uncooked fish, and is associated with eosinophilic granulomatous enteritis (Van Thiel et al., 1960). And second, hypersensitivity reactions to parasite antigen can occur after ingestion of fresh, previously frozen, or cooked fish products (Kasuya et al., 1990; Audicana et al., 1995; Audicana et al., 2002). *Anisakis simplex* and *Pseudoterranova decipiens* are the species most commonly associated with these human diseases (Butt et al., 2004). In fish, living anisakid parasites are associated with little or no inflammatory reaction, but dead parasites become engulfed by granulomatous inflammation (Hauck and May, 1977).

Although anisakid larvae occur in up to 100% of wild marine salmonids (Deardorff and Kent, 1989; Bristow and Berland, 1991; Angot and Brasseur, 1993), until now anisakid larvae have not been documented in farmed salmon fed processed feeds (Deardorff and Kent, 1989; Bristow and Berland, 1991; Angot and Brasseur, 1993; Inoue et al., 2000; Lunestad, 2003). Anisakids have a complex life cycle in which adult parasites lay eggs in the intestines of piscivorous mammals; the eggs develop and hatch as free-swimming L2 larvae, which are ingested by crustaceans and develop into L3 larvae (Butt et al., 2004). After infected crustaceans are ingested by a fish, the L3 larvae pene-

trate the intestinal wall of the fish and remain mostly in the visceral cavity until the fish is consumed by the piscivorous mammal. The cycle is broken in farmed fish because the processing of formulated feeds renders the L3 larvae nonviable. The purpose of this communication is to report the first case of anisakid larvae in a farmed Atlantic salmon (*Salmon salar*).

2. Methods

The British Columbia Provincial Government (western Canada) administers a Fish Health Auditing and Surveillance Program that examines fish cultured in marine net pens. These fish are examined for lesions, cause of death, and pathogens of international significance. In 2006, British Columbia fish farms produced about 78,000 mt of salmon (source: <http://www.env.gov.bc.ca/omfd/fishstats/aqua/index.html>). British Columbia is the fourth largest producer of cultured salmon in the world after Norway, Chile, and the United Kingdom. Each quarter, 30 farms are randomly selected for audit from the 60–70 active farms in the province; farms are systematically selected to be representative of region and species cultured. Samples are collected from fish that die in net pens on the selected farms. During the period of this study, October 2006 through March 2008, sample size varied from 0 to 15 fish per farm visit. Carcasses selected for diagnostic tests are representative of mortalities among the population of fish most likely to be marketed. Diagnostic procedures on each sampled fish include gross examination and histopathology of heart, liver, head kidney, trunk kidney, spleen, intestinal ceca, exocrine pancreas, and mesenteric adipose tissue. Tissues are preserved in 10% neutral buffered formalin, processed routinely into paraffin, sectioned at 3–4 µm, and stained with

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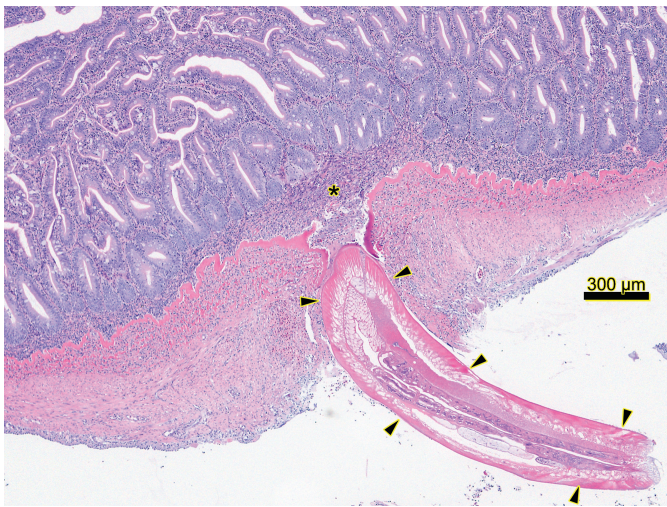


Fig. 1. Micrograph of an oblique section of an intestinal cecum; the outer wall has been penetrated by an anisakid nematode (arrowheads). Mild inflammation (*) is on the end of the nematode. Haematoxylin and Eosin stain.

Haematoxylin and Eosin for light microscopy. During the 18-month study, intestinal ceca and surrounding mesenteries were sampled from 894 Atlantic salmon and 210 Pacific salmon (*Oncorhynchus tshawytscha* and *O. kisutch*). The risk ratio for anisakids in farmed Atlantic salmon was calculated by comparing results from this study with published data from wild Atlantic salmon where 100 out of 155 fish sampled had anisakid parasites (Bristow and Berland, 1991).

3. Results and discussion

One Atlantic salmon collected during a farm visit in the third quarter of 2007 had a single anisakid larva penetrating the wall of an intestinal cecum (Fig. 1). The source farm is in regulatory area 3.3, a region of narrow channels, inlets, and islands east of Port McNeill, British Columbia. The farm reared only Atlantic salmon, and fish were being fed a commercially prepared extruded feed. Fish at this farm had been in the marine environment for about 14 months. The affected fish (about 2.5 kg) was smaller than the mean weight of other fish in the pen (about 4 kg), but it was not emaciated. The parasite was not observed when the tissues were collected, and the diagnosis was made using histopathology. In microscopic section, the parasite is about 400 μm in diameter and nearly 2 mm long. Mild inflammation near the perforated stratum compactum includes plump (activated) fibroblasts in the lamina propria and a small focus of fibrin on the end of the parasite. Numbers of eosinophilic granular cells in the lamina propria are about the same whether near or far from the parasite. Parasite size, anatomic location, prominent intestine and large excretory gland cells are characteristic of anisakids (Hauck and May, 1977). The affected fish might have contracted the parasite by eating crustaceans or small wild fish that are common within net pens in British Columbia fish farms. None of the other 893 Atlantic salmon or any of the 210 Pacific salmon had evidence of anisakid parasites. Further, intestinal ceca and surrounding fatty mesenteries from more than 100 farmed salmon are submitted by fish farmers each year to the Animal Health Centre for diagnostic histopathology, and none of these samples have had anisakid parasites.

The relative risk of anisakids in Atlantic salmon is estimated to be 570 times greater in wild fish than in farmed fish (95% confidence interval, 80 to 4100). The prevalence of anisakid parasites in this study (0.11%) is consistent with the estimated population prevalence

(<0.16%) that was based on 0 anisakids in 3699 farmed Atlantic salmon sampled from Norway and Scotland (Angot and Brasseur 1993).

The diagnostic specificity of histopathology for anisakids is high (i.e., false-positive diagnoses are unlikely). But what is the diagnostic sensitivity of single-section intestinal histopathology for anisakids in the skeletal muscle? This comparison has not been made in Atlantic salmon, but in Pacific herring (*Clupea pallasii*), microscopic anisakid prevalence was 76% in sections of intestine and intestinal ceca and 3.3% in sections of skeletal muscle (Marty et al., 1998). Therefore, the prevalence of anisakids in a section of intestinal ceca probably is a conservative estimate of muscle prevalence. Precision of prevalence estimates of anisakids in skeletal muscle of farmed salmon can be increased using more sensitive diagnostic methods such as enzymatic digestion or UV illumination (Levsen et al., 2005).

In conclusion, anisakid parasites are common in the marine environment, and on rare occasions farmed Atlantic salmon might ingest these anisakids and become infected. However, consumption of farmed salmon results in significantly lower risk of exposure to anisakid parasites than consumption of wild salmon, and application of protocols that kill anisakid larvae (e.g., freezing, Gustafson, 1953) will ensure that farmed salmon products consumed uncooked are free of viable parasites.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.aquaculture.2008.04.006.

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