Chapter 16: Parasites

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Potential Food Safety Hazard

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Parasites (in the larval stage) consumed in uncooked, or undercooked, unfrozen seafood can present a human health hazard. Among parasites, the nematodes or roundworms (*Anisakis spp.*, *Pseudoterranova spp.*, *Eustrongylides spp.* and *Gnathostoma spp.*), cestodes or tapeworms (*Diphyllobothrium spp.*) and trematodes or flukes (*Chlonorchis sinensis, Opisthorchis spp., Heterophyes spp., Metagonimus spp., Nanophyetes salminicola and Paragonimus spp.*) are of most concern in seafood. Some products that have been implicated in human infection are: ceviche (fish and spices marinated in lime juice); lomi lomi (salmon marinated in lemon juice, onion and tomato); poisson cru (fish marinated in citrus juice, onion, tomato and coconut milk); herring roe; sashimi (slices of raw fish); sushi (pieces of raw fish with rice and other ingredients); green herring (lightly brined herring); drunken crabs (crabs marinated in wine and pepper); cold-smoked fish; and, undercooked grilled fish. A recent survey of U.S. gastroenterologists has confirmed that seafood-borne parasitic infections occur in the U.S. with sufficient frequency to make preventive controls necessary during the processing of parasite-containing species of fish that are intended for raw consumption (FDA, 2001).

Nematodes

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Anisakiasis is caused by the accidental ingestion of larvae of the nematodes (roundworms) *Anisakis simplex* and *Pseudoterranova decipiens*. Adult stages of *A. simplex* or *P. decipiens* reside in the stomach of marine mammals, where they are embedded in the mucosa, in clusters.

Eggs produced by adult females are passed in the feces, hatch and yield second stage larvae. Upon ingestion by crustaceans, third stage larvae develop that are infective to fish and squid. After ingestion by the fish and squid hosts, the larvae migrate from the intestine to the peritoneal cavity to (upon the host's death) the muscle tissues. Through predation, the larvae are transferred from fish to fish until they are ingested by the marine mammal. In this definitive host, the larvae develop into adults, thus closing the cycle. Humans become infected by eating raw or undercooked marine fish. After ingestion, the anisakid larvae penetrate the gastric and intestinal mucosa, causing the symptoms of anisakiasis.

Within hours after ingestion of infected larvae, violent abdominal pain, nausea, and vomiting may occur. Occasionally the larvae are coughed up. If the larvae pass into the bowel, a severe eosinophilic granulomatous response may also occur, causing symptoms mimicking Crohn's disease 1-2 weeks following infection.

A. simplex and *P. decipiens* are found worldwide, with higher incidence in areas where raw fish is eaten (e.g., Japan, Pacific coast of South America, the Netherlands). Increasing incidence in the United States due to increased consumption of raw fish (CDC, 1999a).

Anisakiasis is associated with eating raw fish (sushi, sashimi, lomi lomi, ceviche, sunomono, Dutch green herring, marinated fish and cold-smoked fish) or undercooked fish (Ward et al., 1997).

Freezing and cooking may kill *A. simplex*, but may not protect consumers against allergenic reactions to ingested *A. simplex* antigens (Audicana et al., 1997).

***Eustrongylides* spp.** larvae are large, bright red nematodes, 25-150 mm long and 2 mm in diameter. They occur in freshwater fish, brackish water fish, and marine fish. The larvae normally mature in wading birds such as herons, egrets, and flamingos. If the larvae are consumed in raw or undercooked fish, they can attach to the wall of the digestive tract and penetrate the gut wall with accompanying severe pain. Infections are extremely rare and have been associated with consumption of live minnows and sashimi in the U.S. (FDA, 1991).

Gnathostoma spinigerum infects vertebrate animals. In the natural definitive host (cats, dogs, wild animals) the adult worms reside in a tumor which they induce in the gastric wall. They deposit eggs that are immature when passed in the feces. After maturation in water, the egg releases a first stage larva (L1). After ingestion by a small crustacean (Cyclops) (first intermediate host), the L1 develops into a L2. Following ingestion of the Cyclops by a fish, frog or snake (second intermediate host), the L2 develops in their flesh into a L3. When the second intermediate host is ingested by a definitive host, the L3 develops into an adult stage parasite in the stomach wall. Alternatively, the second intermediate host may be ingested by another animal (paratenic host) in which the L3 does not develop further, but remains infective to the next predator. Humans become infected by eating undercooked fish or poultry containing L3s, or reportedly by drinking water containing L2-infected Cyclops.

The clinical manifestations in human gnasthostomiasis are caused by migration of the immature worms (L3s). Migration in the subcutaneous tissues causes intermittent, migratory, painful,

pruritic swellings (cutaneous larva migrans). Migration in other tissues (visceral larva migrans), can result in cough, hematuria, ocular involvement, with the most serious manifestations being eosinophilic meningitis with myeloencephalitis. High eosinophilia is present. *Gnathostoma spinigerum* is found in Asia, especially Thailand and Japan (CDC, 1999b).

Cestodes

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Diphyllobothrium latum (the fish or broad tapeworm), is the largest human tapeworm. Several other *Diphyllobothrium* species have been reported to infect humans, but less frequently; they include *D. pacificum*, *D. cordatum*, *D. ursi*, *D. dendriticum*, *D. lanceolatum*, *D. dalliae*, and *D. yonagoensis*.

The adult *D. latum* tapeworm resides in the small intestine where it attaches to the mucosa. It can reach more than 10 m in length, with more than 3,000 proglottids. Immature eggs are discharged from the proglottids (up to 1,000,000 eggs per day per worm) and are passed in the feces. Under appropriate conditions, the egg matures (in 11-15 days), yields an oncosphere which develops into a coracidium. After ingestion by a suitable freshwater crustacean (copepod) (first intermediate host) the coracidium develops into a procercoid larva. Following ingestion of the copepod by a suitable freshwater fish (second intermediate host), the procercoid larva migrates into the fish flesh where it develops into a plerocercoid larva (sparganum). When the smaller infected fish is eaten by a larger one, the sparganum may migrate into the flesh of the larger fish. Humans (the optimal definitive host) acquire the infection by eating raw or undercooked infected fish. Eggs appear in the feces 5-6 weeks after infection. In addition to humans, many other mammals can also be infected.

Diphyllobothriasis can be a long lasting infection (decades). Most infections are asymptomatic. Manifestations may include abdominal discomfort, diarrhea, vomiting, weight loss. Vitamin B12 deficiency with pernicious anemia may occur. Massive infections may result in intestinal obstruction. Migration of proglottids can cause cholecystitis or cholangitis.

Diphyllobothriasis occurs in areas where lakes and rivers coexist with human consumption of raw or undercooked freshwater fish. Such areas are found in the Northern Hemisphere (Europe, ex-USSR, North America, Asia), and in Uganda and Chile (CDC, 1999c).

Trematodes

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Clonorchis sinensis is the Chinese or oriental liver fluke. The adult flukes (10-25 mm by 3-5 mm) reside in small and medium sized biliary ducts. Embryonated eggs are discharged in the biliary ducts and in the stool. After ingestion by the suitable snail intermediate host, the eggs release miracidia which go through several developmental stages (sporocysts, rediae, and cercariae). The cercariae are released from the snail and encyst as metacercariae in the skin and flesh of freshwater fish. Infection of humans occur by ingestion of undercooked, salted, pickled, or smoked freshwater fish. After ingestion, the metacercariae excyst in the duodenum and ascend the biliary tract through the ampulla of Vater. Maturation takes approximately 1 month. Adult

flukes can survive 20 to 25 years. In addition to humans, carnivorous animals can serve as reservoir hosts.

Most pathologic manifestations result from inflammation and intermittent obstruction of the biliary ducts. In the acute phase, abdominal pain, nausea, diarrhea, and eosinophilia can occur. In long-standing infections, cholangitis, cholelithiasis, pancreatitis, and cholangiocarcinoma can develop, which may be fatal.

Endemic areas are in Asia including Korea, China, Taiwan, Vietnam. Clonorchiasis has been reported in non endemic areas (including the United States). In such cases, the infection is found in Asian immigrants, or following ingestion of imported, undercooked or pickled freshwater fish containing metacercariae (CDC, 1999d).

Opisthorchiasis is caused by *Opisthorchis viverrini* (Southeast Asian liver fluke) and *O. felineus* (cat liver fluke). The adult flukes (*O. viverrini*: 5 mm –10 mm by 1 mm-2 mm; *O. felineus*: 7 mm – 12 mm by 2 mm – 3 mm) reside in the biliary and pancreatic ducts of the mammalian host, where they attach to the mucosa. They deposit fully developed eggs that are passed in the feces. After ingestion by a suitable snail (first intermediate host), the eggs release miracidia, which undergo in the snail several developmental stages (sporocysts, rediae, cercariae). Cercariae are released from the snail and penetrate freshwater fish (second intermediate host), encysting as metacercariae in the muscles or under the scales. The mammalian definitive host (cats, dogs, and various fish-eating mammals including humans) become infected by ingesting undercooked fish containing metacercariae. After ingestion, the metacercariae excyst in the duodenum and ascend through the ampulla of Vater into the biliary ducts, where they attach and develop into adults, which lay eggs after 3-4 weeks.

Most infections are asymptomatic. In mild cases, manifestations include dyspepsia, abdominal pain, diarrhea or constipation. With infections of longer duration, the symptoms can be more severe, and hepatomegaly and malnutrition may be present. In rare cases, cholangitis, cholecystitis, and chlolangiocarcinoma may develop. In addition, infections due to *O. felineus* may present an acute phase resembling Katayama fever (schistosomiasis), with fever, facial edema, lymphadenopathy, arthralgias, rash, and eosinophilia. Chronic forms of *O. felineus* infections present the same manifestations as *O. viverrini*, with in addition involvement of the pancreatic ducts.

O. viverrini is found mainly in northeast Thailand, Laos and Kampuchea. *O. felineus* is found mainly in Europe and Asia, including the former Soviet Union (CDC, 1999e).

Heterophyes heterophyes, a minute intestinal fluke causes heterophyiasis. Adult *H. heterophyes* (1.0 mm - 1.7 mm by 0.3 mm - 0.4 mm) reside in the small intestine, where they are attached to the mucosa. They release fully embryonated eggs that are passed in the feces. After ingestion by a suitable snail (first intermediate host), the eggs hatch and release miracidia which undergo several developmental stages in the snail (sporocysts, rediae, and cercariae). The cercariae are released from the snail and encyst as metacercariae in the tissues of a suitable freshwater fish (second intermediate host). The definitive host becomes infected by ingesting undercooked or salted fish containing metacercariae. After ingestion, the metacercariae excyst, attach to the

intestinal mucosa, and mature into adults. In addition to humans, various fish-eating animals can be infected by Heterophyes.

The main symptoms are diarrhea and colicky abdominal pain. Migration of the eggs to the heart, resulting in potentially fatal myocardial and valvular damage, has been reported from the Philippines. Migration to other organs (e.g., brain) has also been reported.

H. heterophyes are found in Egypt, the Middle East and Far East (CDC, 1999f).

Metagonimus yokogawai, a minute intestinal fluke (and the smallest human fluke), causes metagonimiasis. Adult *M. yokogawai* (1.0 mm - 2.5 mm by 0.4 mm - 0.75 mm) reside in the small intestine, where they are attached to the mucosa. They release fully embryonated eggs that are passed in the feces. After ingestion by a suitable snail (first intermediate host), the eggs hatch and release miracidia which undergo several developmental stages in the snail (sporocysts, rediae, and cercariae). The cercariae are released from the snail and encyst as metacercariae in the tissues of a suitable freshwater fish (second intermediate host). The definitive host becomes infected by ingesting undercooked fish containing metacercariae. After ingestion, the metacercariae excyst, attach to the intestinal mucosa, and mature into adults. In addition to humans, fish-eating mammals and birds can also be infected.

The main symptoms are diarrhea and colicky abdominal pain. Migration of the eggs to extra-intestinal sites (heart, brain) can occur, with resulting symptoms.

M. yokogawai are found mostly in the Far East, as well as Siberia, Manchuria, the Balkan states, Israel and Spain (CDC, 1999g).

Paragonimiasis is an infection in animals and humans caused by more than 30 species of trematodes (flukes) of the genus *Paragonimus*. Among the more than 10 species reported to infect humans, the most common is *P. westermani*, the oriental lung fluke. Human infection with *P. westermani* occurs by eating inadequately cooked or pickled crab or crayfish that harbor metacercariae of the parasite. The metacercariae excyst in the duodenum, penetrate through the intestinal wall into the peritoneal cavity, then through the abdominal wall and diaphragm into the lungs, where they become encapsulated and develop into adults (7.5-12 mm by 4-6 mm). Time from infection to oviposition is 65 to 90 days. The eggs are excreted unembryonated in the sputum, or alternately they are swallowed and passed with the stool. In the external environment, the eggs embryonate, hatch and yield miracidia which enter the first intermediate host, a snail. Cercariae emerge from the snail and invade the second intermediate host, a crustacean (crab of crayfish) where they encyst and become metacercariae. Ingestion of the metacercariae closes the cycle. Infections may persist for 20 years in humans, and occasionally other sites than the lungs are involved. Infection occurs also in many animal species.

The acute phase (invasion and migration) may be marked by diarrhea, abdominal pain, fever, cough, urticaria, hepatosplenomegaly, pulmonary abnormalities, and eosinophilia. During the chronic phase, pulmonary manifestations include cough, expectoration of discolored sputum, hemoptysis, and chest radiographic abnormalities. Extrapulmonary locations of the adult worms result in more severe manifestations, especially when the brain is involved.

While *P. westermani* occurs in the Far East, other species of *Paragonimus* are encountered in Asia, the Americas, and Africa (CDC, 1999h).

Nanophyetus salmincola or ***N. schikhobalowi*** are the names, respectively, of the North American and Russian troglotrematoid trematodes (or flukes). Nanophyetiasis is the name of the human disease caused by these flukes. At least one newspaper referred to the disease as "fish flu." *N. salmincola* is responsible for the transmission of *Neorickettsia helminthoec*a, which causes an illness in dogs that may be serious or even fatal.

Knowledge of nanophyetiasis is limited. The first reported cases are characterized by an increase of bowel movements or diarrhea, usually accompanied by increased numbers of circulating eosinophils, abdominal discomfort and nausea. A few patients reported weight loss and fatigue, and some were asymptomatic. The rickettsia, though fatal to 80% of untreated dogs, is not known to infect humans.

There have been no reported outbreaks of nanophyetiasis in North America; the only scientific reports are of 20 individual cases referred to in one Oregon clinic. A report in the popular press indicates that the frequency is significantly higher. It is significant that two cases occurred in New Orleans well outside the endemic area. In Russia's endemic area the infection rate is reported to be greater than 90% and the size of the endemic area is growing.

Nanophyetiasis is transmitted by the larval stage (metacercaria) of a worm that encysts in the flesh of freshwater fishes. In anadromous fish, the parasite's cysts can survive the period spent at sea. Although the metacercaria encysts in many species of fish, North American cases were all associated with salmonids. Raw, underprocessed, and smoked salmon and steelhead were implicated in the cases to date (FDA, 1999).

Control Measures

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The process of heating raw fish sufficiently to kill bacterial pathogens is also sufficient to kill parasites.

Freezing (-20ºC [-4ºF] or below [internal or external] for 7 d or –35ºC [-31ºF] or below [internal] for 15 h) of fish intended for raw consumption also kills parasites. The Food Code recommends these freezing conditions to retailers who provide fish intended for raw consumption.

Brining and pickling may reduce the parasite hazard in a fish, but they do not eliminate it, nor do they minimize it to an acceptable level. Nematode larvae have been shown to survive 28 d in 80º salinometer brine (21% salt by weight).

Trimming away the belly flaps of fish or candling and physically removing parasites are effective methods for reducing the numbers of parasites. However, they do not completely eliminate the hazard, nor do they minimize it to an acceptable level (FDA, 1998).

FDA Guidelines

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FDA guidelines for freezing fish to kill parasites.

- Freeze and store at -4°F (-20°C) or below for 7 days (total time); or
- Freeze at -31°F (-35°C) or below until solid and store at -31°F (-35°C) or below for 15 hours; or
- Freeze at -31°F (-35°C) or below until solid and store at -4°F (-20°C) or below for 24 hours.

Note: these conditions may not be suitable for freezing particularly large fish (e.g. thicker than six inches) (FDA, 2001).

FDA guidelines for parasites in fish.

Product	Guideline	Reference
Tullibies, ciscoes, inconnus, chubs, and whitefish	50 cysts per 45.45Kg (100 lbs)	FDA, 1996
Blue fin and other freshwater herring averaging 1 lb (454 g) or less	60 cysts per 100 fish, if 20% of the fish examined are infested	FDA, 1996
Blue fin and other freshwater herring averaging more than 1 pound (454 g)	60 cysts per 45.45 kg (100 lbs), if 20% of the fish examined are infested	FDA, 1996
Rose fish (red fish and ocean perch)	3% of fillets examined contain 1 or more copepods accompanied by pus pockets	FDA, 1996

Published Process Studies

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Controlling nematodes

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A literature review and proposed HACCP model for controlling nematodes in fish recommended freezing the fish to below -20ºC (-4ºF) at the thermal center and storage at or below -20ºC (-4ºF) for at least 24 h (Howgate, 1998).

Freezing conditions to inactivate nematodes in fish for raw consumption (Karl and Leinemann, 1989).

Max. Product Core Temp.		Min. Holding Time	Max. Holding Temp.	
(ºC)	(ºF)		(ºC)	(ºF)
-18	-0.4	24 h	-18	-0.4

-18	-0.4	24 h	-20	-4
-20	-4	24 h	-18	-0.4
-20	-4	24 h	-20	-4
-34	-29.2	24 h	-18	-0.4
-34	-29.2	24 h	-20	-4

Controlling trematodes

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Freezing conditions to kill trematodes (*Heterophyes* spp.) in frozen mullet (Hamed and Elias, 1970).

Maximum External Temperature		Minimum Time
(ºC)	(ºF)	(h)
-10	14	30
-20	-4	30

Freezing conditions to kill trematodes (*Clonorchis sinensis*) in frozen cyprinids (*Pseudorasbora parva*) (Fan, 1998).

Maximum External Temperature		Infective After	Not Infective After
(ºC)	(ºF)	(d)	(d)
-12	10.4	18	20
-20	-4	7	N/D

N/D = Not determined

Analytical Procedures

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Parasitic amimals in foods (USFDA) Top

Isolation and identification of anisakid roundworm larvae in fish (HC ExFLP-1) Top

Other analytical procedures Top

- Parasites in fish muscle: Candling procedure (AOAC, 1995).

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