Infections related to the ingestion of seafood. Part II: parasitic infections and food safety

Adeel A Butt, Kenneth E Aldridge, and Charles V Sanders

Parasites are responsible for a substantial number of seafood-associated infections. The factor most commonly associated with infection is consumption of raw or undercooked seafood. People with underlying disorders, particularly liver disease, are more susceptible to infection. In the first part of this review, published last month, we discussed the viral and bacterial agents associated with consumption of seafood. In part II, we discuss the parasites commonly associated with seafood consumption. Parasites readily identifiable from both consumable seafood and infected human beings include nematodes, trematodes, cestodes, and protozoa. The salient features associated with seafood-related parasite infestations are discussed. To provide a safe product for consumers, the seafood industry and the government in the USA have undertaken specific measures, which include good manufacturing practices and hazards analysis and critical control points implemented by the government and regulatory agencies. Consumers should take common precautions including obtaining seafood from reputable sources especially if the seafood is to be consumed uncooked. Adequate cooking of seafood is the safest way of preventing related infections.

Lancet Infect Dis 2004; **4:** 294–300

Following on from the first part of our review, in which we discussed the viral and bacterial agents associated with consumption of seafood,[1] here we discuss parasitic infections and the safety of seafood in general. We also address good manufacturing practices and hazards analysis and critical control points, programmes that have led to increasing safety of seafood.

Parasites

It is estimated that more than 50 million people are infected with food-borne trematodes worldwide. Most of these infections result from the consumption of raw or undercooked freshwater seafood.[2,3] The highest prevalence of these infections is in southeast and east Asia,[4,5] but increasing numbers of infections are being recognised in areas previously considered non-endemic, due largely to increased importation of seafood that may be contaminated and travel from endemic regions.[2,6,7] Nearly all marine animals are infected with parasites, but most do not cause disease in their natural host nor in human beings.[8–10] However, some of these parasites may survive the food preparation process and cause human infection. Additionally, large outbreaks related to protozoa have been reported, though water is more often a vehicle of transmission of such infection than seafood.

A range of conditions allows these infections to become established in endemic areas. Poor sanitation leading to faecal contamination of natural water sources used for fishing, and intentional use of night soil (human excrement used as fertiliser) in aquacultural fish ponds lead to reservoirs of infection. In the life cycles of most digenetic trematodes, cercariae encyst in or on a second intermediate host, forming a metacercariae. When the second intermediate host is eaten the metacercariae excyst in the definitive host's intestinal tract, thus establishing an infection. Consumption of raw or lightly cooked fish or shellfish is responsible for transmission of infection to human beings. Proper cooking will effectively kill metacercariae (if the flesh becomes white or pale in colour and assumes a firm texture).[6] Freezing can be effective, but salting, smoking, and marinating are unreliable in killing the metacercariae. It follows that the infection rates in people are greater in areas where large amounts of fish and shellfish are consumed raw or lightly cooked. For example, about 1000 cases of anisakiasis are reported from Japan each year, whereas in the USA, where seafood is more often thoroughly cooked, only about 50 cases have been reported.

Measures can be taken during harvesting, processing, or post-processing to reduce the risk of infection in people. Two measures taken by the industry and the US government are particularly important in attempts to provide a safe seafood product to consumers—good manufacturing practices and hazard analysis and critical control points (HACCP).[11,12] Before harvesting, measures that can be taken include determining the type and size of fish, their feeding habits, and their environment. For example, many parasites like anisakids and diphyllobothroids accumulate within the host during the lifetime of the fish, with the number of worms increasing in accordance with the age and size of fish. Therefore, harvesting younger fish of such species will reduce the likelihood of large numbers of parasites in these fish. After capture, if the fish are rapidly chilled or gutted,

AAB is at the Division of Infectious Diseases, University of Pittsburgh, the VA Pittsburgh Healthcare System, and the Center for Health Equity Research and Promotion, Pittsburgh, PA, USA; and KEA and CVS are at the Section of Infectious Diseases, Department of Medicine, Louisiana State University Health Sciences Center, New Orleans, LA, USA.

Correspondence: Dr Adeel A Butt, 3601 Fifth Avenue, Suite 3A, Falk Building, University of Pittsburgh, Pittsburgh, PA 15213, USA. Fax +1 412 648 6399; email butta@msx.dept-med.pitt.edu

the number of parasites in the flesh will be lower. A prolonged time before freezing may allow the parasites to migrate from the gut to the flesh, and chilling and freezing will not be effective.

Nematodes

Anisakis *spp*

Anisakiasis is the human nematode infection most commonly associated with consumption of seafood.[13,14] The species most commonly implicated is *Anisakis simplex*, followed by *Pseudoterranova decipiens*.[15,16] *A simplex* is about 2 cm long and is extremely difficult to see within the fish musculature. In addition to many fish species anisakid parasites (*A simplex* and *A pegreffii*) have been reported in cephalopod species (particularly squid) from Japan, China, and Spain.[17] The life cycle of anisakis is illustrated in figure 1.[18,19]

Large outbreaks of human anisakiasis have been reported from countries with a high consumption of raw or undercooked seafood. Ishikura et al[20] reported 11 629 cases of gastric, 567 cases of intestinal, and 45 cases of extraintestinal anisakiasis from Japan. They also reported 355 cases of gastric pseudoterranoviasis. 107 cases of anisakiasis were reported from Korea between 1989 and 1992. More than 500 cases of ileitis have also been reported from other countries.

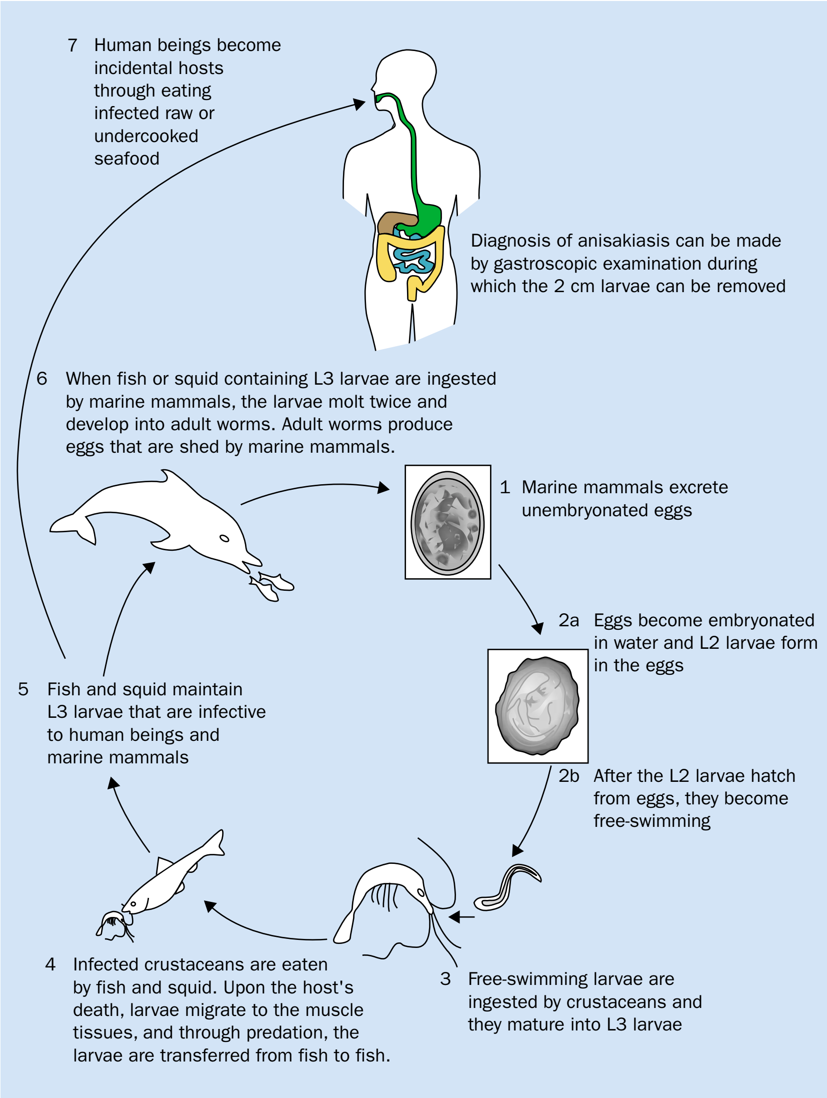

Figure 1. Life cycle of Anisakis simplex. *Adapted from http://www.dpd.cdc.gov/dpdx/HTML/Anisakiasis.htm (accessed March 31, 2004).*

After ingestion, a viable juvenile anisakid nematode may penetrate into or through the wall of the gastrointestinal tract, causing invasive anisakiasis. The disease may present clinically as sudden onset of severe, episodic, epigastric distress, sometimes accompanied by nausea and vomiting. Acute epigastric pain generally occurs within 1–12 h of consumption of the infected seafood, but has been reported up to 14 days.[14,21] Diarrhoea and urticaria have been reported as has oeosinophilia in the chronic stage of the disease. In some cases the larvae may be coughed up in an otherwise symptom-free person.[22] The other common manifestation of human anisakiasis is an allergic reaction in sensitised individuals.[23–31] Such patients are typically advised to avoid seafood after diagnosis. Anisakiasis has also been reported to be a cause of intestinal pseudo-obstruction,[32] occupational asthma,[33] peritonitis in a patient on continuous ambulatory peritoneal dialysis, and rheumatological symptoms (arthralgias/arthritis).[33–35]

A high degree of suspicion is required to make the diagnosis, with history of ingestion of raw fish being a critical clue. Ultrasound findings of small bowel dilatation, focal oedema of Kerckring's folds in patients who develop acute abdominal symptoms[36] are seen in some patients. Threadlike gastric filling defects corresponding to the presence of larvae may be seen in about half the patients.[14]

Many patients will recover spontaneously, but some may require endoscopic removal of the worms. Adequate cooking (60°C) or freezing of fish (–10°C) for 24 h will kill the anisakids and prevent infection in people.

Trematodes

Although 33 species of trematodes have been reported to be transmissible to human beings via consumption of seafood, only a few cause human disease.[6,37] Whereas transmission is most frequently associated with ingestion, sporadic cases resulting from the handling of naturally infected seafood have also been reported.[38]

Liver flukes

Clonorchis sinensis, known as the Chinese liver fluke, is highly prevalent in China, Korea, Taiwan, Vietnam, and Japan. More than 5 million people are thought to be infected in China alone,[2] and further cases are being diagnosed and reported in non-endemic areas. *C sinensis* was reported to be the most common parasitic infection in Hong Kong immigrants to Canada between 1979 and 1981. Of the 29 095 individuals examined, 13·4% had a stool sample positive for *C sinensis* ova. In surveys in the USA in the 1990s, 1226 stool samples out of 216 275 tested were positive for the ova of *Clonorchis* or *Opisthorchis* spp, making this group the most frequently isolated trematode. People of any age may be infected. Infection rates are higher in men, the reasons for which are unclear but may be linked to more opportunities for men to eat at restaurants. Infection has been associated with consumption of raw or undercooked seafood.[39,40] Many fish species from the endemic areas harbour the parasite and have been associated with transmission of infection.

The Canadian liver fluke *Metorchis conjunctus* was reported as a cause of an outbreak in Quebec in 1996. 17 of 19 individuals who consumed raw white sucker fish caught in a small river became symptomatic after an incubation period of 1–15 days. Symptoms reported were abdominal pain, fever, diarrhoea, headache, nausea, and back pain. These symptoms resolved spontaneously or after treatment with praziquantel. Development of symptoms was related to the amount of fish consumed.[41]

Trematodes from the genus *Echinostoma* are small, typically 3–10 mm in length and 1–3 mm wide. At least 16 species have been implicated in human infections, and the infection is endemic in southeast and east Asia.[2] Metacercariae are the infective stage, and are ingested by human beings in raw or undercooked fresh or brackish water molluscs, fish, crustaceans, and amphibians.[42] An outbreak of gastroenteritis was reported in a group of American tourists returning from a trip to Kenya and Tanzania in 1983, and several cases have been reported from Canada.

Clinical findings are related to parasite load, and range from anaemia, headaches, dizziness, stomach pain, and loose stools in light to moderate infections, to eosinophilia, profuse watery diarrhoea, anaemia, and anorexia in heavy infections. Diagnosis is made when characteristic eggs are seen in the stool. Treatment is with mebendazole, albendazole, or praziquantel.[42]

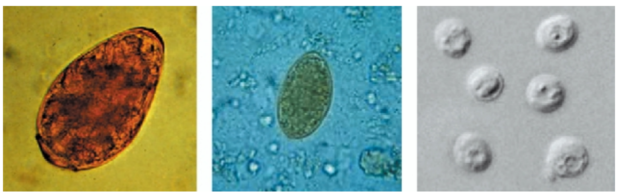

Figure 2. Egg of Paragonimus westermani *(left). Eggs of* Diphyllobothrium latum *(centre). Oocysts of* Cryptosporidium parvum*, in wet mount, seen with differential interference contrast microscopy. Images courtesy of DPDx: the US Centers for Disease Control and Prevention website for laboratory identification of parasites; http://www.dpd.cdc.gov/dpdx.*

General advice on the purchase of seafood

When shopping at a grocery store, select seafood last and put them in separate plastic bags. If the packages are open, crushed or torn, do not buy them

Look for signs of frost or ice crystals inside the bags if the bags are transparent. Being able to see frost or ice crystals or frost inside the bags may suggest that the fish has either been stored for a long time or has been thawed and refrozen

Check for cleanliness at the meat or fish counter and the salad bar. For instance, cooked shrimp lying on the same bed of ice as raw fish could become contaminated

When buying shellfish, buy from markets that get their supplies from state-approved sources; do not buy from vendors who sell shellfish from roadside stands or the back of a truck

If you anticipate that it will take more than an hour to get your groceries home use an ice chest to store the frozen seafood

Lung flukes

Paragonimus spp are lung flukes that occur in human beings and several other mammals. Human paragonimiasis has been reported from many countries and is endemic in Thailand, Japan, Korea, and China. The two species of medical importance are *Paragonimus westermani* and *P skrjabini. P skrjabini* needs three hosts to complete its life cycle: freshwater snails, freshwater crabs, and the definitive hosts including cats, dogs, and other carnivorous animals. In the definitive host the eggs of the parasite (figure 2a) are coughed up and swallowed, and are passed in the faeces before being ingested by the intermediate host. In endemic areas, people acquire infection by eating raw or undercooked freshwater crabs containing live metacercariae.[43] Adult worms have been detected in human tissue.[44] The incubation period is 11–12 days, and the illness is characterised by remittent fever, cough, and chest pain. Some patients may exhibit subcutaneous nodules. Mild leucocytosis may be present and patients generally have a high eosinophil count. Chest radiograph may show patchy infiltrate, usually involving the upper lung fields. *Paragonimus* spp ova may not be seen in the stool or sputum specimen and diagnosis is made by serological tests. Treatment is praziquantel for 3 days.[5,43]

Intestinal flukes

Heterophyes and *Metagonimus* spp are other trematodes that are acquired by eating raw, marinated, or improperly cooked fish. Cases are frequently reported from the Middle East and Asia. One case of *Heterophyes heterophyes* infection was reported in the USA in 1986 in a patient who had never travelled outside the USA but had eaten sushi at a restaurant that served fish flown in from Asia. Two cases of infection with *Metagonimus yokogawai* were reported from Canada in 1978, and one case in a woman in California who had first developed symptoms while travelling in southeast Asia.[2]

Another intestinal fluke that can be recognised by its characteristically large eggs and is associated with consumption of salmon is *Nanophyetus salmincola.* Ten symptomatic patients (ages 6–55 years.) in northwest USA were identified as having *N salmincola* by recognition of the parasite eggs in the stool. Eight of the ten patients recalled

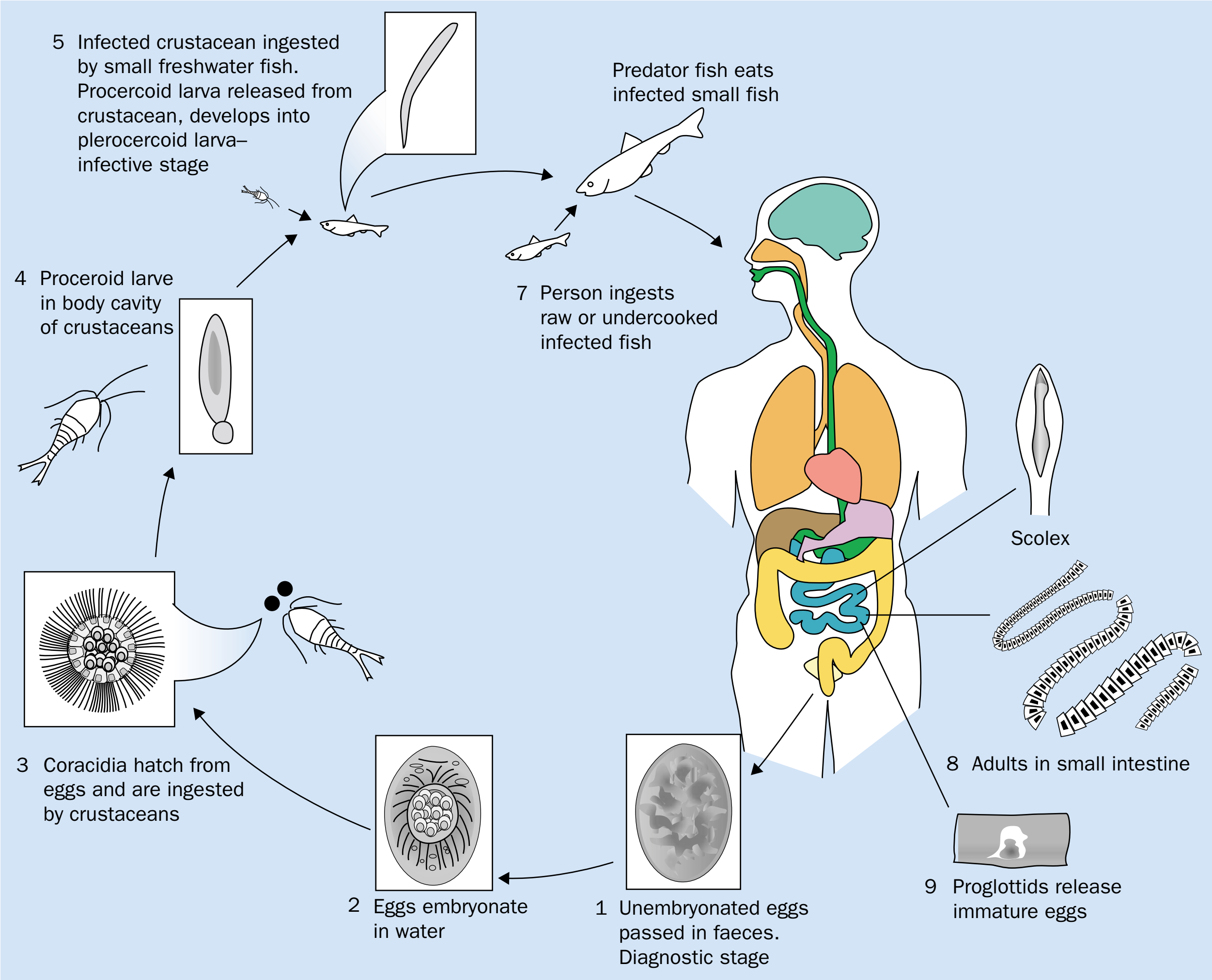

Figure 3. Life cycle of Diphyllobothrium latum. *Adapted from http://www.dpd.cdc.gov/dpdx/HTML/ImageLibrary/Diphyllobothriasis_il.htm (accessed March 31, 2004).*

eating raw, undercooked, or smoked salmon, steelhead trout, or steelhead eggs. Nine of the ten patients resolved their infections with or without antiparasitic agents.[45] *N salmincola* infections have been traced to patients who ingested kippered salmon.[46]

Cestodes

Diphyllobothrium latum

Cestodes associated with seafood consumption are mostly *Diphyllobothrium* spp. Cases are generally reported from areas where fish are eaten raw, marinated, or undercooked. At least 13 species have been implicated in human infection, with *Diphyllobothrium latum* (figure 2b) and *D dendriticum* being the most common. *D latum* is of interest because it competes for vitamin B12 in the human host causing pernicious anaemia. In Japan around 100 cases are reported annually. Of 52 cases reported in 1980 from the US west coast states, salmon was implicated in 82%. Seafood-associated disease due to *Diphyllobothrium* spp has decreased in western Europe over the past 30 years perhaps due to a decrease of the fish species known to be the major host for these organisms.[47] However, six cases reported from Italy were associated with eating raw fish fillets or smoked fish.[47] Most patients presented with abdominal pain and diarrhoea and had eosinophilia. All patients were effectively treated with a single 2 g dose of niclosamide. The life cycle of *Diphyllobothrium* species is incompletely understood (figure 3).[6]

Protozoa

Cryptosporidium

Cryptosporidium was discovered in 1907 by Tyzzer, in the stomach of a mouse. The first human case was reported in 1976 in a child with diarrhoea.[48] Cryptosporidiosis was an uncommon infection until 1982, when the AIDS epidemic began. Many studies have since shown that cryptosporidiosis is the leading cause of diarrhoea due to protozoal infections worldwide. Patients with AIDS, infants attending day-care centres, international travellers, people living in tropical regions of the world, and those who have contact with animals have an increased predilection for infection with this organism.[49] The parasite is most commonly transmitted through waterborne sources. In the largest known epidemic, reported from Milwaukee, Wisconsin, more than 400 000 people were infected.

Oocysts of *Cryptosporidium* spp (figure 2c) are resistant to environmental factors and cause disease when ingested by human beings. Infectious oocysts have been recovered from oysters and mussels from various geographical sites.[50,51] Each shellfish from these areas of high water contamination may carry 5000 oocysts. Since *Cryptosporidium* spp oocytes survive well in the marine environment, have a small median infectious dose of approximately 132 oocysts,[49] and are present in large numbers in shellfish, seafood is a potential source for cryptosporidiosis. Although many waterborne outbreaks of cryptsporidiosis have been reported, no major seafood-related outbreak has been reported (for life cycle see figure 4).

Intestinal cryptosporidiosis is characterised by severe watery diarrhoea but may sometimes be symptomless. Pulmonary and tracheal cryptosporidiosis have been reported and are associated with coughing and frequently a low-grade fever often accompanied by intestinal distress.

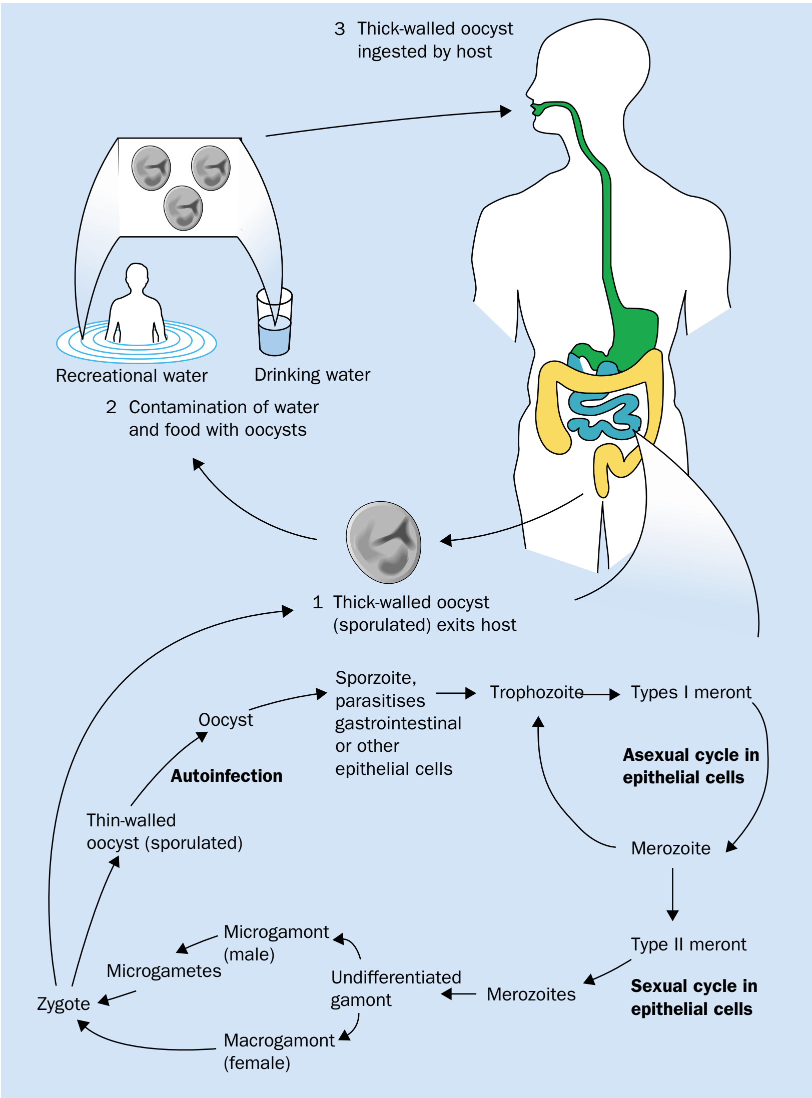

Figure 4. Life cycle of Cryptosporidium *spp. Adapted from http://www.dpd.cdc.gov/dpdx/HTML/ImageLibrary/Cryptosporidiosis_il.htm (accessed March 31, 2004).*

Giardia

Giardia lamblia is a flagellated parasite detected as trophozoites or cysts and represents the most common cause of intestinal parasitosis in human beings worldwide. The route of transmission is predominantly faecal–oral or through contaminated food or water with an inoculum size of 10–100 cysts sufficient to cause disease.[52] The incubation period is generally 12–20 days. In symptomatic patients acute onset of symptoms can include nausea, chills, fever, epigastric pain, and foul-smelling diarrhoea, which may contain mucous and blood. Laboratory diagnosis is through microscopic detection of trophozoites or cysts in diarrhoeic stool samples. Although the majority of transmission is via water or water-associated food stuffs, rare reports have associated transmission of *G lamblia* with fish. In the USA home-canned salmon, and in China soup (koipla) prepared with uncooked freshwater fish have been implicated.[53,54]

Safety of seafood

This section deals primarily with the seafood safety in the USA, a country where specific steps have been taken to increase the safety of seafood, both by the industry and the government. The US Food and Drug Administration (FDA) is the agency responsible for enforcing the code pertaining to the safety of animal and human drugs, medical and radiological devices, and most foods and their additives, including seafood.[55] After several large typhoid fever outbreaks in the 1920s associated with consumption of raw molluscs and shellfish, the National Shellfish Sanitation Program was established. This programme is administered by the FDA and works with local and state agencies and the shellfish industry to ensure that contaminated molluscs are not shipped in for interstate trade and do not reach the retail market.

Good manufacturing practices and HACCP programmes

Two programmes in particular are important in improving the safety of shellfish that is marketed for human consumption. Good manufacturing practices implemented by the shellfish industry can work at several levels to ensure a safe product. Harvesting from approved areas, type and size of fish caught, the method of capture, and processing immediately after capture can all decrease the chances of a contaminated product.

The HACCP programme is designed specifically to address food safety hazards and to eliminate or reduce them to acceptable levels. The first step in HACCP is to identify a food safety hazard; then the processing step best suited for the control of the hazard is determined, and the actual control plan is implemented.[6,11,56]

The method of estimating the risk of infection and the optimum way to eliminate or reduce the hazard to acceptable levels depends on the organism in question. Faecal coliforms are a group of Gram-negative bacteria generally associated with the waste of human beings and animals; their counts in shellfish harvest areas have used as indicators of sanitary quality. These counts provide a reasonable indication of bacterial contamination, and actions taken based on faecal coliform counts have effectively reduced certain bacterial infection related to seafood ingestion.[57,58] Seafood harvest beds are classified according to the faecal coliform counts, and harvesting and marketing decisions are based on those counts (table).[59] Such counts are, however, not always a reliable method for detection of contamination with enteric viruses. Outbreaks of norovirus gastroenteritis have been reported from estuaries where faecal coliform counts were within acceptable standards.[58] Monitoring by newer molecular methods such as PCR may be more sensitive in predicting the level of viral contamination.[60]

Depuration

Depuration is a method in which filter-feeding bivalve molluscs are placed in clean water under controlled conditions for varying periods of time. Just as they accumulate organisms when filtering contaminated water, these organisms may be removed when filtering clean water. Depuration decreases bacterial counts significantly, but is ineffective in substantially reducing concentrations of viruses such as norovirus. In one study depuration for 48 h reduced bacterial counts by 95% but produced only a 7% reduction in norovirus concentrations.[58]

Parasite detection and elimination

Parasites may be detected by visual inspection by putting the fish fillet on a light table—a process called candling. Candling is a subjective process with some limitations: detection is dependent on the thickness of the fillet, presence of skin on the fillet, the oil content, pigmentation, and the expertise of the operator. If seen, the parasites can be removed by forceps or the infected part can be cut out.[6] Heating is the most effective method for eliminating the risk of parasitic disease from seafood. The internal temperature of the thickest part of the product must reach a minimum of 63°C (145°F) for 15 s or longer. Heat smoking is also effective, but cold smoking does not produce temperatures high enough for inactivation of the parasites. Freezing is also an acceptable method for parasite inactivation, but is also temperature and time dependent: 15 h at –35°C (–31°F) or 7 days at –20°C (–4°F) will be effective.[6]

Search strategy and selection criteria

We searched Medline for articles printed in the English language using the search terms “seafood”, “infection”, “parasites” and the specific organisms covered in this review (search period 1980–2003). Articles reporting infections or outbreaks related to seafood consumption were selected, as were articles addressing the safety of seafood. Cited references in the selected articles were also screened and pertinent articles retrieved. Additional articles addressing the safety of seafood, hazards analysis, and critical control points were identified from the references provided in the primary manuscripts.

Restaurant guidelines for seafood handling

The following recommendations have been proposed for seafood handling in restaurants. Fish fillets should be defrosted at room temperature within 14 h. Subsequent refrigerator storage should not exceed 3 days, and once removed from the refrigerator a tray of fish should be used within 3 h. Shrimp should be shelled as soon as possible preferably before they are completely defrosted. Defrosted shrimp should not be kept in the refrigerator for more than 48 h, and they should be removed from the refrigerator during that period only as needed. Clams should be used within 3 days, including the time required for defrosting. The shelf life of clams is very limited even though they are always kept at –5°C or below.[61] FDA recommends that all fish and shellfish intended for raw (or semi-raw such as marinated or partly cooked) consumption be blast frozen to –35°C (–31°F) or below for 15 h, or be regularly frozen to –20°C (–4°F) or below for 7 days.

Advice to the consumer

Individuals consuming a single serving of raw shellfish from an approved harvesting site in the USA may have an estimated probability of one in 100 of becoming infected with a moderately infectious enteric virus.[60] To reduce the chance of infection from seafood, it is recommended that such food be eaten fully cooked and that the catch should be harvested only from approved areas (panel). People with underlying medical problems, especially liver disease, should be particularly careful, and certain US states require the vendors of raw shellfish to post signs warning consumers of the potential hazards associated with such products. While it may be impossible for the consumer to verify the source and date of harvest of their seafood, eating at reputable

Classification of seafood harvest beds according to faecal coliform counts[58]

Category A	<230 *Escherichia coli* per 100 g of seafood	May go for direct human consumption if end product standard are met
Category B	<4600 *E coli* per100 g in 90% of samples	Must be depurated, heat treated or relayed to meet category A
Category C	<60 000 faecal coliforms per 100 g of seafood	Must be relayed for a long period (at least 2 months) to meet category A or B, or heat treated
Category D	>60 000 faecal coliforms per 100 g of seafood	Harvesting prohibited

restaurants with visible cleanliness is a recommended common-sense measure to reduce risk of infection. Detailed information on the safe purchase, handling, and consumption of seafood maybe obtained from governmental internet sites—eg, http://www.state.ak.us/local/akpages/ENV.CONSERV/deh/seafood/safety.htm (accessed November 23, 2003).

Conclusion

Infections related to the ingestion of seafood are common. Fortunately, most of these infections are mild and viral in origin, and resolve without any specific therapy. Both the government and vendors of seafood have a responsibility in ensuring a safe product for the consumer, but the consumers should exercise common-sense precautions to reduce the risk of infection. They should eat their seafood well cooked and at reputable outlets. People with certain medical conditions, especially liver disease, should take extra care to ensure that their seafood is well cooked.

Conflicts of interest

We have no conflicts of interest to declare.

References

1 Butt AA, Aldridge KE, Sanders CV. Infections related to the ingestion of seafood. Part 1: viral and bacterial infections. *Lancet Infect Dis* 2004; **4:** 201–12.

2 Dixon BR, Flohr RB. Fish- and shellfish-borne trematode infections in Canada. *Southeast Asian J Trop Med Public Health* 1997; **28** (suppl 1): 58–64.

3 New D, Little MD, Cross J. *Angiostrongylus cantonensis* infection from eating raw snails. *N Engl J Med* 1995; **332:** 1105–06.

4 Ko RC. Current status of food-borne parasitic zoonoses in Hong Kong. *Southeast Asian J Trop Med Public Health* 1991; **22:** 42–47.

5 Xu ZB. Studies on clinical manifestations, diagnosis and control of paragonimiasis in China. *Southeast Asian J Trop Med Public Health* 1991; **22:** S345–48.

6 Adams AM, Murrell KD, Ross JH. Parasites of fish and risks to public health. *Rev Sci Tech* 1997; **16:** 652–60.

7 Hine PM, Thorne T. A survey of some parasites and diseases of several species of bivalve mollusc in northern Western Australia. *Dis Aquat Organ* 2000; **40:** 67–78.

8 Deardorff TL. Epidemiology of marine fish-borne parasitic zoonoses. *Southeast Asian J Trop Med Public Health* 1991; **22** (suppl): 146–49.

9 Hauck AK. Occurrence and survival of the larval nematode *Anisakis* sp in the flesh of fresh, frozen, brined, and smoked pacific herring, *Clupea harengus pallasi. J Parasitol* 1977; **63:** 515–19.

10 Jackson GJ. Testing for food-borne parasites, their metabolic products and symbionts. *Southeast Asian J Trop Med Public Health* 1991; **22:** S334–S336.

11 Huss HH. Development and use of the HACCP concept in fish processing. *Int J Food Microbiol* 1992; **15:** 33–44.

12 Khamboonruang C, Keawvichit R, Wongworapat K, et al. Application of hazard analysis critical control point (HACCP) as a possible control measure for *Opisthorchis viverrini* infection in cultured carp (*Puntius gonionotus*). *Southeast Asian J Trop Med Public Health* 1997; **28** (S1): 65–72.

13 Deardorff TL, Kayes SG, Fukumura T. Human anisakiasis transmitted by marine food products. *Hawaii Med J* 1991; **50:** 9–16.

14 Sakanari JA, McKerrow JH. Anisakiasis. *Clin Microbiol Rev* 1989; **2:** 278–84.

15 Herreras MV, Aznar FJ, Balbuena JA, Raga JA. Anisakid larvae in the musculature of the Argentinean hake, *Merluccius hubbsi. J Food Prot* 2000; **63:** 1141–43.

16 Piccolo G, Manfredi MT, Hoste L, Vercruysse J. Anisakidae larval infection in fish fillets sold in Belgium. *Vet Q* 1999; **21:** 66–67.

17 Abollo E, Gestal C, Pascual S. Anisakis infestation in marine fish and cephalopods from Galician waters: an updated perspective. *Parasitol Res* 2001; **87:** 492–99.

18 Ma HW, Jiang TJ, Quan FS, et al. The infection status of anisakid larvae in marine fish and cephalopods from the Bohai Sea, China and their taxonomical consideration. *Korean J Parasitol* 1997; **35:** 19–24.

19 Nagasawa K, Moravec F. Larval anisakid nematodes of Japanese common squid (*Todarodes pacificus*) from the Sea of Japan. *J Parasitol* 1995; **81:** 69–75.

20 Ishikura H, Kikuchi K, Nagasawa K, et al. Anisakidae and anisakidosis. *Prog Clin Parasitol* 1993; **3:** 43–102.

21 Bouree P, Paugam A, Petithory JC. Anisakidosis: report of 25 cases and review of the literature. *Comp Immunol Microbiol Infect Dis* 1995; **18:** 75–84.

22 Juels CW, Butler W, Bier JW, Jackson GJ. Temporary human infection with a *Phocanema* sp larva. *Am J Trop Med Hyg* 1975; **6:** 942–44.

23 Alonso A, Moreno-Ancillo A, Daschner A, Lopez-Serrano MC. Dietary assessment in five cases of allergic reactions due to gastroallergic anisakiasis. *Allergy* 1999; **54:** 517–20.

24 Audicana MT, Fernandez de Corres L, Munoz D, Fernandez E, Navarro JA, del Pozo MD. Recurrent anaphylaxis caused by *Anisakis simplex* parasitizing fish. *J Allergy Clin Immunol* 1996; **96:** 558–60.

25 Buendia E. Anisakis, anisakidosis, and allergy to Anisakis. *Allergy* 1997; **52:** 481–82.

26 Del Pozo MD, Audicana M, Diez JM, et al. *Anisakis simplex*, a relevant etiologic factor in acute urticaria. *Allergy* 1997; **52:** 576–79.

27 Esteve C, Resano A, Diaz-Tejeiro P, Fernandez-Benitez M. Eosinophilic gastritis due to *Anisakis*: a case report. *Allergol Immunopathol* 2000; **28:** 21–23.

28 Garcia-Labairu C, Alonso-Martinez JL, Martinez-Echeverria A, Rubio-Vela T, Zozaya-Urmeneta JM. Asymptomatic gastroduodenal anisakiasis as the cause of anaphylaxis. *Eur J Gastroenterol Hepatol* 1999; **11:** 785–87.

29 Lopez-Serrano MC, Gomez AA, Daschner A, et al. Gastroallergic anisakiasis: findings in 22 patients. *J Gastroenterol Hepatol* 2003; **15:** 503–06.

30 Montoro A, Perteguer MJ, Chivato T, Laguna R, Cuellar C. Recidivous acute urticaria caused by *Anisakis simplex. Allergy* 1997; **52:** 985–91.

31 Moreno-Ancillo A, Caballero MT, Cabanas R, et al. Allergic reactions to *Anisakis simplex* parasitizing seafood. *Ann Allergy Asthma Immunol* 1997; **79:** 246–50.

32 Dominguez Ortega J, Cimarra M, Sevilla M, et al. *Anisakis simplex*: a cause of intestinal pseudo-obstruction. *Rev Esp Enferm Dig* 2000; **92:** 132–39.

33 Armentia A, Lombardero M, Callejo A, et al. Occupational asthma by *Anisakis simplex. J Allergy Clin Immunol* 1998; **102:** 831–34.

34 Ohta M, Ikeda K, Miyakoshi H, et al. A very rare case of continuous ambulatory peritoneal dialysis peritonitis caused by *Anisakis* larva. *Am J Gastroenterol* 1995; **90:** 1902–03.

35 Cuende E, Audicana MT, Garcia M, et al. Rheumatic manifestations in the course of anaphylaxis caused by *Anisakis simplex. Clin Exp Rheumatol* 1998; **16:** 303–04.

36 Ido K, Yuasa H, Ide M, Kimura K, Toshimitsu K, Suzuki T. Sonographic diagnosis of small intestinal anisakiasis. *J Clin Ultrasound* 1998; **26:** 125–30.

37 Ekarohit D, Chesdapan C, Thitasut P, Sukonthasan K, Choochote W. Paragonimiasis in Mae Hong Son Province northern Thailand: case report. *Southeast Asian J Trop Med Public Health* 1991; **22** (suppl): 340–41.

38 Harrell LW, Deardorff TL. Human nanophyetiasis: transmission by handling naturally infected coho salmon(*Oncorhynchus kisutch*). *J Infect Dis* 1990; **161:** 146–48.

39 Yu SH, Kawanaka M, Li XM, Xu LQ, Lan CG, Rui L. Epidemiological investigation on *Clonorchis sinensis* in human population in an area of South China. *Jpn J Infect Dis* 2003; **56:** 168–71.

40 Joo CY, Chung MS, Kim SJ, Kang CM. Changing patterns of *Clonorchis sinensis* infections in Kyongbuk, Korea. *Korean J Parasitol* 1997; **35:** 155–64.

41 MacLean JD, Arthur JR, Ward BJ, Gyorkos TW, Curtis MA, Kokoskin E. Common-source outbreak of acute infection due to the North American liver fluke *Metorchis conjunctus. Lancet* 1996; **347:** 154–58.

42 Graczyk TK, Fried B. Echinostomiasis: a common but forgotten food-borne disease. *Am J Trop Med Hyg* 1998; **58:** 501–04.

43 Cui J, Wang ZQ, Wu F, Jin XX. An outbreak of paragonimiosis in Zhengzhou city, China. *Acta Trop Med* 1998; **70:** 211–16.

44 Onuigbo WI, Nwako FA. Discovery of adult parasites of *Paragonimus uterobilateralis* in human tissue in Nigeria. *Trop Med Parasitol* 1974; **25:** 433–36.

45 Eastburn RL, Fritsche TR, Terhune CA Jr. Human intestinal infection with *Nanophyetus salmincola* from salmonid fishes. *Am J Trop Med Hyg* 1987; **36:** 586–91.

46 Farrell RK, Soave OA, Johnston SD. *Nanophyetus salmincola* infections in kippered salmon. *Am J Public Health* 1974; **64:** 808–09.

47 Terramocci R, Pagani L, Brunati P, Gatti S, Bernuzzi AM, Scaglia M. Reappearance of human diphyllobothriasis in a limited area of Lake Como, Italy. *Infection* 2001; **29:** 93–95.

48 Nime FA, Burek JD, Page DL, Holscher MA, Yardley JH. Acute enterocolitis in a human being infected with the protozoan *Cryptosporidium. Gastroenterology* 1976; **70:** 592–98.

49 DuPont HL, Chappell CL, Sterling CR, Okhuysen PC, Rose JB, Jakubowski W. The infectivity of *Cryptosporidium parvum* in healthy volunteers. *N Engl J Med* 1995; **332:** 855–59.

50 Gomez-Bautista M, Ortega-Mora LM, Tabares E, Lopez-Rodas V, Costas E. Detection of infectious *Cryptosporidium parvum* oocysts in mussels (*Mytilus galloprovincialis*) and cockles (*Cerastoderma edule*). *Appl Environ Microbiol* 2000; **66:** 1866–70.

51 Graczyk TK, Farley CA, Fayer R, Lewis EJ, Trout JM. Detection of *Cryptosporidium* oocysts and *Giardia* cysts in the tissues of eastern oysters (*Crassostrea virginica*) carrying principal oyster infectious diseases. *J Parasitol* 1998; **84:** 1039–42.

52 Rendtorff RC. The experimental transmission of *Giardia lamblia* among volunteer subjects. In: Jacubowski W, Hoff JC, eds. Waterborne transmission of giardiasis: EPA 600/9–79-001. Washington DC: Environmental Protection Agency; 1978: 64–81.

53 Cheng HS, Shieh YH. Investigation on subclinical aspects related to intestinal parasitic infections among Thai laborers in Taipei. *J Travel Med* 2000; **7:** 319–24.

54 Osterholm MT, Forfang JC, Ristinen TL, et al. An outbreak of foodborne giardiasis. *N Engl J Med* 1981; **304:** 24–28.

55 Adams AA. Establishing jurisdiction through forensic parasitology. *J Parasitol* 1993; **79:** 459–60.

56 Rippey SR. Infectious diseases associated with molluscan shellfish consumption. *Clin Microbiol Rev* 1994; **7:** 419–25.

57 Burkhardt W 3rd, Calci KR. Selective accumulation may account for shellfish-associated viral illness. *Appl Environ Microbiol* 2000; **66:** 1375–78.

58 Schwab KJ, Neill FH, Estes MK, Metcalf TG, Atmar RL. Distribution of Norwalk virus within shellfish following bioaccumulation and subsequent depuration by detection using RT-PCR. *J Food Prot* 1998; **61:** 1674–80.

59 Wilson IG, Moore JE. Presence of *Salmonella* spp. and *Campylobacter* spp in shellfish. *Epidemiol Infect* 1996; **116:** 147–53.

60 Lipp EK, Rose JB. The role of seafood in foodborne diseases in the United States of America. *Rev Sci Tech* 1997; **16:** 620–40.

61 Venkataramaiah N, Kempton AG. Bacterial growth in seafoood on restaurant premises. *Can J Microbiol* 1975; **21:** 1788–97.