CHAPTER 7

Foodborne Trematodes

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7.1 PREFACE

Digenetic trematodes comprise one of the most common groups of parasitic worms. They have a complex life cycle involving both sexual and asexual reproduction. The parasites require at least two hosts, the first of which is usually a mollusc. Over a 100 species of digenetic trematodes have been recorded from human hosts, but many cases may be spurious or accidental (Crompton, 1999). The association between humans and trematodes is longstanding, with reports of *Schistosoma* eggs in Egyptian mummies and of *Clonorchis* eggs in a 2000-year-old corpse from the Chu Dynasty in China.

In the present chapter, trematodes from six genera will be discussed (Table 7.1), including their geographic distribution, life cycles, epidemiology, and clinical aspects of disease. These trematodes account for approximately 40 million cases of human infections worldwide. Other mammalian hosts may also be infected, often acting as reservoir hosts for the parasites. Infection of the final host occurs from the consumption of foods contaminated with infectious larvae (metacercariae). The implicated sources of infection are usually freshwater or anadromous fishes, freshwater crustaceans, or aquatic vegetation, depending on the species of trematode involved.

Many of the species of food-borne trematodes are endemic in developing nations and have significant impact on public health. Developed countries, including the United States, also have foci of trematode infections. Some species are naturally present in the United States, such as *Nanophyetus salmincola*, *Fasciola hepatica*, and *Paragonimus kellicotti*. Others are introduced by the importation of contaminated foods or infected intermediate hosts, the development of new food habits, and the immigration of peoples from endemic countries.

The control of the trematode populations and prevention of their respective diseases are dependent on understanding the transmission and development of the parasites. Disruption of the life cycles is necessary for the control and eradication of disease, which can be attained through the treatment of infected populations, improved sanitation and agricultural practices, or the alteration of food habits.

7.2 *PARAGONIMUS* SPP.

7.2.1 Introduction

Trematodes of the genus *Paragonimus* invade various organs, but are found primarily within the lungs of the definitive hosts. WHO (1995) estimated that over 20 million people are infected worldwide. Over 40 species of *Paragonimus* have

Table 7.1. Comparison of foodborne trematodes.

Species	*Estimate of human cases*[a] *(millions)*	*Usual infection site for adult parasite*	*Size range, adults (mm)*	*Size range, eggs (μm)*	*First intermediate hosts*	*Second intermediate hosts*
Paragonimus spp.	20.68	Lungs	7–20 × 4–8	80–120 × 45–65	Snails	Freshwater crabs
Clonorchis sinensis	7.01	Bile Ducts	8–25 × 1.5–5	26–35 × 12–19	Snails	Freshwater fishes
Opisthorchis spp.	10.33	Bile Ducts	7–12 × 2–3	19–30 × 12–17	Snails	Freshwater fishes
Fasciola hepatica	2.39	Bile Ducts	20–40 × 8–13	130–150 × 63–90	Snails	Aquatic vegetation[b]
Fasciolopsis buski	0.21	Small Intestine	20–75 × 8–20	115–158 × 63–90	Snails	Aquatic vegetation[b]
Nanophyetus salmincola	0.019	Small Intestine	0.8–2.5 × 0.3–0.5	64–97 × 34–55	Snails	Freshwater and anadromous fishes

[a] WHO, 1995.
[b] Cercariae shed their tails and encyst as metacercariae on aquatic vegetation.

been described although not all may be valid. At least 15 species have been reported from humans, of which eight are considered important (Cross, 2001; Sinniah, 1997). *P. westermani* is the most studied since it has the broadest geographical distribution and accounts for most cases of pulmonary paragonimiasis. This species has been reported from China, Japan, Korea, Thailand, Taiwan, the Philippines, Indonesia, India, Nepal, and Manchuria. In addition to *P. westermani*, other species of medical importance in China are *P. skrjabini*, *P. hueitungensis*, and *P. heterotremus*. The latter is also distributed in southeast Asia, including Thailand. *P. miyazakii* is endemic in Japan in addition to *P. westermani*. Two species are endemic in Africa, *P. africanus* and *P. uterobilateralis*. In Latin America, including Peru and Ecuador, *P. mexicanus* is of greatest concern for human infections, although other species have been reported (e.g., *P. amazonicus* and *P. inca*. Note that *P. peruvianus*, *P. ecuadoriensis*, and *P. caliensis* are considered synonyms of *P. mexicanus*) (WHO, 1995). The North American species, *P. kellicotti*, is primarily known from wild and domestic carnivores, especially cats, dogs, pigs, minks, fishers, foxes, and muskrats (Schell, 1985; Yokogawa, 1965). However, human infections derived domestically have been reported (Castilla *et al.*, 2003; DeFrain and Hooker, 2002; Mariano *et al.*, 1986; Pachucki *et al.*, 1984; Procop *et al.*, 2000) and Weina and England (1990) suggest that the trematode may be more prevalent.

7.2.2 Life Cycle

The adult trematode of *P. westermani* is thick and oval shaped; 7–20 mm long, 4–8 mm wide, and 2.5–5 mm thick (Cross, 2001; Sinniah, 1997). The two suckers are equal in size and the tegument is spinose. The ovary is lobate and anterior of the two deeply lobed testes. The uterus lies opposite of the ovary. The cecae are unbranched and extend to the posterior end of the body. In addition to humans, other definitive hosts of this species include dogs, pigs, cats, tigers, leopards, panthers, and other wild cats (Yokogawa, 1965). Depending on the species of *Paragonimus*, weasels, badgers, monkeys, rats, and other carnivorous mammals in addition to those previously mentioned may also act as definitive hosts. Generally, the adults live in the respiratory tract of their hosts, although extrapulmonary infections can occur and are discussed below.

Two worms are usually present in a cyst although the trematodes are hermaphroditic. Eggs are passed unembryonated from the adults, through the cyst and into the bronchi. They are then either coughed up in thick sputum or are swallowed and passed out in the feces. The eggs are thick-shelled, operculate, and generally oval in shape (Fig. 7.1). If the eggs reach water, they will embryonate and hatch in 2 to 3 weeks. The resulting miracidia swim in search of appropriate snail intermediate hosts, of the families Hydrobiidae, Pleurocercidae, and Thiaridae (Cross, 2001). Numerous species of snails act as hosts for *Paragonimus* spp. in China and southeast Asia, demonstrating little host specificity. In North America however, only the snail *Pomatiopsis lapidaria* is known as the first intermediate host of *P. kellicotti* (WHO 1995; Yokogawa, 1965).

Within the molluscan host, the parasite develops as a sporocyst, then through two generations of rediae, prior to forming cercariae. These cercariae have small, knobby tails with spines (Fig. 7.2) and are poor swimmers. Therefore, after leaving

Figure 7.1. Egg of *Paragonimus westermani*.

the snails, the cercariae crawl along the sediment and rocks in search of crustacea to act as second intermediate hosts. Inside these hosts, the trematodes encyst in the viscera, gills, and muscles and form metacercariae. Numerous species of freshwater crabs and crayfish have been reported as second intermediate hosts of *Paragonimus* spp., again demonstrating little host specificity. Several species of crayfish of the genus *Cambarus*, including *C. propinquus*, are hosts for metacercariae of *P. kellicotti* in North America (Yokogawa, 1965). In Latin America, species of freshwater crabs from three genera (*Hypolobocerca*, *Potamocarcinum*, and *Pseudothelphusa*) are recognized as second intermediate hosts of *P. mexicanus* (WHO, 1995). In addition to penetration and encystment, experimental evidence indicates that crustaceans may also become infected from consuming infected snails (Noble, 1963).

When the infected crustacean is eaten raw or undercooked, the trematodes excyst in the intestine and pass through the intestinal wall. The worms penetrate the diaphragm and pleura, and encyst in the lungs. The trematodes reach maturity and

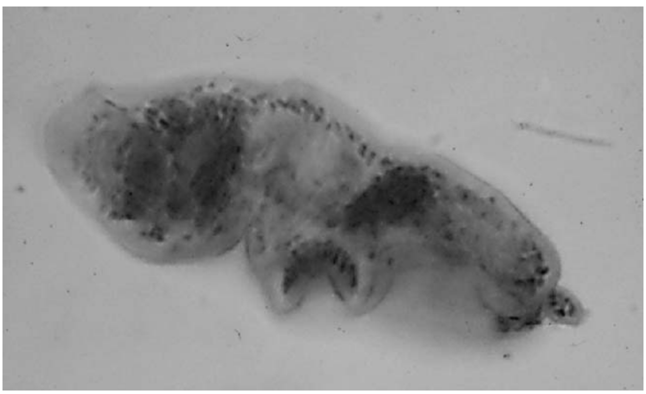

Figure 7.2. Cercaria of *Paragonimus westermani* showing small, knob-like tail.

begin to lay eggs in 5 to 6 weeks after infection. Although infections may persist up to 20 years, most adult worms die in about 6 years (Sinniah, 1997). If the host is not suitable for the trematodes to develop into adults, the worms will encyst in the tissues of that host and remain until consumed by an appropriate host (Sinniah, 1997). For example, people in Japan became infected after eating raw meat from a wild boar. Immature worms of *Paragonimus* were found in the muscle of the boar (Miyasaki and Hirose, 1976).

7.2.3 Epidemiology

Paragonimiasis is more common in children, with a peak prevalence in 10–14 year olds (Kum and Nchinda, 1982; WHO 1995). The prevalence also tends to be higher among males in comparison to females, but the difference is not always statistically significant (Kum and Nchinda, 1982; Moyou-Somo *et al*., 2003). The exception to this observation concerns the use of raw crabs by females in Africa to aid fertility, resulting in higher infection rates. However, Kum and Nchinda (1982) conducted a survey which indicated this practice has decreased, with only 4% of the respondents considering this practice to be effective.

In general, humans become infected from the ingestion of infected crustaceans that are either raw or undercooked. Some traditional preparations of crustaceans by marinating, pickling, or salting may give the appearance of cooked flesh, but the metacercariae maintain their infectivity. In Korea, crab marinated in soy sauce is a major source of paragonimiasis, as are crabs soaked in wine (drunken crabs) in China (Cross, 2001). Throughout Asia, cultures have various dishes consisting of raw crab, shrimp, and crayfish which provide transmission of the trematode.

Improper cooking may account for many infections in cultures that infrequently consume raw crustaceans. Kum and Nchinda (1982) reported that only 12% surveyed in Cameroon admitted eating raw crabs; however, the local delicacy consisted of a preparation of crabs and plantain baked in hot ashes. The period of baking was not always sufficient to inactivate metacercariae. The authors also noted that children would roast crabs directly in the fire, but not long enough, since the metacercariae present were still viable. In a later study by Moyou-Somo *et al*. (2003), also in Cameroon, all children examined reported preparing crustaceans by roasting, boiling, or frying (in oil). However, duration of cooking was not determined by internal temperature or consistency of flesh, but rather by a color change of the crab shell. Sachs and Cumberlidge (1990) related that roasting of crabs by children was also common in Liberia, but that the claws and legs of crabs were often removed prior to roasting and children chewed these raw appendages while food preparation was underway. They proposed that this activity was the predominant route of infection for children and adolescents.

Cross-contamination of cooked foods with raw materials or utensils is another route of transmission for paragonimiasis. In Japan, crab soup is prepared by removing the shells and legs and chopping the bodies with a knife on a chopping block. The crab is strained through a bamboo basket and cooked 10 to 20 minutes with vegetables or noodles. A study of the preparation of the soup demonstrated metacercariae on the knife, on the cook's hands, on the chopping block and table, and on the bamboo basket (Yokogawa, 1965). Metacercariae can survive for weeks outside of the animal host, so contaminated utensils can be a serious source of infection (Cross, 2001).

In addition, juices from crustaceans may be used in food preparation or in traditional medicines. In Korea, raw juice from crabs were used to treat fever and diarrhea. Similarly, in Japan, juice from *Eriocheir japonicus* and *Potamon dehani* were used to treat fever or to make an ointment for urticaria (Yokogawa, 1965). Medicinal use of juice from crabs occurs also in South America, with the supernatant from ground crabs of the genus *Hypolobocera* used to treat children (WHO, 1995).

7.2.4 Clinical Signs, Diagnosis, and Treatment

The severity of symptoms is often determined by the location of the trematodes, the degree of infection, and the progression of the worms through the body of the host. Early in the infection, the human host may be asymptomatic; however, when the worms migrate from the intestine into the abdomen, diarrhea and abdominal pain may be experienced. Migration to the lungs can elicit an allergic response, with fever, chills, chest pain, urticaria, and eosinophilia (Cross, 2001). After maturity in the lungs, eggs may appear in the sputum without the presence of symptoms. Incidental lesions may form in the lungs with a granulomatous reaction that eventually results in fibrotic encapsulation of the adult trematodes. The most common clinical signs of infection are persistent cough, especially in the morning, and the production of gelatinous, odorous, rust-colored sputum. Other symptoms include fatigue, myalgia, fever, and dyspnea (WHO, 1995; Yokogawa1965, 1969). Chronic infections can result in hemoptysis, pleural effusion, persistent rales, clubbed fingers, and pneumothorax (Cross, 2001). Patients with only chronic cough may be misdiagnosed with bronchitis, bronchial asthma, or bronchiectasis. Similarly, the dark sputum, heavy cough, rales, and hemoptysis of pulmonary paragonimiasis can cause confusion with tuberculosis. Chest radiography and CT scans of these infections may add to the possibility of misdiagnosis by mimicking the pleural and parenchymal lesions, and solitary nodular lesions of tuberculosis or other diseases (Mukae *et al.*, 2001). Kum and Nchinda (1982) recommended that chest X-rays be used only to evaluate the extent of damage to the lungs from the infections rather than for diagnosis. The literature is replete with reports of patients with paragonimiasis first being treated for tuberculosis (Pezzella *et al.*, 1981; Weina and England, 1990; Yokogawa, 1965). In Ecuador, 13% of the patients being treated for tuberculosis were actually infected with *Paragonimus* (WHO, 1995). Since treatment for the parasite was estimated to be 100 times less costly, the misdiagnoses had an economic impact as well as medical.

The lungs are the primary site for infections by *Paragonimus*, but the worms can wander and encyst throughout the body of the mammalian host. Reported extrapulmonary locations for encystment include the brain, spinal cord, liver, eyes, reproductive organs, subcutaneous tissues, diaphragm, pancreas, pericardium, and lymph nodes (Cross, 2001; Yokogawa, 1965, 1969). The spinal cord and brain are common sites for encystment outside of the lungs. Spinal involvement can result in paraplegia, monoplegia, limb weakness, and sensory deficiencies. Symptoms of cerebral paragonimiasis, usually manifested about 10 months after the appearance of pulmonary signs, are headache, fever, vomiting, seizures, and visual disturbances. Cerebral hemorrhage may occur, especially in children under 15 years of age (Sinniah, 1997). Oh and Jordan (1967) evaluated the intellectual capabilities of patients with cerebral paragonimiasis in Korea and reported that 90% of afflicted children under 15 years became mentally retarded. Children also frequently experience involvement of the liver. A study in China found 51% of infected children presenting with hepatic involvement (WHO, 1995).

Although *P. westermani* may occasionally demonstrate extrapulmonary infections in the abdomen or in subcutaneous tissues, these manifestations are usually caused by *P. skrjabini*, *P. heterotremus*, and *P. mexicanus* because humans are not the most suitable hosts for these parasites (Rim *et al.*, 1994). As a result, migratory subcutaneous lesions may form in the chest, abdominal wall, and extremities.

Microscopic examination of the sputum or stool for the characteristic eggs of *Paragonimus* is the usual approach for the clinical diagnosis of the infection. However, eggs may be intermittently discharged by the patient, decreasing the sensitivity of the procedure (Kong *et al.*, 1998). In light infections, sputum might not be produced or the patient may habitually swallow it, thereby eliminating the analysis of the sputum as a means of diagnosis. Pezzella *et al.* (1981) reported the treatment of 11 patients (15 to 39 years of age) in Korea with pulmonary paragonimiasis, of which only two had eggs in their sputum. In an earlier study, eggs were detected in sputum 72% of the time and in stools, 63% (Yokogawa, 1965). In those cases (13%) of which eggs were found in the stool specimens but not in the sputa, the patients were primarily children or the elderly.

Serological testing, in addition to the microscopical procedures, are of value for confirmation of other tests and for diagnosis in chronic infections of which eggs are difficult to isolate. The interdermal test using the veronal buffered saline (VBS) extract of adults of *P. westermani* is recommended as a screening method (Cross, 2001; Yokogawa, 1965). The test may be used in surveys or to differentiate paragonimiasis from tuberculosis, tumors, or other nonparasitic conditions. This method will provide positive results for up to 20 years after complete recovery; therefore, follow-up testing with complement fixation tests, ELISA, or immunoblot is recommended (Cross, 2001). Earlier work with these sero-diagnostic techniques indicated cross-reactivity with antigens from other trematodes, including *Clonorchis*, *Schistosoma*, and *Fasciola* (Hillyer and Serrano, 1983; Yokogawa, 1965). Advances in these procedures have increased the sensitivity and specificity, such that the cross-reactivity inherent in the earlier procedures have been decreased or eliminated. For example, ELISA methods have been developed using cysteine protease antigens which have high specificity and can differentiate between paragonimiasis and fascioliasis (Ikeda, 1998; Ikeda *et al.*, 1996). Similarly, immunoblot for paragonimiasis has been developed and refined such that sensitivity is estimated at 96% and specificity at 99% (Slemenda *et al.*, 1988). In a study of 40 separate cases of paragonimiasis caused by three species of *Paragonimus*, sera from patients with other trematodes or cestodes infections, with lung cancer, or from healthy subjects failed to react in the immunoblot (Kong *et al.*, 1998).

Several regimens are available for treatment. The drug of choice is praziquantel administered at 25 mg/kg, three times a day for 1 to 2 days (Medical Letter Inc, 1984). Dosage is the same for children as for adults. Bithionol is also efficacious for all forms of paragonimiasis (Yokogawa, 1969). The drug is given at a dose of 30–50 mg/kg every other day for 10 to 15 days (Sinniah, 1997). Niclofolan is also effective in a single oral dose of 2 mg/kg of body weight (Kum and Nchinda, 1982). When treating cerebral paragonimiasis, corticosteroids should be given to ameliorate the localized immune response to the dead parasites (Cross, 2001).

Surgical treatment may be necessary for severe pleural paragonimiasis; not to specifically remove the worms, but rather to alleviate some of the damage incurred during the infection (Pezzella *et al.*, 1981). In these cases, patients were treated pharmologically 10 to 14 days before surgery. Surgical treatment of cerebral paragonimiasis could eliminate seizures with the removal of encapsulated abscesses, often including a trematode (Yokogawa, 1965). Only 30% of those patients operated upon were considered improved or cured after 2 years and 21% still experienced seizures after surgery.

7.3 *CLONORCHIS SINENSIS*

7.3.1 Introduction

Clonorchis sinensis, also known as the Chinese Liver Fluke, is widely distributed in China, Hong Kong, Taiwan, Japan, Korea, Vietnam (northern), and the far east of the Russian Federation. Stoll (1947) estimated that less than 19 million people were infected with *C. sinensis*, and that the disease was confined to Asia. Although some sources still support that estimate (e.g., Sun, 1997), the generally accepted estimate is approximately 7 million at present (Crompton, 1999; Rim *et al.*, 1994; WHO, 1995).

The trematode has been present in China for over 2000 years, as demonstrated by the discovery of eggs in an excavated corpse from the Chu Dynasty of 206 B.C. (WHO, 1995). The parasite remains as a major public health issue in China with 4 million people infected, although efforts are underway to lower the prevalence of infection. A control program was implemented from 1983 to 1989 in the Sichuan Province, with a resulting drop from 21 to 24% prevalence to less than 1%. Similarly, 10.6% of the population were infected in the Henan Province in 1973, but only 0.7% in 1983 (WHO, 1995). Efforts in Korea have also resulted in a decrease in prevalence. A survey in the 1950s found an incidence of 11% of the population. From 1971 to 1992, clonorchiasis decreased from 4.6 to 2.2%, and although the trematode hasn't been eradicated, the mean fecal egg counts have decreased. The number of people currently infected is approximately one million (Cross, 2001; WHO, 1995). Japan has been very successful in the control of the trematode, such that infections are no longer detected in children and only sporadic cases are diagnosed in adults. In 1971, 780 cases were diagnosed; in 1976, 26 cases; in 1986, one case; and no cases in 1991 from an examination of one million stool specimens (WHO, 1995).

Migrations of people from endemic areas may present medical personnel with unexpected foci of infections. For example, clonorchiasis has been reported from Chinese immigrant communities in Canada and the United States. Among 150 immigrants in New York City, the prevalence of infection was 26%; 15.5% of Chinese immigrants examined in Montreal were infected (Sun, 1980). All were immigrants

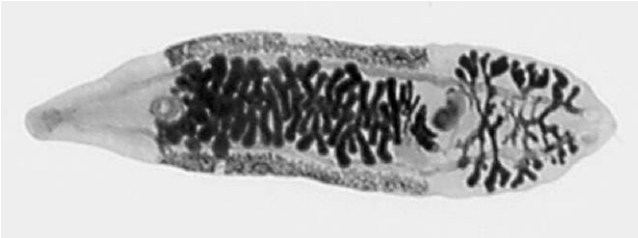

Figure 7.3. Adult of *Clonorchis sinensis*. Note the distinctly branched testes in the posterior portion of the body. Photograph by Charles Sterling, University of Arizona.

from Hong Kong. During 1979 to 1981, 13.4% of Chinese residents of Hong Kong applying for emigration to Canada had clonorchiasis (WHO, 1995).

7.3.2 Life Cycle

The adult trematode (Fig. 7.3) is small, 8–25 mm long, 1.5–5 mm wide, with a smooth cuticle (Chen *et al.*, 1994; Sun, 1997). The size of the parasite varies according to the species and size of the host, intensity of infection, the age of the trematode, and its location within the bile ducts (Chen *et al.*, 1994; Komiya, 1966). Adults have a single rounded ovary and two extensively branched testes posteriad of the ovary and in the lower third of the body. Although some place the parasite in the genus *Opisthorchis*, most parasitologists support the retention of the original taxonomic designation based on the distinctive structure of the testes. Eggs are small, ovoid, operculated with a prominent shoulder, and have a terminal knob at the abopercular end (Fig. 7.4). They measure 26–35 μm long and 12–19 μm at their widest point (Cross, 2001; Sun, 1997). Differentiation between eggs of *C. sinensis* and those of *Opisthorchis* is difficult because the dimensions and general appearance are similar, however, Ash

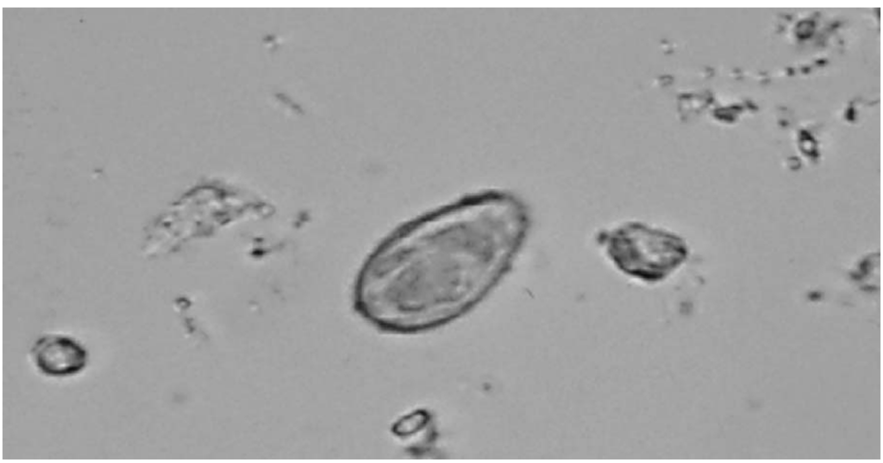

Figure 7.4. Egg of *Clonorchis sinensis*, with distinctive shoulders on egg for the operculum.

and Orihel (1997) state that the eggs of the former species have more prominent shoulders and well seated opercula. Eggs of *Clonorchis* are sometimes confused with those of *Heterophyes heterophyes*, *Metagonimus yokogawi*, and *Haplorchis taichui*, but these heterophyid eggs usually have inconspicuous opercula that appear to be flush with the surface of the shell (Ash and Orihel, 1997; Cross, 2001).

Adult trematodes reside in the intrahepatic bile ducts of humans and other fish-eating mammals, including dogs, cats, pigs, rats, tigers, mink, camels, and civets. Fish-eating birds may also be infected, such as the night heron, *Nycticorax nycticorax* (Komiya, 1966). Cats, dogs, pigs, and rats are probably the most important reservoir hosts in endemic areas. Cats are particularly susceptible. For example, in Japan from 1955 to 1961, the prevalence of *C. sinensis* in cats ranged from 45% to 87% (Komiya, 1966). Although the prevalence of infection in humans is generally lower than that in the reservoir hosts, the intensity of infection in humans is usually greater. As a result, infected humans drive the dynamics of transmission of clonorchiasis rather than reservoir hosts (WHO, 1995).

Adult trematodes produce eggs which are embryonated (each contain a ciliated miracidium) when passed into the bile ducts and excreted with the feces of the host. The eggs do not hatch when deposited in fresh water, rather they must be eaten by one of nine species of operculate snails for further development. (Rim *et al.*, 1994; WHO, 1995). *Parafossarulus manchouricus*, *Bithynia fuchsiana*, and *Alocinma longicornis* are the primary molluscan hosts for *C. sinensis* and are commonly found in ponds used for aquaculture (Cross, 2001). After ingestion by the snail, the miracidium hatches from the egg and develops into a sporocyst. Redia develop later and then cercariae form. After leaving the snail, the cercariae hang upside-down in the water and sink gradually to the bottom. They then vigorously rise in the water to resume their previous position. Cercariae do not actively search for the next host, rather, they react to disturbances in the water with sporadic movements. When a cercaria makes contact with a fish, it attaches with its suckers and penetrates within 6 to 15 minutes (Komiya, 1966). Within hours of penetration, the trematode encysts and forms a metacercaria. Metacercariae become infective to final hosts in 3 to 4 weeks. *C. sinensis* infects 113 species of freshwater fish belonging to 13 families; 95 species are members of the family Cyprinidae (Rim *et al.*, 1994; WHO, 1995). In the Fukien Province of China, some species of crayfish have been implicated in the transmission of clonorchiasis, including experimental infections of guinea pigs with metacercariae from crayfish (Komiya, 1966). When an infected fish is ingested, the trematodes excyst in the gastrointestinal tract and migrate through the common bile duct to the intrahepatic bile ducts. Migration through the ducts take 1 to 2 days and the trematodes mature into adults in about 30 days. Eggs thus appear in the stool about 4 weeks postinfection. In untreated infections, some trematodes can live up to 30 years (Cross, 2001; Sun, 1980). Unlike *Fasciola hepatica*, *C. sinensis* does not invade hepatic tissues.

7.3.3 Epidemiology

Generally, clonorchiasis is a disease of adults, with the incidence of infection increasing with age. The greatest prevalences of infection in Japan were found in those 30–50 years of age; whereas in some endemic areas of China, children under

15 years of age were also found to be infected (Komiya, 1966). In the latter circumstances, children tended to eat insufficiently cooked fishes and raw crayfishes. Early work in Vietnam indicated that in the heavily endemic region of the Red River delta, 40% of the adults and 8% of the children were infected (Komiya, 1966). Children are less likely to be infected than adults in Korea (Cross, 2001).

Depending on cultural practices, gender differences may also be present in regard to clonorchiasis. No difference was found in infection rates between males and females in Japan, but males were more often infected in China and Taiwan. In Korea, a social custom persists during which men gather to drink rice wine and eat raw fish, resulting in a higher prevalence of clonorchiasis in men 41–50 years of age (Cross, 2001; Komia, 1966). Women rarely participate in this custom.

Food habits are a primary component in the transmission of *C. sinensis*. As noted above, the improper cooking of fish or the consumption of raw fish and crayfish play important roles in the incidence of the trematode in endemic areas. In China, Taiwan, and Hong Kong, people eat a preparation called congee consisting of thin slices of raw fish over watery rice. Although neither the fish nor the snails are present in Hong Kong, this preparation from fish imported from the Chinese mainland has been implicated (Cross, 2001). Similarly, residents of Hawaii and the west coast of the United States who have been diagnosed with clonorchiasis but haven't been to the Orient probably acquired the parasite from eating imported fish containing viable metacercariae (Ash and Orihel, 1997).

Aquacultural practices in endemic areas directly impact the prevalence and persistence of the trematode. Freshwater fish, especially cyprinids, are commonly raised in ponds in Asia. These ponds also provide excellent habitat for snails which act as the first intermediate host for the parasite. In China and elsewhere, latrines were often placed over the ponds to allow immediate introduction of fecal material to the system (Komiya, 1966). Although efforts have been taken to restrict the use of human and animal wastes as fertilizer in aquaculture, the inadvertent contamination of ponds with human or animal excreta continues (WHO, 1995).

7.3.4 Clinical Signs, Diagnosis, and Treatment

In endemic areas, only about a third of those with clonorchiasis present with symptoms. Factors in the presentation of symptoms appear to be the numbers of worms and the length of infection. Moderate infections consist of 100–1000 trematodes, but infections of up to 21,000 have been reported (Komiya, 1966). The mean intensity of infections in endemic areas is 20 to 200 worms. Repeated exposure most likely results in an increase in intensity because antibody response by the host is not protective (Sun, 1980; Sun and Gibson, 1969).

Acute infections are usually asymptomatic and rarely reported. If food with a large number of metacercariae is ingested, symptoms of acute clonorchiasis can occur 10 to 26 days afterwards (Cross, 2001). Symptoms include fever, diarrhea, epigastric pain, indigestion, anorexia, eosinophilia, hepatosplenomegaly, and leucocytosis. Patients with heavy infections may also experience weakness, weight loss, feelings of abdominal fullness, anemia, and edema. Chronic infections may present with jaundice, portal hypertension, ascites, and upper gastrointestinal bleeding. Children with repeated or heavy infections with *C. sinensis* have been reported to suffer dwarfism with retardation of sexual development (WHO, 1995). In China, a study of 85 children with clonorchiasis found 40% experienced retarded growth (Sun, 1997).

The pathology of clonorchiasis is believed to be caused by the mechanical irritation from the suckers of the adults in the bile ducts and by toxic metabolic substances produced by the parasite. The presence of the worms in the ducts elicit proliferation of the epithelium, an increase in goblet cells, and an increase in the secretion of mucus. The cellular proliferation and infiltration of leucocytes result in the thickening of the ductal walls and the lumen becomes dilated, with possible fibrosis. The liver parenchyma and hepatic cell function are unaffected by *C. sinensis*.

An important complication of clonorchiasis is recurrent pyogenic cholangitis caused by secondary bacterial infections. Symptoms include repeated febrile attacks, abdominal pain, and jaundice. The high proportion of mucus in the bile and the presence of the eggs and adult trematodes form a favorable environment for bacteria, primarily *Escherichia coli*. Occasionally a patient with *C. sinensis* and cholangitis caused by *Salmonella* organisms can act as a typhoid carrier (Sun, 1980). Cholecystitis may also be caused by *C. sinensis*. The eggs of the trematode act as nuclei for the formation of stones in the bile ducts and the gall bladder (WHO, 1995). Although adults may be found in the gall bladder, they cannot survive in bile and do not directly cause cholecystitis (Sun, 1997); however, calculi may form around dead worms. Stones in the gall bladder and bile ducts were found in 70% of infected patients autopsied in Hong Kong (Cross, 2001). If adults enter the pancreatic ducts, dilation and hyperplasia of the ductal epithelium occur. Symptoms of acute pancreatitis may present, usually 1 to 3 h after a meal.

Cholangiocarcinoma is strongly associated with clonorchiasis, in that the cancer is more prevalent in areas where the parasite is endemic than in nonendemic regions such as Europe and North America (Chen *et al.*, 1994; Patel, 2002). Similarly, examination of the ratio of cholangiocarcinoma to hepatocellular carcinoma in areas considered to have high rates of primary liver cancer provides further support. In Hong Kong, which has a high incidence of clonorchiasis, the ratio is 1:5; in the Pusan area of South Korea, 1:4. In contrast, ratios in areas in which the trematode is absent are: Java, 1:56; Africa, overall, 1:38; and Johannesburg, South Africa, 1:20 (Chen *et al.*, 1994; Flavell, 1981). The development of cholangiocarcinoma requires long term and persistent infections by the liver fluke, although the presence of the trematode does not appear to be the sole factor in the development of the malignancy. Rather, the parasite most likely alters the biliary habitat, which becomes more susceptible to carcinogenic stimuli (Chen *et al.*, 1994; Flavell 1981; Parkin *et al.*, 1993; WHO, 1995).

Diagnosis is usually by direct microscopic examination of stool for the presence of eggs. A more sensitive test is the recovery of eggs from duodenal drainage, but patients tend to be reluctant. Single examinations of stool specimens have a high false-negative rate. Although repeated testing increases sensitivity, obtaining multiple stool specimens is difficult (Chen *et al.*, 1994). As mentioned above, differentiation of eggs of *C. sinensis* from those of *Opisthorchis* spp., *Metagonimus* spp., or other heterophyid trematodes is difficult and requires intense study of specimens. Information such as food habits and past travel, in addition to the morphology of the eggs, may be needed to arrive at a diagnosis. For example, Canadian patients presented with abdominal pain, anorexia, and abnormalities in their liver function after eating raw fish. Further study indicated they had consumed the flesh of the white sucker, *Catostomus commersoni*, a fish common in North America, and that they were infected with *Metorchis conjunctus* (Ash and Orihel, 1997). Definitive diagnosis can be made from the identification of the worms passed by the patient after treatment.

Identification from histological specimens can also be done. In tissue sections, *C. sinensis* usually appears as 1 or 2 sections of an adult worm in the lumen of a bile duct, whereas *Opisthorchis* spp. is usually smaller and appears unfolded (Sun, 1997). Sections with *F. hepatica* show the branched digestive tract and cuticular spines of the trematode.

In early stages of study and treatment, the populations in endemic areas often willingly submitted stool specimens for testing. As *C. sinensis* has come under control and has a lower prevalence, the submission of stool specimens has decreased (WHO, 1995). Coupled with the rates of false-negatives with microscopical methods, other means of diagnosis are needed. Serologic tests are commonly used in surveys, but are not used in routine diagnosis. In Korea, screening is done with an enzyme-linked immunosorbent assay (ELISA) using a crude antigen of *C. sinensis*, followed by stool examinations for sero-reactive patients. Those found to be shedding eggs are then treated (WHO, 1995). Further development of ELISA techniques is ongoing to improve the sensitivity and specificity of the technology. Some approaches provide specificity, such as the use of serum IgG4 antibody, but lack sensitivity (Hong *et al.*, 1999). Other work involving excretory-secretory antigens indicated that patients with active infections can be differentiated from past cases, but further development is necessary (Kim, 1998). An ELISA technique utilizing cystatin capture with multiple cysteine proteinases appears to be sensitive and more specific than ELISA using crude extracts for serodiagnosis (Kim *et al.*, 2001). Other serologic techniques include the indirect fluorescent antibody test (IFAT), indirect hemaglutination test (IHA), complement fixation test (CFT), and counter-immunoelectrophoresis (CIEP), all of which have varying sensitivities and specificities (Chen *et al.*, 1994).

Indirect evidence of parasitic infection of the bile ducts may be provided by radiological techniques (Cross, 2001; Sun, 1997). Ultrasonography is noninvasive and may detect the movements of the trematodes. Computed tomography has been used to diagnose clonorchiasis, particularly in endemic regions.

Praziquantel is the drug of choice for treatment of clonorchiasis, at a dose of 25 mg/kg of body weight, three times in one day (Liu, 1996; Medical Letter, Inc., 1984). Treatment for 2 days provides cure rates of 97.7 to 100%, and is suggested for moderate infections (Sun, 1997; WHO, 1995; Yangco *et al.*, 1987). Praziquantel is suitable for adults and children older than 4 years of age. Although differential diagnoses of clonorchiasis, opisthorchiasis, and infections by other heterophyids can be difficult, treatment with praziquantel is similar such that the infections can be effectively treated. For those patients with cholangitis, antibiotics should also be administered. Prognosis of light infections is good; chronic infections may result in hepatobiliary disease.

7.4 *OPISTHORCHIS VIVERRINI* AND *OPISTHORCHIS FELINEUS*

7.4.1 Introduction

Several species of *Opisthorchis* infect humans and cause disease similar to clonorchiasis. *O. viverrini* is distributed in southeast Asia, including Thailand, Laos, and Kampuchea. In northeastern Thailand, the prevalence of *O. viverrini* rose from 3.5 million people infected in 1965 to 5.4 million in 1981 (Sun, 1997). The most recent estimate is approximately 7 million people infected in Thailand and 1.7 in Laos (Rim *et al.*, 1994). *O. felineus* (synonym: *O. tenuicollis*) is endemic in southern, central, and eastern Europe, including Poland and Germany, in the Russian commonwealth, Turkey, and western Siberia. Stoll (1947) estimated 1 million people were infected with the latter species. Sun (1997) suggests that several million people worldwide are presently infected. Approximately 1.2 million are infected in Russia and 1.5 million in the former USSR (Rim *et al.*, 1994). Western Siberia comprises the largest endemic region for *O. felineus*, with prevalences ranging from 40 to 95% in the Tyumen and Tomsk districts (WHO, 1995). A third species, *O. guayaquilensis* (synonym: *Amphimerus pseudofelineus*) infects humans in an isolated area of Ecuador (Sun, 1997).

Similar to that seen with *C. sinensis*, migrations of people from endemic areas can result in cases of opisthorchiasis being diagnosed far from the original foci of infections. The prevalence of opisthorchiasis among refugees from Asia range from 5 to 20% (WHO, 1995). Laotian immigrants in the United States and France are frequently found to have opisthorchiasis (WHO, 1995; Woolf *et al.*, 1984). Migrant labor can be a contributory factor in the introduction and spread of disease, including opisthorchiasis, as indicated by the movement of workers among the oil fields of Siberia. Similarly, Thai workers in Kuwait, Taiwan, and China were found to be infected with *O. viverrini*, but no transmission to the local population has been documented (WHO, 1995).

7.4.2 Life Cycle

Adults of *Opisthorchis* spp. are generally smaller than those of *C. sinensis*. The former trematodes are 7–12 mm long and 2–3 mm wide, with lobate testes. Adults of *O. viverrini* differ from those of *O. felineus* in that the former species has more indentations in the testes, the ovary is in greater proximity to the testes, and the vitellaria are clustered (Cross, 2001). The eggs of the two species closely resemble those of *C. sinensis* in appearance but are smaller, ranging from 19–30 μm in length and 12–17 μm in width (Ash and Orihel, 1997; Sun, 1997). Eggs of the opisthorchid species may be difficult to differentiate from eggs of the heterophyid trematodes.

The life cycle and development of the larval stages of the *Opisthorchis* spp. are nearly identical to those of *C. sinensis*. The adults reside in the biliary ducts of humans or other fish-eating mammals. In addition to humans, cats and dogs are commonly infected with *O. viverrini* in Thailand and Laos and are important in maintaining transmission of the parasite. Of the 28 species of mammals in the former USSR determined to be involved in the transmission of *O. felineus*, cats, dogs, foxes, and muskrats are the most important reservoir hosts (WHO, 1995). The trematode eggs pass in the feces of the host and must reach freshwater for further development. Eggs do not hatch in the water, rather they are ingested by the appropriate snails prior to hatching. Molluscan hosts for *O. viverrini* are *Bithynia siamensis goniomphalus* (synonym: *B. s. siamensis*), *B. s. funiculata*, and *B. s. laevis*. Snails of the species *Codiella* (*Bithynia) inflata*, *C. troscheli*, and *C. leachi* act as first intermediate hosts for *O. felineus* (WHO, 1995). The miracidia develop into sporocysts which in turn produce rediae and then cercariae. Cercariae of *Opisthorchis* spp. find and penetrate fishes similar to those of *C. sinensis*. Carp or cyprinoid fish commonly serve as the second intermediate hosts. In Thailand, 15 species of small freshwater fishes are the primary sources of infection by *O. viverrini*, with the fish eaten raw, pickled, smoked, or fermented. Infections by *O. felineus* in Russia are acquired from 22 species of cyprinids consumed raw, frozen, pickled, or smoked (Rim *et al*., 1994).

7.4.3 Epidemiology

The age distribution of opisthorchiasis mirrors that of clonorchiasis. Generally, the incidence of the disease increases with age, from childhood to adulthood. In endemic areas such as Tyumen in the Russian Federation, chronic infections are highly prevalent; infections have been reported in 1-year-old infants and nearly 100% of children by 10 years of age (WHO, 1995). Infants and children become infected from eating raw or incompletely cooked fish. The intensity of infection as reflected by fecal egg counts also increases with age and then plateaus for those over 20 years of age, however, the number of worms in infections usually peaks between 30 and 40 years of age (Cross, 2001; Haswell-Elkins *et al*., 1991).

No gender differences are observed in cases of opisthorchiasis, indicating similarities in diets and food habits between males and females in those countries in which *Opisthorchis* spp. are endemic. In early studies, the incidence of hepatobiliary disease as a result of infection appeared to be approximately equal for the sexes (Elkins *et al*., 1990; Haswell-Elkins *et al*., 1991). Later studies in northeast Thailand indicated a high prevalence of cholangiocarcinoma among heavily infected males (Haswell-Elkins *et al*., 1994).

Food habits and aquacultural practices directly contribute to the transmission and persistence of the disease. Infection occurs from consuming infected fish raw or incompletely cooked, incompletely frozen, salted, or smoked. An outbreak of opisthorchiasis in Europe was traced to fish eaten after only 1 day of salting (Cross, 2001). In many endemic areas, traditional preparations of raw fish are eaten, such as koi-pla in Thailand. In some cultures, rituals involving food preparation may require that fish remain raw.

Aquacultural practices are common throughout Asia. Similar to China, ponds in Thailand are fertilized with human and animal excreta, ensuring the introduction of trematode eggs to freshwater. As aquaculture increases, the incidence of the disease may also increase if poor sanitation persists. Irrigation can also facilitate transmission of the trematode as a result of improved snail habitat and increased populations of freshwater fishes. *O. viverrini* is more prevalent among residents of the Nam Pong Resources Development Project, an irrigation district in Thailand, than in the population in the surrounding nonirrigated areas (WHO, 1995).

7.4.4 Clinical Signs, Diagnosis, and Treatment

Although Rim *et al.* (1994) stated that occurrence of symptoms did not appear to be related to the fecal egg count, others report a correlation between the presence of symptoms and the intensity and duration of infection (Elkins *et al.*, 1990; Upatham *et al.*, 1984). About 10% of those infected present with symptoms, with a higher occurrence of symptoms in heavy infections. Most individuals with light to moderate infections will be asymptomatic. Symptoms of acute infections include fever, eosinophilia, myalgia, and lymphadenopathy. Patients with chronic opisthorchiasis may present with diarrhea, flatulence, fever, dyspepsia after meals, anorexia, right upper quadrant pain, jaundice, and hepatomegaly. Although the liver may become enlarged and painful, the trematodes usually have no measurable effect on liver function. In areas with high prevalences of infection, opisthorchiasis has been shown to significantly contribute to malnourishment of children. In southern Laos, almost 70% of children weigh less than 80% of normal for their age (WHO, 1995).

Patients with chronic infections may also suffer from cholecystitis, cholangitis, biliary stones, and cholangiocarcinoma. Occurrence of these conditions are more common with opisthorchiasis than with clonorchiasis. In particular, the association of *O. viverrini* with cholangiocarcinoma is stronger than with *C. sinensis* (Cross, 2001; Sun, 1997). Cholangiocarcinoma is the leading cancer in northeast Thailand, but the cancer is rare in other areas of Thailand where *O. viverrini* is uncommon (Vatanasapt *et al.*, 1990). In the Khon Kaen province of northeast Thailand, the annual rate of cholangiocarcinoma was about 135 per 100,000 among males and 43 per 100,000 among females (Green *et al.*, 1991). Although fewer studies have been completed involving *O. felineus*, a correlation between the trematode and cholangiocarcinoma has also been reported in the central part of the Tyumen region of the Russian Federation. The prevalence of *O. felineus* in the central area was 45% and the rate of the cancer was almost 50 per 100,000. In the area south of Tyumen, 0.5% of the population was infected and the average prevalence of cholangiocarcinoma was approximately 4 per 100,000 (WHO, 1995).

Diagnosis of opisthorchiasis, like clonorchiasis, is usually by direct microscopic examination of eggs in a fecal sample. The eggs of *Opisthorchis* spp. are slightly different in size and have less prominent shoulders than those of *C. sinensis*, otherwise the eggs appear indistinguishable. Diagnosis may require additional information such as travel to endemic areas by the patient or food habits. Care must also be taken to differentiate the opisthorchid eggs from those of heterophyid trematodes.

Efforts to develop other means of diagnosis reflect approaches similar to those for clonorchiasis. Serological techniques detect antibodies to the trematodes, but active infections cannot be differentiated from those past. Antibodies can persist long after opisthorchiasis has been resolved through antihelminthic treatment. Specificity could also be an issue for serological tests. An indirect immunofluorescent antibody (IFA) technique for serology indicated 81% of patients had antibodies to the adult trematodes or eggs, and that about 5% of patients had a possible cross-reaction between the parasite antigens and "self" antigens (Boonpucknavig *et al.*, 1986). ELISA techniques and DNA probes have been continuously studied and pursued. A sandwich ELISA with a mixture of three IgG_1 monoclonal antibodies was used to detect as little as 0.05 ng of antigens of *O. viverrini*. A probe to detect DNA from the trematode eggs was specific in a dot blot hybridization procedure. Both techniques displayed good specificity and their sensitivity was similar to that of microscopy (Sirisinha *et al*., 1991). The development of a polymerase chain reaction (PCR) technique to detect *O. viverrini* in experimentally infected hamsters was expanded to test for eggs in human feces. The PCR method was highly specific, with a sensitivity somewhat less than the microscopical techniques (Wongratanacheewin *et al*., 2002). The difference in sensitivity was suggested to be the effect of the amount of sample analyzed in PCR (0.1 g) versus that in microscopy (1 g or 2 g). The PCR method may be suitable for screening large numbers of samples in epidemiological studies.

The drug of choice, in the treatment of opisthorchiasis, is praziquantel at 25 mg/kg of body weight, three times in one day (Lui, 1996). Mebendazole at 30 mg/kg/day for 3 to 4 weeks or albendazole at 400 mg twice a day for 3 to 7 days are also effective (Cross, 2001). Patients with cholangiocarcinoma were not treated with praziquantel to prevent possible worsening of obstruction by dead trematodes (Elkins *et al*., 1990). However, surgery may be necessary to alleviate jaundice caused by obstructions (Cross, 2001). Patients with cholangitis should also be treated with antibiotics.

7.5 *NANOPHYETUS SALMINCOLA*

7.5.1 Introduction

Nanophyetus salmincola is an intestinal trematode of the family Nanophyetidae and is present in the Pacific Northwest of the United States and in eastern Siberia. Skriabin and Podiapolskaia (1931) described the trematode from humans in Siberia as *N. schikhobalowi*, based on smaller body size and eggs, and on the absence of a cirrus pouch. However, subsequent work has shown that the two species are synonymous and the Siberian parasite is considered a subspecies (Filimonova, 1966; Gebhardt *et al*., 1966; Witenberg, 1932).

7.5.2 Life Cycle

The adult trematode is small, about 1–2.5 mm in length, and oval or pyriform in shape (Fig. 7.5). Definitive hosts are primarily piscivorous mammals, including dog, wolf, fox, bear, raccoon, spotted skunk, otter, and bobcat (Millemann and Knapp, 1970; Schlegel *et al*., 1968). Piscivorous birds such as the Great Blue Heron, Hooded

Figure 7.5. Adult of *Nanophyetus salmincola*. Photograph by Thomas R. Fritsche.

Merganser, and Belted Kingfisher were also found to be naturally infected, but other birds such as domestic mallards and chickens were refractive to experimental infection (Schlegel *et al.*, 1968). In eastern Siberia, human infections are common among the native peoples, with up to 80% prevalence in some villages along the Amur River (WHO, 1995). Until a report of infections in Oregon (Eastburn *et al.*, 1987), only the subspecies *N. s. schikhobalowi* was known to infect humans, although according to Gephardt *et al.* (1966) a parasitologist successfully infected himself with the North American subspecies by eating raw trout from Oregon. His infection was asymptomatic, but small numbers of eggs were observed in his stool 10 days after ingestion of the fish. Therefore, one can assume that people were infected with *N. salmincola* previously in the Pacific Northwest, but the infections were not diagnosed because of vague symptoms or none at all.

The life cycle of *N. salmincola* is complex, requiring two intermediate hosts. The eggs are passed from the definitive host and must be introduced to fresh water to hatch. The resulting miracidia are not attracted to snails, the first intermediate hosts, but have been observed to repeatedly bump into the snails and swim away (Bennington and Pratt, 1960). The trematode has high specificity for its snail hosts and its geographic distribution is limited by the presence of the snails in rivers and streams. In the Pacific Northwest, *Jugo plicifera* (*Oxytrema silicula* and *Goniobasis plicifera* are synonyms) acts as the molluscan host; whereas, in Siberia, *Semisulcospira cancellata* and *S. laevigata* fulfill that role (WHO, 1995). The miracidium penetrates the snail and develops into redia. The prevalence of rediae in snails depends on the time of the year, ranging from 9 to 52% in Oregon and 9 to 17% in Siberia (Millemann and Knapp, 1970).

Cercariae develop within the rediae and are then shed. The cercariae are somewhat elongate, measuring 0.31 to 0.47 mm long by 0.03 to 0.15 mm wide, with a small blunt tail. Although cercariae rhythmically contract, they do not actively search for the second intermediate host. When fish come in contact with the cercariae, the trematodes immediately begin to penetrate the skin, taking no more than 2 minutes (Bennington and Pratt, 1960). The cercariae penetrate quickly into the muscles and into the blood vessels and spread throughout the tissues of the piscine host where they encyst and form metacercariae (Fig. 7.6).

The trematode does not display the same degree of host specificity for the second intermediate host. Both salmonid and non-salmonid fishes are known to be naturally infected, as well as the Pacific giant salamander, *Dicamptodon ensatus* (Filimonova, 1963; Gebhardt *et al.*, 1966; Millemann and Knapp, 1970; WHO, 1995). Millemann and Knapp (1970) summarizes the pathology of infection by *N. salmincola*. Experimental infections confirmed the pathogenicity of the trematode to fish when exposed to large numbers of cercariae in a limited time period, although different tolerances to infection were displayed by different species of fish. Salmon and trout can have high numbers of metacercariae. For example, up to 2000 trematodes in salmon from Siberia; 14,062 in a cutthroat trout and 2002 in a steelhead trout, both from Oregon. The prevalence and numbers of metacercariae in salmonids are of concern, since most human infections reported by Eastburn *et al.* (1987) and Fritsche *et al.* (1989) resulted from the ingestion of salmon or steelhead trout. The growth of infected fish was also retarded in comparison to control fish. Heavy infections in fish

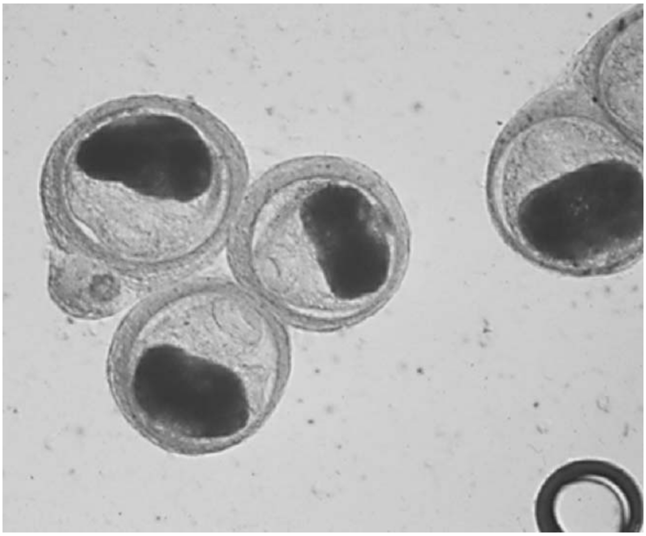

Figure 7.6. Metacercariae of *Nanophyetus salmincola*. Photograph by Thomas R. Fritsche.

with metacercariae can result in tail curvature, increased respiration, decrease in movement or erratic swimming, drifting, and loss of equilibrium.

In the Pacific Northwest, some hatcheries experience losses of heavily infected salmon when the fish begin to migrate to saltwater, probably due to the inability to adapt to the saline environment. Initially, the migration of salmon to the ocean was thought to eliminate the trematode, but this was probably a reflection of the loss of heavily infected fish. Weiseth *et al*. (1974) examined three species of ocean-caught salmon from along the coast of Oregon; king (*Oncorhynchus tshawytscha*), coho (*O. kisutch*), and pink (*O. gorbuscha*). King and coho salmon had overall infections of 31 and 53% respectively. Numbers of metacercariae were generally low, with only 5% of the king salmon and 18% of the coho having more than 24. The pink salmon were free of *N. salmincola*. Although some spawning of pink salmon occurs along the Pacific Northwest coast, the more abundant runs occur throughout the Alaskan coastal waters. In addition, after emergence from the gravel, the fry of pink salmon quickly migrate downstream to the ocean (Heard, 1991), thereby decreasing exposure to cercariae. These two factors most likely explain the lack of the metacercariae in the pink salmon (Weiseth *et al*., 1974).

7.5.3 Salmon Poisoning Disease

N. s. salmincola is unique in that it acts as a vector for a rickettsial disease of canids referred to as "salmon poisoning disease." The Siberian subspecies is not known to transmit the rickettsia. The correlation between the consumption of fish, usually salmon, and the often fatal disease for dogs was well known to settlers and Indians of the Pacific Northwest (Philip, 1955; Simms *et al*., 1931). Donham (1925) found small trematodes in the intestines of necropsied dogs and later reported the association of the disease with the cysts (metacercariae) in fish (Donham *et al.*, 1926). In the 1950s the etiological agent for salmon poisoning was identified and named *Neorickettsia helminthoeca* (Millemann and Knapp, 1970).

Dogs, foxes, coyotes, and other canids are susceptible to the disease. Raccoons may experience a slight temperature after consuming infected fish, but are otherwise without symptoms. However, Philip (1955) injected a dog with a suspension made from trematodes recovered from a raccoon, resulting in the characteristic fatal infection. Therefore, although raccoons are refractive to infection by the rickettsia, they can perpetuate the disease in the environment.

The course of infection by *N. helminthoeca* in canids is well documented (Millemann and Knapp, 1970; Philip, 1955; Simms *et al*., 1931). The incubation period is generally 5 to 7 days after ingestion of the infected fish, followed by a sudden onset of fever. Anorexia usually occurs within 24 h of the fever. The fourth or fifth day of symptoms results in persistent vomiting with excessive thirst. Diarrhea ensues on the fifth to seventh day. The stools become dark with blood. Although the temperature may drop to normal, the dog becomes dehydrated and weak from loss of weight. Death usually occurs by the tenth day. Untreated infections are 90% fatal. *N. helminthoeca* is susceptible to a broad spectrum of antibiotics including sulfonamides, penicillin, tetracycline, and streptomycin (Millemann and Knapp, 1970; Philip, 1955). Dogs that recover, either spontaneously or through treatment, are immune to further infection by the rickettsia.

7.5.4 Clinical Signs, Diagnosis, and Treatment

Although we now know that both subspecies of *Nanophyetus* can infect humans, the presentation of symptoms is dose-dependent, requiring approximately 100 or more worms (WHO, 1995). The most common complaint is vague, nonlocalized abdominal pain or discomfort, often associated with diarrhea (Eastburn *et al*., 1987; Fritsche *et al*., 1989). Patients also reported bloating, nausea and vomiting, weight loss, fatigue, and decreased appetite. Symptoms are often described as resembling those of influenza, resulting in the general description of the disease as "fish flu." At least half of those infected also presented with peripheral blood eosinophilia ranging from 6 to 43% (Eastburn *et al*., 1987). Untreated infections can persist for 8 months or more (Filimonova, 1965; Fritsche *et al*., 1989).

With the presentation of the above symptoms, plus a history of eating raw or undercooked fish, particularly salmon or trout, a diagnosis of nanophyetiasis should be contemplated. Further consideration of the geographic distribution of the trematode in the Pacific Northwest of the United States or in eastern Siberia may also indicate infection by this parasite. However, the latter information should not preclude the consideration of nanophyetiasis in the differential diagnosis of patients. Infection by this trematode has been reported in New Orleans, Louisiana (US) and attributed to the consumption of raw salmon that had been shipped fresh from the Pacific Northwest (Adams and DeVlieger, 2001).

Diagnosis is made from the observation of eggs in stool specimens, but routine laboratory procedures may be insufficient to identify all cases. Eggs generally appear in the stool 1 week after ingestion of infected fish. However, adult worms contain few eggs, which probably results in the small number of eggs in stool specimens from

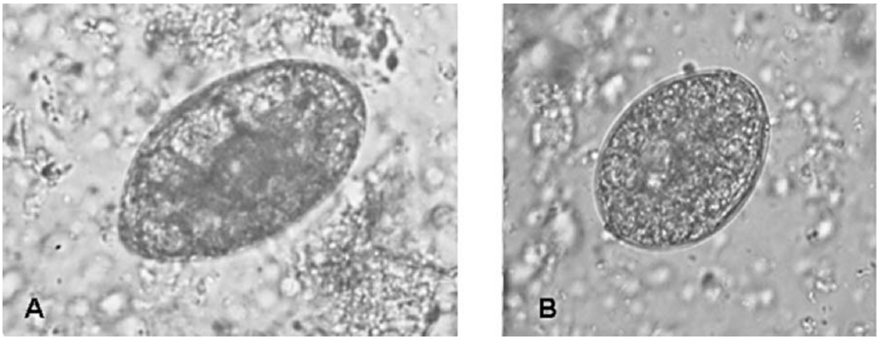

Figure 7.7. (a) Egg of *Nanophyetus salmincola*. Photograph by Thomas R. Fritsche. (b) Egg of *Diphyllobothrium* sp. Photograph by Robert L. Rausch.

patients with light infections. Eastburn *et al.* (1987) also indicated that the clinical preparation of specimens greatly impacts the detection of the trematode eggs. Most of their diagnoses were first made from trichrome stained preparations rather than from the typical formalin-ethyl acetate method. The reason for this discrepancy is unknown.

Because the eggs of *Nanophyetus* (Fig. 7.7 a) resemble those of tapeworms of the genus *Diphyllobothrium* (Fig. 7.7 b), misdiagnosis may occur (Adams and Rausch, 1997). This may be compounded in that several species of *Diphyllobothrium* are transmitted to humans through the consumption of salmonids. Recovery of segments from the cestodes or of whole worms would obviously clarify the etiological agent involved. Pharmacological treatment for both the trematode and the cestodes is similar (Table 7.2), except that nanophyetiasis usually requires a higher dosage for efficacy. This is in contrast to veterinary use of praziquantel to treat nanophyetiasis in canids such that dosages effective against cestodes (6.68–38.73 mg/kg) were adequate for the treatment of the trematode (Foreyt and Gorham, 1988). Note that treatment for the trematode in canids, is separate from that for the rickettsial infection.

Table 7.2. Comparison of Pharmacological Treatments for *Nanophyetus salmincola* and *Diphyllobothrium* spp.

Drug	*Dosage for* N. salmincola	*Dosage for* Diphyllobothrium *spp.*[a]
Bithional	50 mg/kg on alternate days ×2 doses[b]	30 mg/kg total in 1 or 2 doses
Niclosamide	2 g on alternate days ×3 doses[b]	2 g, single dose
Praziquantel	20 mg/kg ×3 daily for 1 day[c]	5–10 mg/kg for 1 day

[a] Adams and Rausch, 1997.
[b] Eastburn *et al.*, 1987.
[c] Fritsche *et al.*, 1989.

7.6 *FASCIOLA* SPP.

7.6.1 Introduction

Fasciola hepatica and *Fasciola gigantica* are both known to infect humans, although they are primarily parasites of ruminants. The former species has a worldwide distribution, but *F. gigantica* is found in Asia, Africa, and Hawaii. The latter trematode was probably introduced to Hawaii with the importation of water buffaloes from Asia (Alicata, 1964). Of the two species, *F. hepatica* more commonly infects humans. Stoll (1947) estimated that there were less than 100,000 cases of fascioliasis worldwide. Fifty years later, estimates are given of 2.4 million human infections in over 56 countries, primarily distributed in China, Egypt, Europe (especially France and Portugal), Iran, and South America (especially Bolivia, Ecuador, and Peru) (Crompton, 1999; Rim *et al.*, 1994; WHO, 1995). Although no consistent quantitative correlation has been shown, areas with high prevalences in domestic animals also tend to have high rates of human infections. For example, in the Altiplano region of Bolivia, infection rates of sheep and cattle range from 25 to 95%. In some villages, 65% of the people were found to pass eggs in their stool and 92% were serologically positive (WHO, 1995).

Fascioliasis is a serious disease in cattle and sheep throughout the world with an enormous economic impact. In 1969, Boray (1969) reported that one quarter of the sheep and cattle (40 million and 5 million, respectively) were grazing on pastures in which the infective metacercariae were potentially endemic. In Great Britain, 53% of the farms had livestock with fascioliasis (Froyd, 1975), with adult stock affected more than twice that of young animals. In the United States, 17% of cattle slaughtered in Montana were found to be infected (Knapp *et al.*, 1992). In a survey conducted by McKown and Ridley (1995), 33% of 278 veterinarians in Kansas reported diagnosing at least one case of liver fluke disease in their practice. Foreyt and Todd (1976) disclosed that 2.1% of beef livers examined in Kansas were condemned because of damage by the trematodes. Fascioliasis causes decreased wool production in sheep, decreased milk production and weight gain, increased numbers of condemned livers at slaughter, and decreased reproductive performance. In the United States alone, financial losses due to these trematodes were estimated at $30 million in 1973 (McKown and Ridley, 1995). Similarly, total losses due to fascioliasis in animals amounted to US $11 million in Peru (WHO, 1995).

7.6.2 Life Cycle

The adult trematodes are large, somewhat leaf-shaped, with a "cone-shaped" projection on the anterior. *F. hepatica* is generally 30 mm long and 13 mm wide (Fig. 7.8); *F. gigantica* may measure 73 mm long. The ventral sucker is larger than the oral sucker and located anteriad, near the base of the "cone." The intestinal ceca are highly branched, as are the two testes. The ovary is smaller, located near the ventral sucker. The operculate eggs of *F. hepatica* measure 130 to 150 μm by 63 to 90 μm (Fig. 7.9); eggs of *F. gigantica* are typically larger, 160 to 190 μm by 70 to 90 μm. Eggs of the former may be differentiated from those of *Fasciolopsis buski* by the roughened or irregular area at the abopercular end of the shell of *F. hepatica* (Ash and Orihel, 1997). Although humans may be infected, definitive

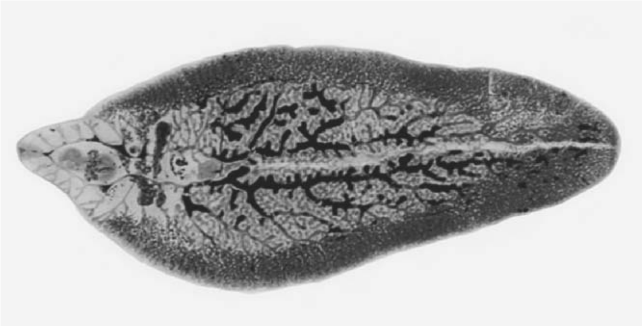

Figure 7.8. Adult of *Fasciola hepatica*, showing the distinct shape of the body and the "cone-shaped" projection on the anterior. Photograph by Charles Sterling, University of Arizona.

hosts are generally ruminants, including cattle, sheep, goats, and swine. Horses may also be hosts, but infections generally lack clinical symptoms and produce low egg counts (Alicata, 1964, Boray, 1969). Rabbits, mice, rats, and guinea pigs are all susceptible to infection. The rabbit has been recognized as a natural reservoir host.

Fasciola adults reside in the bile ducts of the liver. Operculated eggs are passed out in the feces of the host and must reach freshwater for continued development. When the egg hatches, a miracidium escapes and searches for a snail host. Suitable snails belong to several genera including *Lymnaea*, *Fossaria*, *Pseudosuccinea*, and *Austropeplea* (WHO, 1995). The miracidium penetrates the snail and develops into a sporocyst. Two generations of rediae ensue. Cercariae develop in the daughter rediae and later emerge from the snail and become free-swimming. Unlike other trematodes, these cercariae do not infect a second intermediate host. When they encounter vegetation or submerged bark, they shed their tails and encyst on the

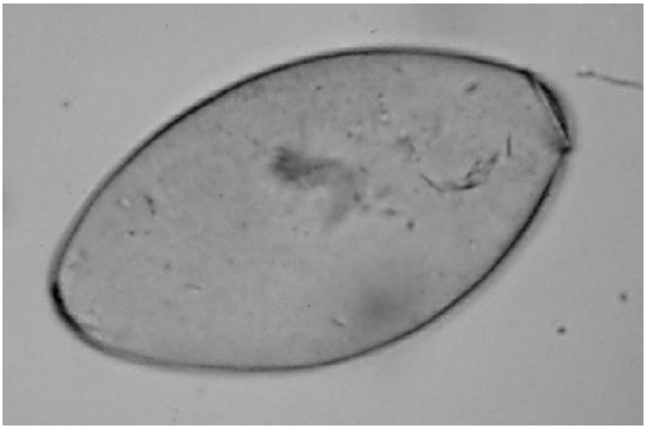

Figure 7.9. Egg of *Fasciola hepatica*.

plants and become metacercariae. If they do not find an object to encyst on, the cercariae will drop their tails and encyst free in the water. When the contaminated vegetation or water is consumed, the trematodes excyst in the small intestine and penetrate the intestinal wall. They travel around the viscera to the liver, where they burrow in and continue to move and feed for a couple months before entering the bile ducts. Approximately 4 months (range 3–18 months) after ingestion, the worm has matured and begun passing eggs (WHO, 1995).

7.6.3 Epidemiology

Within populations in endemic areas, adults are more commonly infected than children. Similarly, women have a higher prevalence of fascioliasis than men (Binkley and Sinniah, 1997). Women more often pick and gather vegetation and may consume more salads and raw vegetation than men.

In general, people become infected from eating raw vegetation contaminated with *Fasciola* metacercariae. Watercress (e.g., *Nasturtium officinale*) is the most common source of infections for people, although mint, lettuce, parsley, and wild watercress may also be contaminated with metacercariae (Rim *et al.*, 1994; WHO, 1995). Food habits often play a role in the transmission of parasites. For example, southern Europeans frequently consume watercress. When they migrated to Algeria, an outbreak of fascioliasis occurred among the Europeans, even though no infections of native Algerians were reported. Unlike the Europeans, the Algerians did not eat watercress (WHO, 1995).

Agricultural practices contribute to the contamination of vegetation by the metacercariae. The use of animal excreta as fertilizer helps distribute the eggs in the environment and increases the chances that the parasites may encounter freshwater and snails. The use of effluent from slaughterhouses or livestock pens as fertilizer for watercress plots increases the concentration and distribution of metacercariae on the plants. Emphasis on "organic" or natural healthy foods has increased the collection and consumption of wild watercress and other vegetation, introducing the infection to higher economic classes in some countries.

Agricultural practices may help maintain or increase fascioliasis among livestock. For example, many farmers allow their livestock to wander freely to graze and to drink from streams and ponds. This provides the parasite eggs ready access to freshwater in order to develop and hatch and gives the livestock direct access to vegetation that may be contaminated with metacercariae. Many farmers also cut forage from wet or swampy areas to feed cattle; in essence feeding the infecting metacercariae to their livestock (Alicata, 1964).

7.6.4 Clinical Signs, Diagnosis, and Treatment

The onset of symptoms usually occur 4 to 6 weeks after ingestion of the metacercariae, but can vary depending on the number of parasites ingested and the host immune response (WHO, 1995). Light infections may be asymptomatic. Patients often complain of fever, sweating, abdominal pain, dizziness, cough, bronchial asthma, fatigue, general malaise, loss of weight, and loss of appetite (Binkley and Sinniah, 1997; WHO, 1996). Patients may have gastrointestinal complaints including nausea and vomiting. Allergic reactions such as urticaria and pruritis may also occur.

The liver may be tender and enlarged, with jaundice. During the acute infection, children may have severe clinical manifestations such as right upper quadrant or general abdominal pain, fever, and anemia (WHO, 1995). These infections in children can be fatal.

The chronic phase of the infection begins when the trematodes reach the bile ducts. Often, this stage of the infection is asymptomatic. After arrival in the bile ducts, the trematodes mature into adults, and may cause irritation and inflammation of the ducts. The resulting symptoms may include dyspepsia, diarrhea, jaundice, biliary colic, cholecystitis, and cholelithiasis. If juvenile worms do not migrate properly into the liver, they may cause ectopic fascioliasis in other organs, including the intestines, lungs, heart, brain, and skin (the most common extrahepatic site). Nodules may form in the skin around the abdomen, sometimes reaching 6 cm across (Binkley and Sinniah, 1997).

In an endemic region, fascioliasis is suggested if a patient presents with fever, hepatomegaly, and eosinophilia (Binkley and Sinniah, 1997). Patients have been misdiagnosed as having visceral larval migrans or toxoplamosis when the fever is intermittent (WHO, 1995). Diagnosis is made either by observation of eggs in a fecal sample or from sero-immunological tests. However, diagnosis dependent on the former may be problematic for several reasons. Passage of eggs may be inconsistent, resulting in negative exams. In a study involving patients with a history of consuming watercress, 79% tested positive by serology but only 4% had positive stool specimens during their hospital stay (Chen and Mott, 1990). Symptoms of infection during the acute phase arise within 4 to 6 weeks (see above), but worms do not mature and pass eggs until approximately 4 months after ingestion of metacercariae. Therefore, the clinical findings cannot be supported by parasitological exams until months later. Patients with fascioliasis may be subjected to exploratory surgery to establish the cause of their disease because of the difficulties in diagnosis (WHO, 1995). In contrast, people who consume infected livers of cattle, sheep, or water buffalo may be incorrectly diagnosed as having fascioliasis when eggs are passed in their stools (Ash and Orihel, 1997). To prevent the diagnosis of spurious fascioliasis, the patient should be placed on a liver-free diet for at least 3 days and then retested. If eggs are still observed in the feces, the diagnosis of fascioliasis can be supported (Binkley and Sinniah, 1997).

Given the lag between the onset of symptoms and the production of parasite eggs, the use of serologic/immunodiagnostic methods are invaluable, particularly during the acute phase. Immunodetection of coproantigens of *Fasciola* rather than the observation of eggs in stool specimens is a viable alternative for diagnosis. Youssef *et al.* (1991) used partially purified *F. gigantica* worm antigens in counter-immunoelectrophoresis to test stool extracts. They found that the test was capable of detecting both early and chronic infections and that false-positives from spurious fascioliasis or cross-reactions with other parasitic infections were avoided. Espino *et al.* (1998) developed a sandwich enzyme-linked immunosorbent assay (ELISA) to detect coproantigens of *F. hepatica*; which was especially useful if patients with prepatent infections (e.g., not excreting eggs) did not have circulating antigens in their sera. If circulating antigens are present, various forms of ELISA are available for diagnosis, including the sandwich ELISA (Espino *et al.*, 1990), the Falcon assay

Table 7.3. Pharmacological Treatments for *Fasciola hepatica* and *F. gigantica*. Dosages are the same for adults and children.

Bithional	30–50 mg/kg on alternate days × 10–15 doses[a]
Praziquantel	25 mg/kg × 3 daily for 1 day[a] or 25 mg/kg × 3 daily for 3 to 7 days[b]
Triclabendazole[c]	10 mg/kg, single dose after overnight fast[d] or 5 mg/kg × 2 postprandially on same day with 6–8 h interval[e] (total dose 10 mg/kg)

[a]Medical Letter Inc., 1984.
[b]Binkley and Sinniah, 1997.
[c]Not approved for use in the United States or Canada.
[d]Apt *et al.* (1995).
[e]WHO, 1995.

screening test (FAST-) ELISA (Hillyer *et al.*, 1992), and Micro-ELISA (Carnevale *et al.*, 2001). Those that use excretory-secretory antigens appear to be more sensitive and specific. In particular, those tests that use worm cysteine proteinases such as cathepsin L1 (O'Neill *et al.*, 1998) or the Fas1 and Fas2 antigens isolated by Cordova *et al.* (1997) have excellent sensitivity and specificity. In addition, these antigens are excreted by the worms at all stages of development. If the trematodes themselves (or portions thereof) have been recovered, differentiation between *F. hepatica* and *F. gigantica* is possible with isoelectric focusing of soluble proteins from the adult worms (Lee and Zimmerman, 1993).

Praziquantel is the drug of choice for most trematode infections. Some consider the drug to also be effective against fascioliasis, however, the WHO (1995) and others (Binkley and Sinniah, 1997; Lui and Weller, 1996; Wessely *et al.*, 1988) recommend the use of other pharmaceuticals (Table 7.3). The current drug of choice is bithionol, but this is expected to be replaced by triclabendazole after appropriate testing. The latter is a benzimidazole antihelminthic and is effective against the adults in the bile ducts and the immature worms which migrate through the hepatic parenchyma (Wessely *et al.*, 1988;WHO, 1995). Usually, a single oral dose is effective, but a second dose may be administered for those infections which persist. Apt *et al.* (1995) treated 24 patients, with 19 testing negative after 2 months. Three of the remaining five were treated again with positive resolution. The drug was well tolerated and effective. Triclabendazole is also effective in the treatment of cattle, reducing the worm burden and improving weight gain (Fuhui *et al.*, 1989). The drug was considered to be safer and more efficacious than other drugs against fascioliasis, but has not been approved by the US Food and Drug Administration for use in the United States.

7.7 *FASCIOLOPSIS BUSKI*

7.7.1 Introduction

Fasciolopsis buski is another fasciolid trematode infectious to humans. Although it is similar in many ways to *Fasciola hepatica*, it also has some striking differences. Rather than residing in the bile ducts and liver, adults of *F. buski* are located in the small intestine. The geographic distribution of the latter species is generally restricted to Asia, but particularly in India, Thailand, China, Indonesia, Taiwan, east Pakistan, Laos, Vietnam, Cambodia, and Bangladesh (Crompton, 1999; WHO, 1995). Other countries, such as Israel and Hong Kong, have been concerned about the possible establishment of the parasite through the importation of foods and animals, and the arrival of infected immigrants from endemic countries (Sinniah and Binkley, 1997). The concern is legitimate. Prior to the 1940s, reports of fasciolopsiasis in Bangladesh were at best sporadic. However, after substantial immigrations from India, more cases were diagnosed until Gilman *et al.* (1982) considered the parasite to be endemic.

Earlier, Stoll (1947) estimated 10 million infections by this trematode, 100 times more than he reported for fascioliasis. Interestingly, Crompton (1999) reported somewhat the opposite situation, with only 210,000 human cases of fasciolopsiasis versus 2.1 million infections by *Fasciola*, a 10-fold difference. His estimates are taken from WHO (1995), which provided numbers of fasciolopsiasis from only China (204,000 cases) and Thailand (10,000). No data are provided for the other endemic countries. The prevalence of infection can vary greatly within the endemic areas, with fasciolopsiasis ranging up to 70% in some villages of Bangladesh, India, and China (Gilman *et al.*, 1982; WHO, 1995).

7.7.2 Life Cycle

The adult trematode is large, 20 to 75 mm long and up to 20 mm wide. Unlike the adult of *Fasciola*, *F. buski* does not have a conical projection on the anterior of the body and does not have branched cecae. The eggs are operculated and large (Fig. 7.10), 115 to 158 μm by 63 to 90 μm (Ash and Orihel, 1997; Hadidjaja *et al.*, 1982). The eggs may be difficult to differentiate from *F. hepatica*. The life cycle of *F. buski* is also very similar to that of *Fasciola*. The eggs pass out in the feces of the host and must reach freshwater. Miracidia hatch from the eggs and penetrate snails of the genera *Hippeutis*, *Segmentina*, *Gyraulus*, and *Polypylis* (Sinniah and Binkley, 1997; WHO, 1995). Development within the snails mirrors that of *F. hepatica*. Cercariae emerge from the snails and encyst on underwater vegetation. Infection of the mammalian host occurs when these plants are eaten raw or when people

Figure 7.10. Egg of *Fasciolopsis buski*.

use their teeth to crack or peel the edible portions. In addition, a small number of cercariae, up to 4%, may encyst free in the water rather than attach to vegetation. Ingestion of the contaminated water may also result in fasciolopsiasis. In a study of case histories, 13% of people and 40% of pigs were found to be infected by this mechanism in China (Sinniah and Brinkley, 1997). The metacercariae excyst in the small intestine and develop into adults without migrating within the host. Once mature, each trematode may produce about 25,000 eggs daily and live for about 6 months in humans (Sinniah and Binkley, 1997). In addition to humans, pigs are commonly infected, although dogs and water buffalo may also be hosts for the parasite.

7.7.3 Epidemiology

In endemic regions, the trematode has a higher prevalence and intensity in children, particularly those between 2 and 10 years of age (Gilman *et al*., 1982). Children often consume vegetation such as water lotus while playing in water. Socioeconomic status may also be a factor in the prevalence of infection. In Bangladesh, water plants are cheap and readily available and thus, are a common food source for the poor (Gilman *et al*., 1982). Plants that are commonly implicated in the transmission of fasciolopsiasis include water chestnut, water caltrop, lotus, bamboo, water hyacinth, water mimosa, and water spinach (Sinniah and Binkley, 1997; WHO, 1995). Water caltrop (*Trapa natans* or *T. bicornis*) is a favorite snack, particularly for children in rural areas, and is the most important plant vector of *F. buski* in east Asia (WHO, 1995).

Similar to *F. hepatica*, agricultural practices directly contribute to the persistence of *F. buski* in the environment and the transmission of the parasite to humans and domestic animals. The use of untreated manure as fertilizer for the cultivation of plants, including water plants in ponds, perpetuate the life cycle of the trematode. In China, the drainage systems from pig farms are often physically linked to ponds where the various edible plants mentioned above are raised (WHO, 1995). In Thailand and elsewhere, ponds used for cultivation are often fertilized with human feces.

Human fasciolopsiasis is generally correlated to a high prevalence of *F. buski* in pigs (WHO, 1995). An exception to this observation occurs in Bangladesh, a predominantly Muslim country, where the numbers of pigs are small. In those villages in which fasciolopsiasis were reported, no pigs were present, and therefore humans were responsible for the introduction and transmission of the trematode (Gilman *et al*., 1982).

7.7.4 Clinical Signs, Diagnosis, and Treatment

Most infections with *F. buski* are light and asymptomatic. However, in heavy infections, the patient may first present with epigastric pain and diarrhea; with vomiting, nausea, and possibly anorexia ensuing. Edema, particularly of the face may occur in heavy infections, especially in children (Cook, 1986). Toxemia and allergic symptoms may occur. Localized inflammation occurs at the site of attachment which in turn causes excess mucus secretion. Some ulceration can occur as well as the occasional formation of abscesses. Although heavy infections may sometimes result in the partial obstruction of the intestine, no clear evidence exists of general malabsorption (Cook, 1986; WHO 1995). Intense infections, defined as greater than 300 worms, are associated with malnutrition and increased mortality (Gilman *et al.*, 1982). Toxemia occurs from the absorption of metabolites from the trematodes, resulting in sensitization and possibly death of the patient. Mortality of children from profound intoxication has been reported in China, India, and Thailand (WHO, 1995).

Diagnosis is primarily by detection of eggs in feces. Rarely, a patient may simplify diagnosis by vomiting intact adult worms (Hadidjaja *et al.*, 1982). Peripheral eosinophilia and a slight macrocytic anemia may be present (Cook, 1986; WHO, 1995). However, no immunologic method for diagnosis is presently available. Care must be taken in diagnosis since the epigastric pain can simulate a peptic ulcer. In both acute and chronic fasciolopsiasis, the diarrhea does not respond to antibiotics. If the infection is thought to be caused by bacteria or viruses, the misdiagnosis can be costly because of repeated treatments (WHO, 1995).

Unlike *F. hepatica*, fasciolopsiasis responds well to treatment with praziquantel. The dose is similar to that of other trematodes: 25 mg/kg, three times in 1 day (Lui and Weller, 1996; Medical Letter Inc., 1984). Niclosamide, tetraclorethylene, and hexylresorcinol are also known to be effective (Cook, 1986).

7.8 PREVENTION AND CONTROL

The prevention and control of infections caused by these foodborne trematodes can be achieved primarily by the disruption of the life cycles of the parasites. Since the cycles are complex, involving multiple hosts and developmental stages, disruption of development and transmission can occur at multiple sites within the cycles.

The elimination of adults and any resulting eggs can be pursued through treatment of populations at risk. A control program may be implemented in an endemic area which relies heavily on drug treatment. WHO (1995) recommends that a program for community-based treatment provides annual drug therapy for the parasite for up to 3 years. In an endemic area of Thailand, 90% of people treated for opisthorchiasis were negative for eggs 2 years after a single treatment. Although infected, the remaining patients had light infections and were essentially asymptomatic. As mentioned previously, control programs for *C. sinensis* in Japan, Korea, and China have been successful at reducing the incidence of the parasite. Additionally, the broad efficacy of praziquantel, the drug of choice for many parasitic infections, allows the targeting of multiple species of parasites, even when the specific identity of a helminth cannot be determined. The general safety of the drug and the accepted use for pediatric patients as well as for adults underscore the value of this treatment for helminthiases.

Generally, patients do not become refractive to reinfection by these foodborne trematodes. Therefore, once treated for the parasites, changes in food preparation and food habits are required to keep patients from acquiring new infections. In addition to drug therapy, the community should receive education as to the life cycle of the parasite and dietary changes that could prevent transmission. Mothers in particular should receive training on the importance of children consuming only fully cooked or adequately processed foods. However, changes in traditional preparation and consumption of foods are difficult and reinfection is not uncommon. For example, in Bangladesh aquatic plants are particularly attractive to and consumed extensively by children (Gilman *et al.*, 1982). Therefore, educational programs to prevent or reduce infections by *F. buski* in children were considered unlikely to be effective.

Infections by foodborne trematodes can be prevented if the intermediate hosts are not consumed raw, undercooked, or improperly (or incompletely) pickled, salted, dried, or smoked. Fully cooking fishes and crabs would neutralize any metacercariae present. This process is time-temperature dependent, e.g., cooking time decreases with an increase in temperature. For example, metacercariae of *O. viverrini* survives for 5 h at 50°C, but only 5 minutes at 80°C (WHO, 1995). Immersion of aquatic vegetation in boiling water for a few minutes kills metacercariae of *F. buski*.

Freezing is also very effective at neutralizing the metacercariae. The texture of the flesh may change after freezing and therefore, many people are reluctant to eat raw previously-frozen fish. Freezing is also time-temperature dependent, with colder temperatures requiring less time. Metacercariae of *O. felineus* in fish are killed after 32 h at −28°C; 14 at −35°C; and 7 at −40°C (WHO, 1995). The type of freezer, thickness of the fish, and the form of the product (e.g., whole, headed and gutted, fillets) can affect the time needed to attain the required temperature and thereby increase the time in the freezer.

Food preparation requiring pickling, salting, drying, or smoking may be insufficient to kill the metacercariae. During pickling, the acidity of the solution and the thickness of the fish may be factors in the continued viability of the larvae. Generally, the higher the acidity the more effective the control measure. However, pickling solutions are usually about 4% acid; similar to vinegar. Metacercariae of *O. viverrini* can survive only 1–1.5 h when placed free in the solution (WHO, 1995), but would require much longer if still situated within the flesh of the fish. Conditions are similar for salting in that the effectiveness of the salt is decreased (1) with a decrease in the concentration, and (2) with an increase in the thickness of the flesh. Consumption of fish salted for only 1 day resulted in an outbreak of opisthorchiasis in Europe (Cross, 2001). The smoking process may include a substantial increase in temperature. If the fish is cooked during the smoking process (also known as hot smoking), the parasites and other pathogens will be killed. However, if the temperature of the product is not raised sufficiently during smoking (e.g., cold smoking), the product is still raw and the parasites remain viable and infectious (Adams and DeVlieger, 2001). Safety of these food preparations can be enhanced considerably by freezing the fish prior to pickling, salting, drying, or smoking (Adams *et al.*, 1997). The previously-mentioned change in texture caused by freezing is usually not noticeable after further processing.

Agricultural practices, particularly those involving sanitation, have a direct impact on the completion of the life cycles of these trematodes. The use of human feces as fertilizer, also known as night soil, assists in the distribution of parasites and their infective stages. In China, latrines were often placed above ponds to enrich the system for fish-rearing. In so doing, eggs of *C. sinensis* were efficiently introduced to the necessary habitat of snails and fishes. Runoff from areas fertilized with human and animal excreta can inadvertently contaminate nearby ponds and waterways with the parasites and other pathogens. Alternatives include composting or sterilizing the excreta prior to use as fertilizer and the use of other compounds for enrichment, such as rice bran. The diversion of runoff and the use of cleaner water sources for fish-rearing ponds also reduces or prevents contamination.

Another approach is the reduction of infected intermediate and reservoir hosts. Regular antihelminthic treatment for farm and companion animals can reduce the available pool of trematode eggs in endemic areas. Restricting access for animals to streams and ponds may also prevent the consumption of metacercariae and the direct introduction of eggs from animal feces into freshwater. Livestock will readily graze on aquatic vegetation, exposing them to metacercariae of *F. hepatica*. The control of snails is difficult. The use of molluscicides is often ineffective since snails can climb up on vegetation to avoid the chemicals and the products can become diluted. Molluscicides are also harmful to fish and the water environment (Cross, 2001).

The increase in aquaculture can inadvertently lead to larger trematode populations. An increase in ponds also creates additional snail habitat. The expansion in fish-farming increases the number of potential fish hosts. In 1990, 46% of the freshwater fish produced in Thailand came from aquaculture. In 1984, approximately 49,000 tons of freshwater fish were produced by Thai aquaculture; increasing to almost 99,000 in 1991 (WHO, 1995). Expanded aquaculture may also result in the fishes being sold over a larger area or exported to nonendemic areas. The parasite may then become established in areas with suitable intermediate hosts and insufficient sanitation. Under these circumstances, the diagnosis and treatment of resulting helminth infections may be difficult because medical personnel would have little experience with these parasitic diseases.

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