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CHAPTER 123

Liver, Lung, and Intestinal Fluke Infections

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This chapter is dedicated to the memory of J. Dick MacLean, a valued colleague, an outstanding teacher, and an inspiration to a new generation of tropical medicine practitioners.

INTRODUCTION

More than 40 million people are estimated to have trematode (commonly referred to as flukes) infections. These parasites infect liver, lung, and intestine, with 21 million individuals harboring lung flukes, 20 million infected with liver flukes, and unknown millions with intestinal flukes.[1] These trematodes are all foodborne zoonoses, with reservoirs in a wide range of domestic and wild animals, transmitted to humans through freshwater fish, crustaceans, and aquatic vegetation. With a worldwide distribution, the highest prevalences are in East and Southeast Asia, determined as much by local eating habits as by the presence of the obligatory intermediate hosts. All of these flukes can produce serious clinical disease, especially with heavy infections, because of the sites of infection and longevity of the parasite.

These flukes are hermaphroditic, bilaterally symmetrical, and flattened dorsoventrally with an anterior oral and a ventral sucker. Their length can range from 1 mm to 12 cm with shapes described as spatulate, piriform, lanceolate, or leaflike (***Fig. 123.1***). Different fluke species share some common features in their life cycles. In general, adult flukes in the mammalian host produce eggs that, after passage in feces or sputum, are ingested by the appropriate first-intermediate-host snails or hatch and subsequently penetrate the snail host as ciliated miracidia. Within the snail, asexual multiplication through sporocyst, redia, and cercaria stages occurs. Free-swimming cercaria leave the snail and penetrate fish or shellfish or attach to aquatic vegetation to encyst as metacercaria. When eaten by the mammalian final host, the metacercaria excyst, migrate to liver or lungs, or stay in the small intestine and develop into adults.

LIVER FLUKES

Human liver flukes are members of two families, the Opisthorchiidae and the Fasciolidae, distinguished by differences in life cycle and pathogenesis (***Fig. 123.2***). In human Opisthorchiidae there are three major species (*Clonorchis sinensis* in East Asia, *Opisthorchis viverrini* in Southeast Asia, and *O. felineus* in some countries in Europe and the former Soviet Union) and two minor species (*O. guayaquilensis* in North and South America and *Metorchis conjunctus* in North America). In the Fasciolidae the species are *Fasciola hepatica*, which has a worldwide distribution, and *F. gigantica* in South Asia, Southeast Asia, and Africa.

OPISTHORCHIASIS AND CLONORCHIASIS

THE AGENT

The three major Opisthorchiidae species – *C. sinensis*, *O. viverrini*, and *O. felineus* – have similar life cycles and pathogenic processes. Differentiation among species is usually based on adult fluke morphology or geographic distribution, as differences in egg morphologies are small.[2,3] Adults, which live in the intrahepatic bile ducts of their host, are flat, spatulate to lanceolate, aspinous, and reddish to brown in color. *C. sinensis* is the largest (10–25 × 3–5 mm) (***Fig. 123.3A***), in contrast to the smaller *O. viverrini* (5–10 × 1–2 mm) and *O. felineus* (7–12 × 2–3 mm). The adults produce ovoid eggs that are yellowish-brown, have opercula, and are of such overlapping and variable size (*O. viverrini*, 30 × 12 μm; *O. felineus*, 30 × 12 μm; *C. sinensis* 28–35 × 12–19 μm) (Fig. 123.3B) that speciation by the egg is very difficult.

The transmission of these trematodes follows the general schema outlined above for flukes, with susceptible freshwater fish species serving as the source of infection in humans. After ingestion in uncooked fish by the final human host, excystation occurs in the duodenum, followed by rapid maturation into adults, and migration through the sphincter of Oddi and up the common bile duct to become wedged in the intrahepatic biliary radicles. The prepatent period is 3–4 weeks, and the life span in the human host can be as long as 30 years.

EPIDEMIOLOGY

C. sinensis is endemic in China, Japan, Korea, Taiwan, Vietnam, and Asian Russia. In China, infection is endemic in 24 provinces, with prevalence rates between 1% and 57%; the greatest number of cases is in the southeastern province of Guangdong and the southern region of Guangxi Zhuangzu.[4] Hong Kong is not an endemic area for the parasite; infections are acquired by eating fish imported from the mainland of China. In Korea, rates of 8–40% were reported in the past, but prevalence rates in the 1990s dropped to 1.5%.[5] People living along river basins are more commonly infected. This parasitosis is reported from all areas of Taiwan, with the highest infection rates of 52–57% from three widely separated areas in northern, central, and southern counties of the island.[5] Although clonorchiasis was found in up to 3% of the Japanese population prior to 1960, by 1991 the disease had almost disappeared. Endemic areas in Russia are in the Amur river region (***Fig. 123.4***).

Opisthorchis spp. are prevalant in Kazakhstan, Russian Federation, Siberia, Ukraine, Germany, and Italy (for *O. felineus*), and Cambodia, Laos, South Vietnam, and Thailand (for *O. viverrini*). Prevalence rates for *O. viverrini* are reported to be >24% in Thailand, and 40–80% in Laos,[1,6]

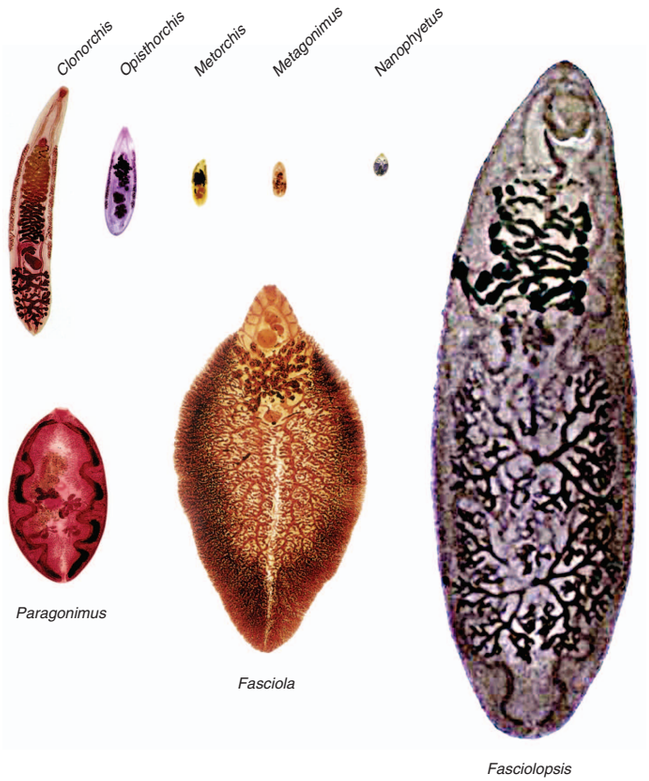

Figure 123.1 Threefold magnification of selected flukes illustrating relative sizes. Actual lengths: *Metagonimus yokogawai* 1.0–2.5 mm, *Nanophyetus salmincola* 0.8–2.5 mm, *Metorchis conjunctus* 1.5–7.0 mm, *Opisthorchis viverrini* 5–10 mm, *Paragonimus westermani* 7–16 mm, *Clonorchis sinensis* 10–25 mm, *Fasciola hepatica* 20–30 mm, *Fasciolopsis buski* 20–75 mm. (*Metagonimus yokogawai* image from Centers for Disease Control and Prevention, Division of Parasitic Diseases, Atlanta, GA; *Nanophyetus salmincola* and *Fasciolopsis buski* images courtesy of Steve J. Upton, Kansas State University; *Opisthorchis viverrini* image from Ash LR, Orihel TC. Atlas of Human Parasitology. Chicago: ASCP Press; 1990: plate 73, 2, p. 213; *Paragonimus westermani, Clonorchis sinensis,* and *Fasciola hepatica* images from Orihel TC, Ash LR. Parasites in Human Tissues. Chicago: ASCP Press; 1995, figures 72, 60, and 58, pp. 272, 268, and 264.)

and ranging from 0.3% to 37% in Vietnam.[7] The epidemiology and clinical presentation of *Opisthorchis* infections are similar to those described for clonorchiasis.[8] *O. felineus* has been reported from Italy[9] and from regions in the former USSR, with endemic foci in western Siberia, the Russian Federation, Kazakhstan, and Ukraine and prevalences in these regions ranging from 40% to 95%.[1]

Other opisthorchiids reported to cause human infections are *O. guayaquilensis* (*Amphimerus pseudofelineus*) and *Metorchis conjunctus*. These have been reported from animals and humans in Latin America and North America. An epidemic of metorchiasis occurred in 19 persons in Canada who had eaten freshly caught white suckers (*Catostomus commersoni*) near Montreal.[10]

A variety of freshwater hydrobid snails, abundant in fish-raising ponds, serve as first intermediate hosts for *C. sinensis*, *O. viverrini*, and *O. felineus*. *Bithynia fuchsiana*, *Parafossarulus manchouricus*, and *Simisulcospica libertina* are important vectors of *C. sinensis* in most endemic areas, while *B. siamensis* is a vector of *O. viverrini* in Thailand; *Melanoides tuberculatus* is an important vector in Vietnam;[7] and *Codiella inflata*, *C. troscheli*, and *C. leachi* are vectors of *O. felineus* in the former USSR.

Over 100 species of fish, many of them synonyms, and most belonging to the carp (Cyprinidae) family, are reported as second-intermediate hosts of *C. sinensis*. *Ctenopharyngodon idellus* in China, *Cyprinus carpio* in Japan, and *Pseudoraspora parva* in Korea are the predominant sources of human infections, often eaten raw. Many of the fish are cultivated in ponds inhabited by snail hosts, and contaminated or intentionally fertilized with human and animal feces. Aquaculture is now playing an important role in transmitting foodborne trematodiasis.[11,12] Twenty-two species of cyprinids are intermediate hosts for *O. felineus* in the former USSR. The fish, such as *Barbus barbus* and *Tinca tinca*, may be eaten raw, dried, salted, and sometimes frozen. In endemic areas of opisthorchiid liver fluke infections, a myriad of mammalian hosts such as dogs, cats, pigs, rats, rabbits, and other wild fish-eating animals serve as reservoir hosts.

THE DISEASE

The biologic and pathologic characteristics of *Opisthorchis* and *Clonorchis* are considered to be essentially the same.[11,13] Variations in clinical presentations seen in different geographic areas are thought to reflect the duration and intensity of infection as well as the genetics and nutrition of the host rather than parasite-specific characteristics. Acute disease has been recognized most frequently in *O. felineus* infections in Russia. The risk of cholangiocarcinoma appears greatest in *O. viverrini* infections in northern Thailand. Intrahepatic pigment stones are reported more frequently in association with *C. sinensis*. Chronic infections are usually asymptomatic, although symptoms may occur in heavier infections. The complications of chronic infection include acute cholangitis, frequently bacterial, and cholangiocarcinoma.

Acute Opisthorchiasis and Clonorchiasis

Acute illness due to new infections with *C. sinensis* has rarely been reported except for a large outbreak of acute clonorchiasis in Shanghai in the 1940s.[14,15] The illness lasted several weeks and was characterized by persistent fever, abdominal pains, fatigue, an enlarged and tender liver, high eosinophil counts, and opisthorchiid eggs in the stool after 3–4 weeks.[15] In Russia acute opisthorchiasis, presenting as fever, abdominal pain, and urticaria, has been seen frequently in migrant populations settling in regions endemic to *O. felineus*.[16,17] In Canada an outbreak of acute illness due to *M. conjunctus* presented with upper abdominal pain, moderate fever, anorexia, high eosinophil counts, and opisthorchiid eggs in the stool late in the second week of illness.[10]

Chronic Opisthorchiasis and Clonorchiasis

Light to moderate infections, lasting for years or decades, are almost always asymptomatic.[18] Case-control and community-based studies have revealed no differences in the signs, symptoms, or laboratory findings between light infections and uninfected controls, but cases with heavy infections (>10 000 eggs/gram) show significantly more abdominal pain, fatigue, dyspepsia, and hepatomegaly.[19–22] There is a correlation between stool egg counts, adult fluke counts, and host disease in *Opisthorchis* infection. But even in heavily infected persons, abdominal symptoms occur in only 10%. These studies are difficult to interpret because raw fish consumption in many communities is frequent and reinfection likely.[21–24] Chronic infection generally reflects the worm burden, and manifests variously as recurrent pyogenic liver cholangitis, cholecystitis, obstructive jaundice, hepatomegaly, cholecystitis, multiple hepatic tumors,[25] and cholelithiasis.[26,27] Many uncontrolled hospital-based studies in endemic regions demonstrate a variety of intermittent symptoms that increase in frequency in those with heavy infections.[28,29] These symptoms include intermittent fatigue, abdominal pain and fullness, anorexia, weight loss, and diarrhea. In these studies, physical signs, such as liver enlargement and tenderness, are more frequent in the heavily infected, and eosinophil counts are higher. Uncontrolled treatment trials with praziquantel have demonstrated a decrease in symptoms of upper abdominal pain, diarrhea, distention, dizziness, fatigue, and insomnia from 72% to 45%.[30]

Ultrasonographic studies have revealed a high frequency of gallbladder enlargement, sludge, dysfunction, and stones in asymptomatic moderately to heavily infected patients. Treatment appears to reverse these parasite-associated gallbladder abnormalities.[31–33]

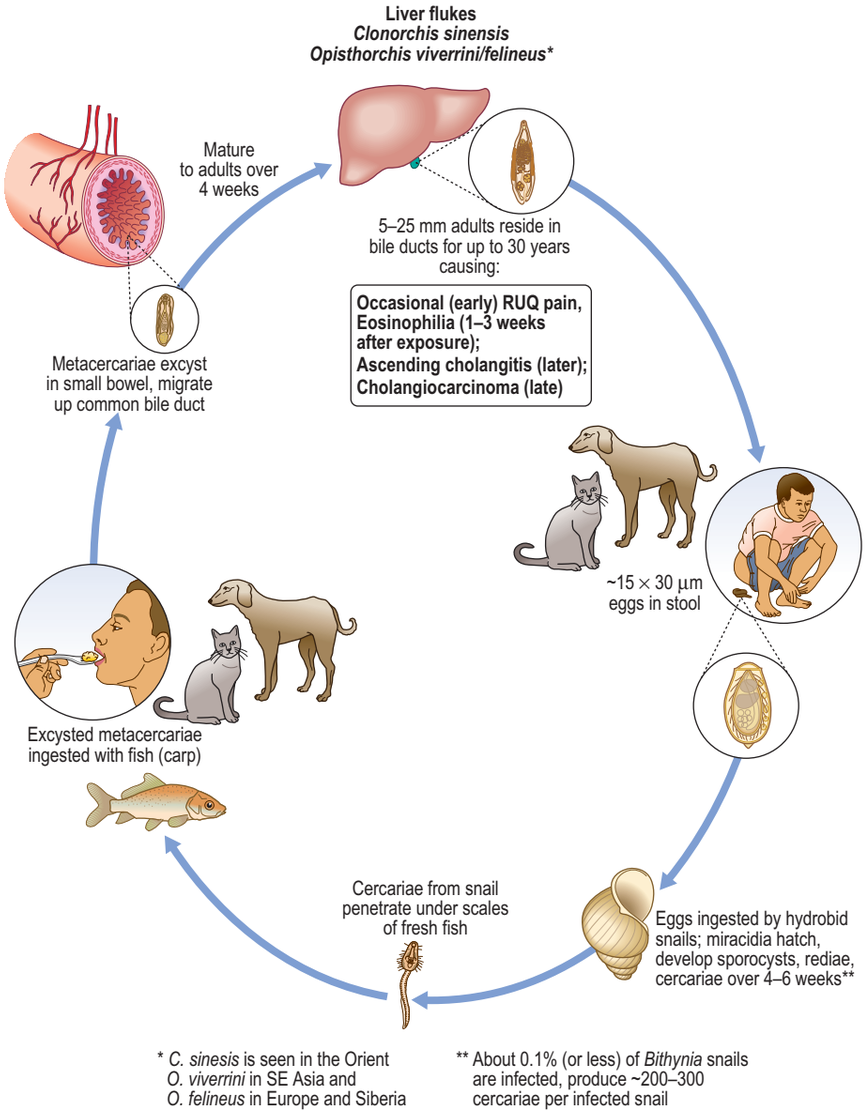

Figure 123.2 Life cycle of *Clonorchis sinensis* and *Opisthorchis viverrini/felineus*. RUQ, right upper quadrant.

Pathologic changes observed on necropsy and biopsy relate to intensity and duration of infection. Early infections reveal bile duct proliferation and pseudostratification of the biliary epithelium. Later, metaplastic squamous cells and glandular proliferation appear, suggesting adenomatous hyperplasia.[34] A small percentage of patients with chronic infection will develop complications, which include recurrent ascending cholangitis, pancreatitis, and cholangiocarcinoma.

Recurrent Ascending Cholangitis and Pancreatitis

Recurrent ascending cholangitis is characterized by repeated episodes of fever, chills, jaundice, right upper-quadrant pain, Gram-negative sepsis, and leukocytosis. Soft, muddy pigment stones are found in the biliary radicles and common bile duct and are associated with dilated intrahepatic bile ducts, ectasia, strictures, and multiple pyogenic abscesses, most notably of the left lobe of the liver.[35] Recurrent exacerbations and remissions can occur over years.[36,37] Pancreatitis at times is found on endoscopic retrograde cholangiopancreatography (ERCP), or at the time of surgery or autopsy, but it is rarely symptomatic or found in isolation without liver involvement.[38,39]

Cholangiocarcinoma

Worldwide, cholangicarcinoma is much rarer than hepatocellular carcinoma, except for those areas in East Asia where infection with *O. viverrini* or *C. sinensis* is widespread and highly prevalent in humans.[40] An increased frequency of cholangiocarcinoma of the liver is seen in northern Thailand, where case-control studies reveal a fivefold increased risk in those infected.[41] The risk increases to 15-fold in persons with heavier infections. In one endemic province of Thailand, the rate of cholangiocarcinoma in males and females was 10- and six-fold higher, respectively, than in a nonendemic area.[41,42] In animal studies, nitrosamines increase the incidence of cholangiocarcinoma in *Opisthorchis*-infected animals.[43–45] High levels of such substances have been noted in the northern Thai diet.[46] An interesting recent discovery has been of a human granulin homolog in *O. viverrini*, termed Ov-GRN-1, that is secreted by the parasite and is able to promote fibroblast proliferation through a mitogen-activated protein kinase-dependent pathway.[47] In recognizing the role of trematode parasites in the induction of cholangiocarcinoma, the International Association for Research on Cancers has recently listed *O. viverrini* as a group 1 agent, a classification that assigns proven risk to an agent.[48] The mechanism of carcinogenesis is thought to be due to the presence of chronic

inflammation at the site of infection, which results in the generation of free radicals and nitrogen species that damage DNA, initiate DNA mutations, and lead to genetic instabilities and malignant transformation.[49]

PATHOGENESIS AND IMMUNITY

The pathologic changes seen in the liver and biliary system in clonorchiasis and opisthorchiasis have been attributed to mechanical injury by the suckers of the flukes and host interactions with their secreted metabolic products,[50–52] such as excess proline production by adult flukes.[53] Eggs probably serve as nidi for biliary stones in the bile ducts and gallbladder.[34,54] Immunohistochemical studies indicate that the excretory-secretory proteins from the digestive and excretory organs (i.e., the intestines and bladder) are the most potent antigens and likely induce the dominant immunologic response.[4] Periductal infiltration with eosinophils and round cells with fibrosis of portal areas – a common finding – implicate immune-mediated tissue damage in the pathogenesis of disease.[4] The local reactions to eggs and migrating parasites are driven by T-lymphocyte effector mechanisms and are regulated by the $CD4^+$ subset of T lymphocytes in infections with related trematodes.[55] The presence of apparently uninfected persons in endemic regions with significantly higher levels of parasite-specific IgM, IgG, and IgA than egg-excreting persons has been used as evidence of protective immunity.[52,56,57]

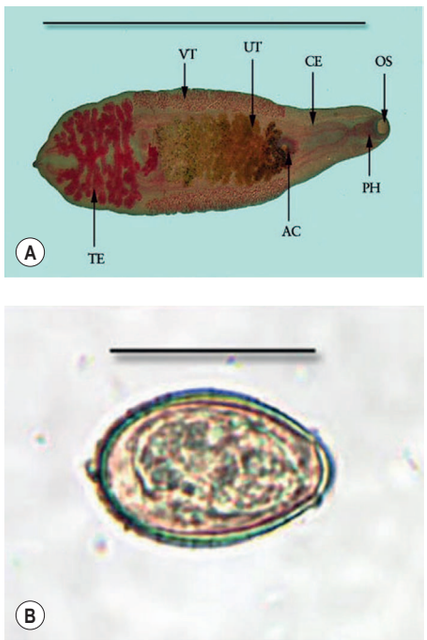

Figure 123.3 *Clonorchis sinensis*. **(A)** Adult of *C. sinensis* stained with carmine. Features of the adult trematode image identified in the photomicrograph include the oral sucker (OS), pharynx (PH), ceca (CE), acetabulum, or ventral sucker (AC), uterus (UT), vitellaria (VT), and testes (TE). The bar represents 1 cm. **(B)** Egg (size 29 × 16 μm). The bar represents 15 μm. (Courtesy of DPDx, Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA).

DIAGNOSIS

Asymptomatic infections with Opisthorchiidae are diagnosed by the presence of characteristic findings on ultrasound, computed tomography (CT), or magnetic resonance imaging (MRI), or by the detection of eggs in stool. In contrast, acute infection typically presents with a history of raw freshwater fish consumption (salted, fermented, or smoked fish, fish sauces, fish condiments), followed within several weeks by upper abdominal pain, high-grade eosinophilia, liver enzyme elevation, and the appearance of compatible eggs in the stool. The combination of cystic or mulberry-like dilations of intrahepatic bile ducts on ultrasound is pathognomonic of opisthorchiasis. With M-mode ultrasound, numerous spotty echoes and thin linear and moving intraductal echoes may be seen. Examination of multiple stool specimens may be necessary in lighter infections, but in infections of <20 adult flukes, no eggs may be found.[58] While egg counts in stools are relatively stable over time and such egg counts have prognostic significance, paradoxically, low egg counts may be seen in the heaviest infections because of blockage of biliary radicles or because pyogenic ascending cholangitis has killed the adults.[59–61] The eggs of *Clonorchis*, *Opisthorchis*, and *Metorchis* are essentially indistinguishable from one another by routine microscopy and can be confused with other fluke eggs as well. A definitive diagnosis may be made by examining the adult flukes in the stool immediately after a praziquantel treatment and purge or at the time of surgery. Recent advances in molecular

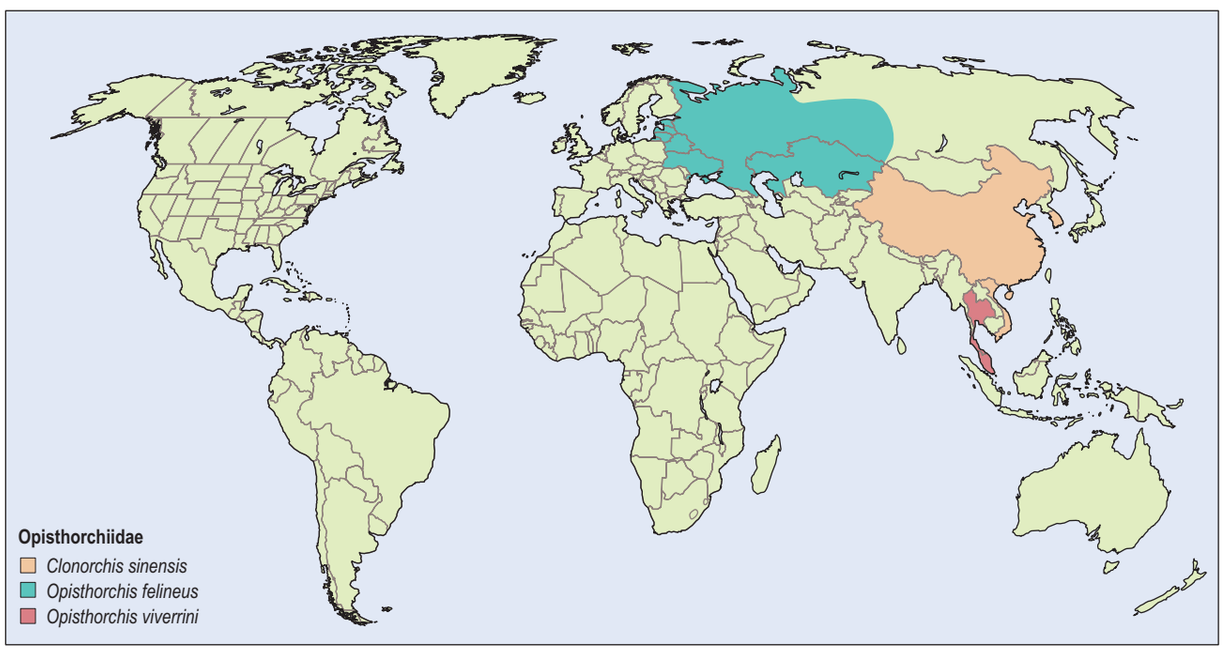

Figure 123.4 Geographic distribution of Opisthorchiidae.

techniques applied to stool samples have allowed considerable improvement in the detection of *Opisthorchis* (and *Clonorchis*) in stool.[62–64] These techniques are at least as sensitive as microscopic examination[63] and have the added advantage of high throughput. While these tests are useful as research tools, they are not yet available for routine use and further work will be required to adapt the techniques to laboratories in endemic regions.

Immunodiagnosis

Immunologic tests generally complement parasitologic testing and until recently have not had a primary role in the diagnosis of opisthorchiasis and clonorchiasis because they do not distinguish between active and old infections.[65] The preferred assay for immunodiagnosis in recent surveys has been enzyme-linked immunosorbent assay (ELISA). When compared with egg-positive stools, sensitivity can be high (79–96%).[4,66] However, use of crude worm extracts in ELISA is associated with significant lack of specificity; antibody positivity is seen in cases of paragonimiasis (33%), schistosomiasis japonica (5–25%), cysticercosis, hepatitis, liver cancer, and tuberculosis. Specificity can be enhanced somewhat by using immune affinity-purified antigens. Monoclonal antibodies have boosted the specificity in an ELISA inhibition test, which has proved to be as sensitive (77%) and more specific (virtually no cross-reactivity with other trematode infections) than the ELISA using crude worm extracts.[4] The use of excretory-secretory antigens as plate antigens has been reported to achieve sensitivities and specificities of >95% in smaller serologic surveys, but their utility in large-scale surveillance is yet to be proven. After treatment, antibody levels return to normal by 6 months in more than half of cases.[67,68] Circulating antigen detection with a monoclonal antibody-based capture ELISA has been reported to detect as little as 30 ng/mL of *C. sinensis* antigen in serum.[69,70] Antigen positivity is seen in 95% of antibody-positive infected patients. This test was reported to be positive in 95% of seropositive infected patients, declining to undetectable levels after 3 months in 81% of those parasitologically cured.[69,70] Stool antigen detection techniques show similar promise.[60]

A metabolic profiling strategy has been utilized successfully for biomarker discovery of the two trematodes, *Schistosoma mansoni* and *S. japonicum,*[71,72] and this has raised hopes for its application in the diagnosis of other trematodes, including *Opisthorchis* and *Clonorchis*. This approach uses a combination of analytical tools, including high-resolution nuclear magnetic resonance spectroscopy and mass spectrometry, with multivariate statistical analysis to measure quantitatively biochemical responses of organisms to physiological or pathological stimuli.[73]

TREATMENT

Praziquantel has been the drug of choice for opisthorchiasis and clonorchiasis since the 1970s because of ease of administration, lack of side effects, and demonstrated effectiveness. Its mechanism of action is thought to be through disruption of calcium homeostasis in parasite cells.[11,12] The recommended dosage of 25 mg/kg three times daily for 2 days has produced cure rates up to 100%, but patients with heavy infections (>5000 eggs/gram of stool) and in some geographic regions where praziquantel cure rates are low (Vietnam) may require retreatment.[1,4,74,75]

Albendazole has produced cure rates of 93–100% at a dosage of 10 mg/kg daily for 7 days.[4,76] Although some studies have suggested that it may not be as effective as praziquantel, it has fewer side effects.

Preliminary studies using rodent models have demonstrated that the artemisinins (e.g., artemether and artesunate) and synthetic peroxides (e.g., synthetic trioxolanes) known for their antimalarial properties,[77] and the Chinese anthelminthic drug tribendimidine[78] show promise for use against foodborne trematodiases.[12] With all treatments, success of therapy is defined as the disappearance of fluke-induced symptoms and fecal egg output, reduction in liver size, and a reversal of biliary tract abnormalities.[31] Recurrent pyogenic cholangitis is primarily a surgical problem, requiring relief of intrahepatic obstructions due to strictures, stones, and sludge, and drainage of the associated abscesses. Antibiotics may be necessary to treat the associated sepsis.

FASCIOLIASIS

Two flukes of the family Fasciolidae infect humans: *Fasciola hepatica*, the most common and widely distributed, and *F. gigantica*, a fluke of much more focal distribution. Both have similar life cycles and produce similar human disease, but *F. gigantica* can be recognized by its larger adult and egg sizes. *F. hepatica* was the first intestinal fluke to be described,[79] causing a significant burden of illness in domestic sheep since antiquity. The life cycle was described in 1883.[79]

THE AGENT

The adult *F. hepatica* is a large fluke (30 × 15 mm), flat and leaflike along the margins, with a cephalic cone (***Fig. 123.5A***). As for other flukes, size, shape, and integumental and internal morphology are species-defining features. The adult fluke lives in the common and hepatic bile ducts of the human or animal host, and eggs reach the exterior via the sphincter of Oddi and the intestine. The eggs are large (130–150 × 60–90 μm), ovoid, and inconspicuously operculate (Fig. 123.5B). In water, miracidia hatch from the eggs and penetrate suitable snail hosts where, after multiplying as sporocysts and redia, they leave the snail as free-living cercaria (***Fig. 123.6***). These attach to suitable plants, evolve into metacercarial cysts, and, when ingested by the human final host, excyst in the duodenum. The larvae migrate through the small intestinal wall and through the peritoneal cavity where they penetrate the liver capsule and slowly migrate to the large hepatic ducts. This prepatent period lasts 3–4 months. Anecdotal reports suggest that the life span in the human host can be up to 10 years.

Figure 123.5 *Fasciola hepatica*. **(A)** Adult (size 30 × 15 mm). The bar represents 3 cm. **(B)** Egg (size 130–150 × 60–90 μm). The bar represents 100 μm. (Courtesy of DPDx, Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA.)

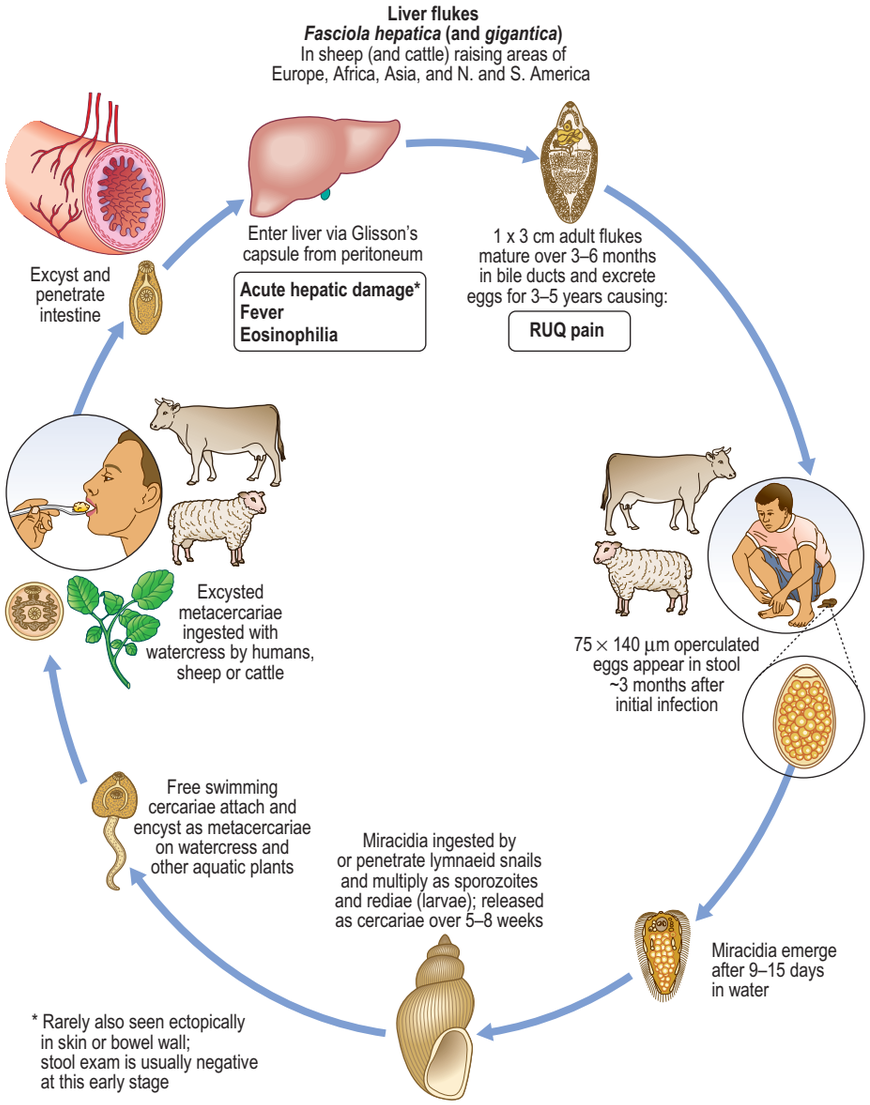

Figure 123.6 Life cycle of *Fasciola hepatica* (and *F. gigantica*) in sheep (and cattle)-raising areas of Europe, Africa, Asia, and North and South America. It is rarely also seen ectopically in skin or bowel wall; stool exam is usually negative at this early stage. RUQ, right upper quadrant.

EPIDEMIOLOGY

F. hepatica is believed to be of European origin, and remains highly prevalent throughout Europe, but its ability to infect many different species and of the intermediate snail host to adapt to a wide range of ecological niches has allowed the parasite to achieve a cosmopolitan distribution.[80] It is now also found in the southern states of the United States, Mexico, North Africa, New Zealand, and small regions within Australia (***Fig. 123.7***). The parasite primarily infects cattle and sheep, although other domesticated animals such as goats, horses, and pigs can be infected. The major natural reservoirs for *F. hepatica* are cattle, sheep, goats, buffalo, camels, llamas, deer, pigs, horses, rabbits, and other wild animals, with prevalence rates of 25–92% in Bolivia, 20–40% in Ecuador, 10–100% in Peru, and 20–40% in Iran. In humans, stool- or antibody-positive prevalence rates in these countries can be similarly high (65–92% in Bolivia, 24–53% in Ecuador, 2–17% in Egypt, and 10% in Peru).[1,12] Since the introduction of *F. hepatica* to South America by Europeans in the eighteenth century it has become a major problem in Brazil, Argentina, Uruguay and in several Andean countries such as Bolivia, Peru, and Ecuador.[80–82] Fasciolosis is now an important veterinary disease and the cause of significant economic loss to the global agricultural community, estimated at >$2000 million annually, as >600 million animals are infected.[83,84] In terms of human disease burden, more than 2 million people are infected, mostly in Bolivia, Peru, Iran, Egypt, Portugal, and France. A variety of freshwater plants upon which metacercariae encyst, such as watercress, water lettuce, mint, and parsley, are important sources of human infection because they are often eaten raw in salads.[82] Over 25 species of amphibious lymnaeid snails that live in wet mud along the shoreline, and rarely in fast-moving or deep waters, serve as the first intermediate host for *F. hepatica*, the most important being *Lymnaea truncatula*. Climate change and manmade modifications of the landscape may influence the spread of fascioliasis.[85]

THE DISEASE

Acute Hepatic (Invasive) Stage

The clinical presentation of infection with *F. hepatica* reflects its peregrinations in the human host. Hepatic transit, variably called the hepatic, larval, invasive, or acute stage, lasts several months. This is followed by

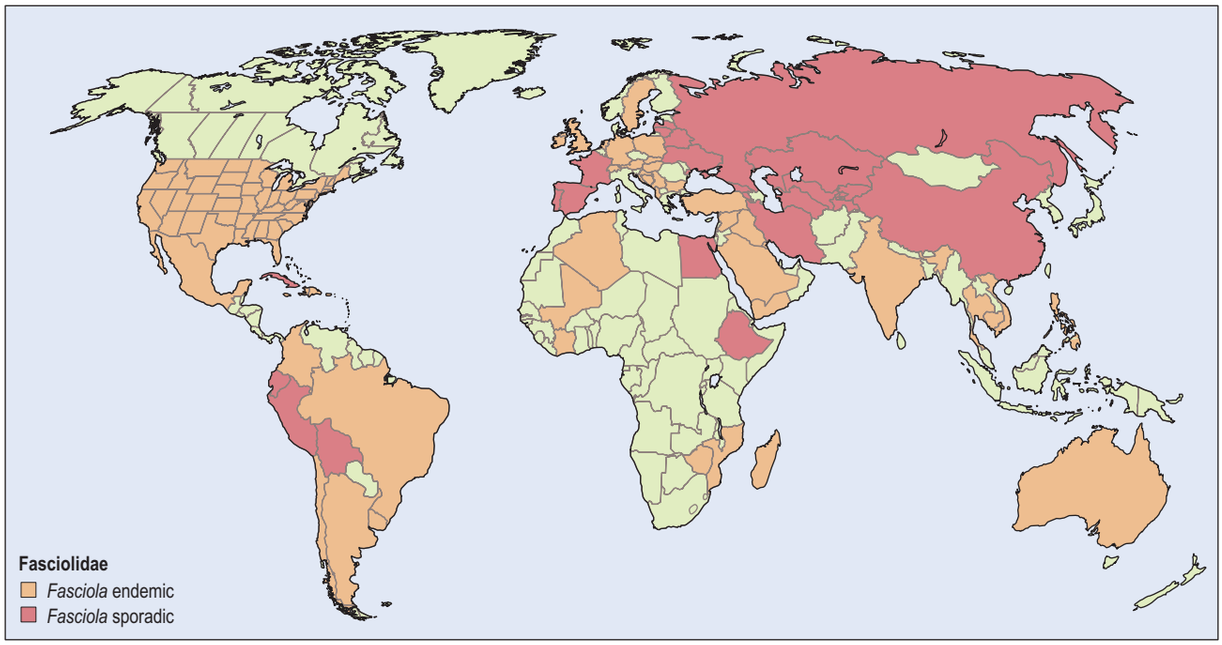

Figure 123.7 Geographic distribution of fascioliasis.

the biliary, adult, or chronic stage, which can persist for years. Where repeated ingestion of metacercaria occurs over an extended period, these two stages can overlap. Within 6–12 weeks of ingestion of metacercariae, symptoms occur that reflect larval migration through the small intestinal wall, the peritoneal cavity, and liver capsule.[86] This acute stage can last for 2–4 months. Typical findings include marked eosinophilia (95%), abdominal pain (65%), intermittent fever (60%), malaise and weight loss (35%), urticaria (20%), and cough, dyspnea, and chest pain (15%). A change in bowel habits, anorexia, and nausea may occur.[87,88] The abdominal pain may be generalized but frequently becomes localized to the right hypochondrium.[12,89] Hepatomegaly is a variable finding, and the liver may be tender on palpation. In some cases, mild elevations of hepatic enzymes are noted. The pulmonary symptoms may be associated with right-sided pleural effusions, which, on aspiration, reveal increased eosinophils.[90] Anemia has been reported.[91,92]

Ultrasound examination of the liver in the acute stage is usually normal, although small amounts of ascites have been found.[90] CT scans frequently reveal single or, more frequently, multiple small hypodense lesions 2–10 mm in diameter.[87] In addition, tunnel-like, branching, hypodense lesions (best delineated with contrast), most frequently situated peripherally within the liver, are relatively specific for fascioliasis, representing the pathologic changes created by the migration of the immature fluke through the liver.[26,93] The hepatic lesions are remarkable in that, on sequential CT scans, the position, attenuation, and shape of the lesions change over time.[94] On laparoscopy, multiple gray-white and yellow nodules 2–20 mm in diameter and short vermiform cords are noted on the liver surface and at times on the adjacent peritoneal surface.[95] Liver biopsies reveal microabscesses and tunnel-like areas of parenchymal necrosis surrounded by inflammatory infiltrates containing abundant eosinophils.[93,96] Necropsies reveal multiple subscapular cavities 5–10 mm in diameter filled with necrotic material from which necrotic tracts radiate. Increasing fibrosis is seen in older lesions.[96,97]

Rarely, immature flukes may migrate to nonhepatobiliary locations such as the skin, lung, intestinal wall, brain, and genitourinary tract, where granulomatous nodules or small abscesses lead to local clinical findings. A variant form of cutaneous larva migrans with migrating erythematous 1.5–6.0 cm cutaneous nodules has been reported.[67,87,97,98]

Chronic Biliary (Obstructive) Stage

F. hepatica has a propensity to migrate to the lumen of the common bile duct, where it reaches maturity. Eggs appear in the stool after a prepatent period of 3–4 months. Clinical findings reflect this new luminal location in that the liver-destructive phase of the infection ends. Fever, anorexia, and abdominal pain resolve, and the patient may become asymptomatic. Eosinophilia is infrequent. An unknown percentage of these cases develop intermittent biliary obstruction presenting with intermittent pain in the epigastrium or right hypochondrium, mimicking biliary colic or acute cholecystitis. At times the presentation is that of ascending cholangitis with fever, jaundice, and upper abdominal pain.[26,27] Ultrasound examination (more sensitive than CT examination) often reveals a soft intraluminal mass obstructing the extrahepatic biliary tree. Lithiasis of the common bile duct and gallbladder is a common sequela.

PATHOGENESIS AND IMMUNITY

Morbidity from *F. hepatica* is dependent on the number of worms and stage of infection.[99] The characteristic hepatic (and extrahepatic) changes of fascioliasis result largely from the anatomic location and large size of the parasite, a foreign body that induces eosinophilic and mononuclear infiltration around the eggs and adult worms.[50] As in other tissue-invasive helminthic infections, fascioliasis is associated with prominent eosinophilia, particularly in the early stages of infection,[87,99] and immune responses to *F. hepatica* appear to be regulated by Th2 subpopulations of T-helper cells, that are characterized by secretion of interleukin (IL)-4, IL-5, and IL-10.[100,101] This subset of T-helper cells also appears to regulate granuloma formation and liver disease in schistosomiasis.[102] IL-10 plays a dominant role in the downregulation of inflammatory responses to *F. hepatica* infection.[103] The role of T cells and other nonantibody-mediated effector systems in killing of the parasite and in the development of pathologic changes in humans is not well understood.[104,105] The role of eosinophils in parasite killing is also unclear, although it has been noted that the invasive phase in the liver is associated with peripheral eosinophilia and eosinophilic infiltrates around the sites of parasites and eggs.[67,96]

Immune evasion mechanisms are likely to play an important role in the survival of this long-lived parasite, and several evasion strategies have

been proposed based on observations in animal models.[106,107] The surface glycocalyx may mediate immune evasion in several ways. First, the glycocalyx changes in composition during development of the parasite. Second, the glycocalyx is continuously sloughed off by the maturing juvenile worm, by one estimate every 3 hours, thus presenting a moving target.[108] Third, glycocalyx released from the surface can mop up circulating antibodies, interfering with antibody-mediated immune effector functions, such as antibody-dependent cellular toxicity.[109] Other evasion strategies include migration away from inflammatory cells, inhibition of oxygen radical generation by macrophages, and inhibition of T-cell function.[110] Natural resistance to fatal infection with *F. hepatica* has been observed in sheep and several strains of mice. Relative resistance to infection in mice correlates with type 1 T-cell responses (interferon-γ), whereas type 2 responses are associated with susceptibility.[106] Protection from challenge infection in mice and rats can be transferred by passive transfer of serum, but this protective effect is limited to sera collected 7–8 weeks post donor infection; after 25 weeks, serum from infected rats gave no protection, attributed to a decline in titers that accompanies the entry of the parasite into bile ducts.[108] Recombinant parasite-derived molecules, particularly peptidases of the cathepsin family (type L1 and B), have been demonstrated to induce protective immunity against challenge with infective stages of the parasite in animals.[83,111,112] Several potential vaccine antigens have been identified from animal models of *F. hepatica* infection. These include fatty acid-binding proteins, glutathione-*S*-transferase, cathepsin-L, and fluke hemoglobin.[8,110,113] Two of these molecules, glutathione-*S*-transferase and a 14.7-kDa polypeptide (Fh15) that has significant homology to, and cross-reacts with, *Schistosoma mansoni* fatty acid-binding protein, appear to confer partial resistance to infection in experimental infections.[105,114,115] Vaccination studies, using cocktails of recombinant antigens in animal models of fascioliasis, have shown that significant reductions in worm burdens (31–72%) and egg production (69–98%) can be achieved.[105,116] However, the success of vaccination and advances in our knowledge of immunological responses to *F. hepatica* detailed above have been largely limited to veterinary infections; the immunology of human disease requires further elucidation.

DIAGNOSIS

F. hepatica eggs are not found in stool specimens during the acute phases of infection, so the diagnosis must be based on the clinical findings of persistent pain and tenderness in the right hypochondrium or epigastrium, altered intestinal function, mild to moderate fever, and high levels of eosinophilia, often in the thousands/μL.[94] CT scans (ultrasound is less sensitive) contribute to the diagnosis, since the majority of symptomatic patients have visible hypodense lesions and tracts in the liver that migrate and change contour over time. The differential diagnosis of this clinical and radiologic syndrome includes visceral larva migrans caused by *Toxocara canis*, in which pulmonary symptoms also occur (see Chapter 109). Needle biopsies of the liver have not been helpful in diagnosis, but laparoscopy may reveal elongated nodules in the liver capsule.

In the acute invasive period, lasting 3–4 months, immunodiagnostic techniques are valuable. Tests that have been employed with varying success include complement fixation, immunofluorescence assays, indirect hemagglutination, countercurrent electrophoresis, and ELISA.[4,67,117–122] ELISAs have largely replaced other techniques because they are sensitive, rapid, and quantitative.[120,121,123] The preferred ELISAs employ excretory-secretory products of the adult worm as an antigen.[124,125] Antibodies to excretory-secretory antigens are elevated early in infection (based on studies in animal models) and remain elevated for years after infection, and successful treatment correlates with a decline in ELISA titers.[126,127] More recently, the Falcon assay screening test-ELISA (FAST-ELISA), a simple and rapid assay based on the ELISA and enzyme-linked immunoblot transfer assay, has been used for serodiagnosis, achieving sensitivities of 95–100% compared with parasitologic diagnosis. However, the specificity of this test is not known and may limit its utility.[87]

An ELISA antigen capture technique to detect circulating antigens has demonstrated a sensitivity of 100% and specificity of 98%.[128] Antigen detection techniques can detect parasite antigens in stool specimens 3–4 weeks before the appearance of eggs.[92] Immunodiagnostic tests continue to evolve, and the use of genus-specific antigens is likely to improve diagnostic accuracy.[87,127,129] Other attempts to improve the specificity of immunodiagnosis have used IgG subtype antibody levels instead of total IgG. Subtype analysis of antibody responses to excretory-secretory antigens such as the cathepsin protease (cathepsin L1) demonstrated that the predominant subtypes induced in human infections are IgG_1 and IgG_4, consistent with a predominant type 2 T-cell response.[26,106,127,129–131] The detection of subtype-specific antibodies in ELISAs may improve the specificity of the diagnostic immunoassays and make it possible to distinguish recent from remote infections.[132] A large study using a FAST-ELISA (a cathepsin (L1)-based antibody detection assay) in 634 children from an endemic region in Peru yielded a sensitivity of 92%, specificity of 84%, and a negative predictive value of 97%.[133]

In chronic biliary fascioliasis, the diagnosis is made on finding *F. hepatica* eggs in stool specimens or at the time of surgery for bile duct obstruction when eggs or adult flukes are removed from the biliary tree. Because egg production tends to be low, it is advisable to examine multiple stool specimens using the AMS iii (Tween 80) method or the Weller-Dammin modification method (since the formalin–ethyl acetate concentration technique appears to be less sensitive).[67,134] Eggs of *F. hepatica* can be confused with those of the intestinal flukes *Fasciolopsis buski* and echinostomes. Recovery of adults after anthelminthic treatment often allows species identification. False-positive "spurious" stool results can occur after consumption of liver of infected animals and can be ruled out by repeated stool examinations.

TREATMENT AND VACCINATION

In the past bithionol was considered the drug of choice.[135–137] Although side effects were mild, constituting anorexia, nausea, vomiting, abdominal pain, and pruritus, more than one course was often necessary. Triclabendazole, a benzimidazole, is now the drug of choice as a single 10 mg/kg oral dose or two doses 12 hours apart. Bioavailability is increased when triclabendazole is taken with food.[138,139] Efficacy has been as high as 92% in humans, but significant resistance has been seen both in animal and *in vitro* studies and repeat treatment may be necessary.[140–142] The most frequent side effect was colicky abdominal pain between days 3 and 7 post-treatment, compatible with fluke expulsion through the bile ducts. Unlike other trematodes, *F. hepatica* is frequently resistant to praziquantel, although some studies have shown effectiveness.[26,87,94,99,143–145] Animal studies show a lack of effectiveness of praziquantel against both immature and adult flukes in cattle and sheep.

Acute ascending cholangitis must be treated with antibiotics and surgery. A patient with a severe acute hepatic stage may benefit from the short-term use of systemic steroids. Other drugs used in the past include emitine, dehydroemetine, chloroquine, albendazole, and mebendazole, but all have been dropped because of toxicity or lack of effectiveness. Surgical approaches, such as ERCP, have been successfully used to relieve obstruction of the biliary tract.[146,147]

Fasciola is one of the few trematodes for which vaccines have been developed and used to protect against veterinary disease. The *F. hepatica* cathepsin-L protein, an important virulence determinant that was identified as a dominant antigen in excreted-secreted proteins, is a first-generation vaccine. There have been a number of trials using this molecule in cattle and sheep, with protection against challenge infection ranging from 38% to 79%.[107,112,148,149] In natural and experimental infections, a polarized Th2 response induces the generation of IgG_1 but little or no IgG_2 antibody subtypes, whereas vaccination induces antibody responses to cathepsin-L, the immunogen, that include high titers of both IgG_1 and IgG_2, indicating a mixed Th1/Th2 response.[106,107] These observations have been interpreted to indicate that protection is associated with a Th1 or a mixed Th1/Th2 response.[83,84,106] However, some vaccine trials with the same antigen have demonstrated little or no protection, suggesting that other factors, such as adjuvant and antigen formulation, may be

important in generating protective immune responses.[83] No vaccines have yet been developed for human infections.

LUNG FLUKES

INTRODUCTION

Lung flukes are members of the genus *Paragonimus* and, while more than 40 species have been described, only eight are presently considered of human importance. Most of the 40 species are parasites of animals, of which 28 are considered distinct species (the remaining may be synonymous species), with 21 from Asia, two from Africa, and five from the Americas; most are in tropical areas.[150] *P. westermani* is the best-known species and is found in humans and animals throughout the East, from India to Japan and the Philippines. *P. heterotremus* is reported from China and Southeast Asia, *P. skrjabini* and *P. hueitungensis* from China, *P. miyazakii* from Japan, *P. uterobilateralis* and *P. africanis* from central and western Africa, *P. mexicanus* from Central and South America, and *P. kellicotti* from North America.[1,12,151]

THE AGENT

P. westermani was first found in a Bengal tiger that died in an Amsterdam zoo and was named after the zoo director, G.F. Westerman. The first human infection was found in a Portuguese sailor who died in Taiwan in 1879. He had earlier been a patient of Patrick Manson's in Amoy, China, and Manson later concluded that the hemoptysis seen in this man and his Chinese patients was due to this parasite.[152]

Adult *P. westermani* is reddish-brown in color, coffee bean-shaped, 7–16 mm in length, 4–8 mm in width, and 5 mm thick. The integument is spiny, and the anterior and ventral suckers are of equal size (***Fig. 123.8A***).

The eggs are yellow-brown in color, thick-shelled with a large operculum, and measure 80–120 × 50–65 µm (Fig. 123.8B). The eggs embryonate in water, and the miracidia hatch in 3 weeks and search for specific snail hosts. Development in snails yields free-swimming cercariae, which penetrate a crab or crayfish as second intermediate host and encyst as metacercariae. When these are eaten raw, partially cooked, pickled, or salted, the metacercariae excyst and penetrate the intestinal wall of the definitive hosts and enter the peritoneal cavity. The larval worms remain here for several days, then cross the diaphragm, and enter the pleural cavity and eventually the lung parenchyma to mature to adults in ~2 months. A fibrotic cyst wall develops around paired (or tripled) adults, but eggs that are produced escape through cyst-bronchial fistulas and are coughed up in sputum or swallowed and passed in the feces.

Other species of *Paragonimus* have life cycles similar to *P. westermani* but develop in different snail and crustacean intermediate hosts. Species differentiation is based on adult fluke rather than egg morphology.

EPIDEMIOLOGY

Paragonimus transmission occurs worldwide (***Fig. 123.9***), most notably in China (*P. westermani*, *P. skrjabini*, *P. heterotremus*, and *P. hueitungensis*), Korea (*P. westermani*), Japan (*P. westermani*, *P. miyazakii*), Vietnam (*P. heterotremus*), Cameroon (*P. africanus* and *P. uterobilateralis*), Ecuador (*P. mexicanus*), and Peru (*P. mexicanus*).[1,85]

In China, human disease caused by *P. westermani*, *P. skrjabini*, and *P. heterotremus* has been reported from 21 provinces with prevalences of up to 10.4% in some areas. Based upon a national survey the total prevalence was about 1.7% in 2005.[153] In Korea, a national skin test survey revealed an overall prevalence of 13% in 1959; however, recent estimates are that no more than 1000 people are infected.[154] Control measures, disruption of the ecosystem, and pollution have reduced crab and crayfish populations, and only 16 of 16 million stools were egg-positive in 1990.[1] Taiwan had several endemic foci in the past, but today human infections

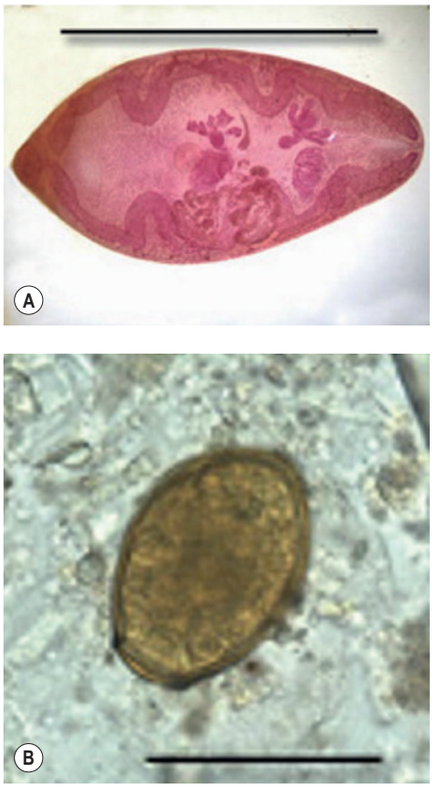

Figure 123.8 *Paragonimus westermani*. **(A)** Adult (size 7–16 × 4–8 mm). The bar represents 1 cm. **(B)** Egg (size 80–120 × 50–60 µm), unstained wet mount. The bar represents 100 µm. (Courtesy of DPDx, Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA.)

are rare owing to changes in eating habits and the effect of water pollution and industrialization on the intermediate hosts.[155] Fewer than 300 human cases of paragonimiasis have been reported from a few areas of the Philippines, although infected crustaceans are easily found in endemic areas.[153,154] Despite the reductions in Southeast and East Asia, it has been estimated that there are 20.5 million cases worldwide.[1,11,12]

More than 15 species of snails in the families Hydrobiidae, Thiaridae, and Pleurocercidae serve as the first intermediate hosts of *P. westermani*. The important second intermediate hosts are crabs in the genera *Eriocheir*, *Potamon*, and *Sundathelphusa*, and crayfish of the genus *Cambaroides*. Individuals become infected by eating these crustaceans raw or insufficiently cooked. The range of culinary artistry is wonderful. In China there is wine-soaked freshwater crab, crayfish curd, raw crab juice, and crab jam; in Thailand, raw freshwater shrimp salad or crab sauce; in Korea, raw crab in soy sauce; in the Philippines, roasted or raw crabs and crab juice seasoning. Crabs and crab juice have been used for medicinal purposes.[150,156]

Paragonimus species can cause abortive infections in many mammalian species, but when humans consume these paratenic hosts, the larvae survive stomach acid and penetrate the small-intestine wall, completing their life cycle in the human host (***Fig. 123.10***). Paratenic wild boars have served as a source of infection when eaten raw.[156]

THE DISEASE

The spectrum of disease caused by *Paragonimus* is species-dependent (determined by host–fluke compatibility), with *P. westermani* representing one clinical pole, with, most commonly, pleuropulmonary disease and relatively infrequent extrapulmonary disease. *P. heterotremus*, *P. africanus*, and *P. uterobilateralis* appear to be similar in presentation to *P.*

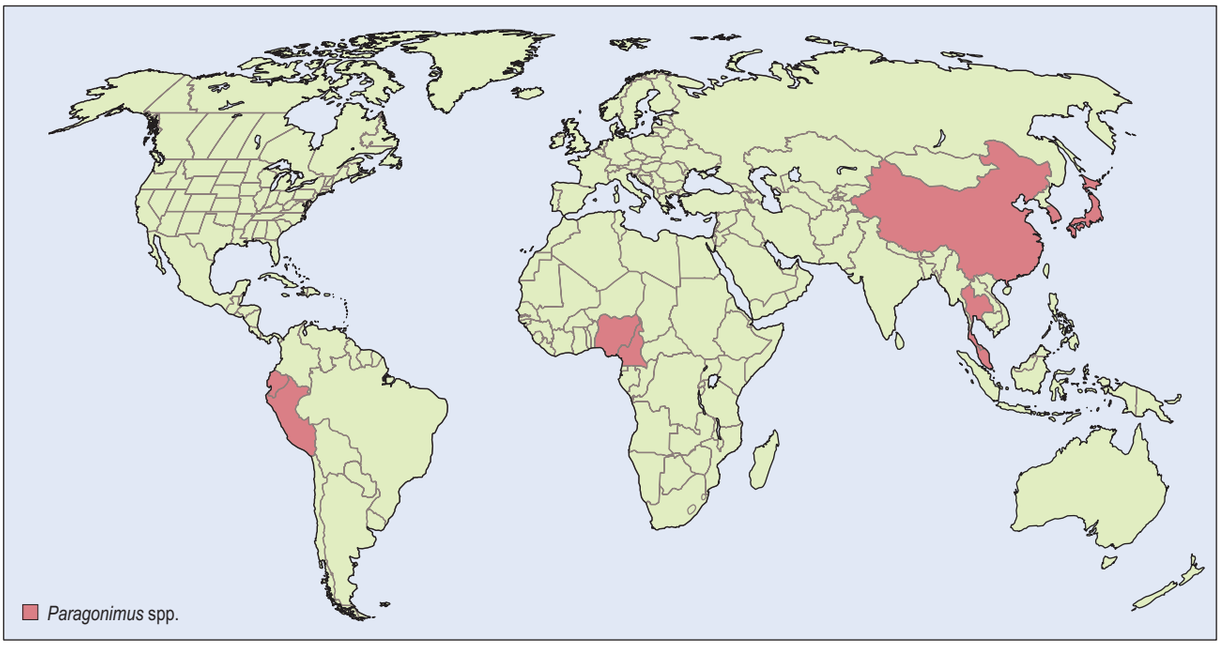

Figure 123.9 Geographic distribution of *Paragonimus* spp.

westermani.[157–160] The other clinical pole, represented by *P. skrjabini*, is mainly extrapulmonary, with cutaneous lesions the most frequent clinical presentation. Pulmonary disease appears to be caused by adult flukes and cutaneous disease by immature flukes.[60,161]

Acute Paragonimiasis

After an incubation period of 2–15 days, the initial symptoms are diarrhea and abdominal pain, followed several days later by fever, chest pain, fatigue, urticaria, eosinophilia, or cough, or any combination of these, lasting several weeks.[12,162]

Pleuropulmonary Paragonimiasis

Although acute paragonimiasis may occur, most infections are either silent or insidious in onset. The initial clinical presentation occurs early in the 5–10-year life span of the adult fluke, but in some cases it may occur many years after acquisition of the infection.[163,164] In *P. westermani* infections, the initial presentation is often an abnormal chest film in an asymptomatic patient. Early clinical symptoms include cough or chest pain. The cough, initially dry, often becomes productive of viscous and rusty-colored or blood-tinged sputum and appears to be worsened by exertion.[165] The sputum may be peppered with rusty-brown flecks consisting of clumps of eggs.[60] Charcot–Leyden crystals are frequent. Occasionally there is profuse hemoptysis following paroxysmal coughing. The chest pain is often pleuritic. Fevers are infrequent and, in spite of a prolonged clinical history, the patient's health usually remains relatively unimpaired. Eosinophilia may be present initially but is usually absent in chronic infections.

Radiographic findings include pulmonary lesions such as focal, segmental, or lobar air space consolidation, small cysts (5–30 mm), calcified spots, linear opacities, or nodules. The earliest infiltrates and nodules may show some limited migration.[161,163,166] About 10–40% of egg-positive patients will have normal chest films.[163] As the fluke matures, cavitary lesions of 1–4-cm diameter are seen; as the fibrotic reaction increases with time they appear as nodules, but their cavitary nature and associated burrow tracts of 0.5–1.0 cm diameter can be visualized by CT scan. Eventually these lesions are replaced by oval to round calcifications.[163] Bronchoscopy, other than as a means of retrieving eggs, does not reveal any diagnostic findings.[163]

Pleural lesions have been found in 5–71% of patients in different clinical series of *P. westermani* infections and include effusions, hydropneumothorax, and pleural thickening, which can be bilateral.[163,167,168] The frequency of pleural disease appears to be greatest in *P. skrjabini* infections.[169] Pleural fluid is sterile, contains a leukocyte count over 1000/μL, many eosinophils, and elevated protein and lactate dehydrogenase, and decreased glucose values. Eggs are rarely found in sputum or pleural fluid.

Excised pulmonary lesions reveal a wide variety of histopathologic changes characterized by the presence of adult worms within fibrous cysts up to 1.5 cm in size, juxtaposed and often communicating with bronchioles or bronchi. Egg-induced granulomas are easily confused with tuberculosis. Adjacent to the cysts are bronchiectases, various pneumonic processes, and vasculitis. Both acute and chronic cellular reactions can coexist within the same lesions.[165,168,170]

Extrapulmonary Paragonimiasis

A small percentage (~1%) of patients with paragonimiasis will develop lesions in locations other than the lung.[154] The frequency is dependent on the species of *Paragonimus*, the intensity of the infection, and possibly the duration. The diagnosis of these ectopic infections depends on the organ involved; cerebral infection produces the most frequent morbidity.

Cerebral Paragonimiasis

Cerebral paragonimiasis is the most frequent form of extrapulmonary disease diagnosed, possibly reflecting the sensitivity of the central nervous system (CNS) to such an insult rather than a predilection of the parasite for that site. Cerebral involvement occurs in <1% of cases in community-based studies and up to 24% in hospital-based studies.[171] Cerebral paragonimiasis most often occurs in younger age groups: 90% of patients are <30 years of age.[172] Clinical findings in cerebral paragonimiasis range from meningitis, arachnoiditis, to cerebral and spinal space-occupying lesions. Meningitis tends to be acute in onset and to be the initial presentation of cerebral paragonimiasis in up to a third of cases. Intracerebral lesions occur usually in occipital or temporal lobes, or both. The clinical presentation, usually insidious in onset, includes a history of seizures (80%), visual disturbances (60%), headache (55%), motor weakness (48%), sensory disturbances (40%), and vomiting

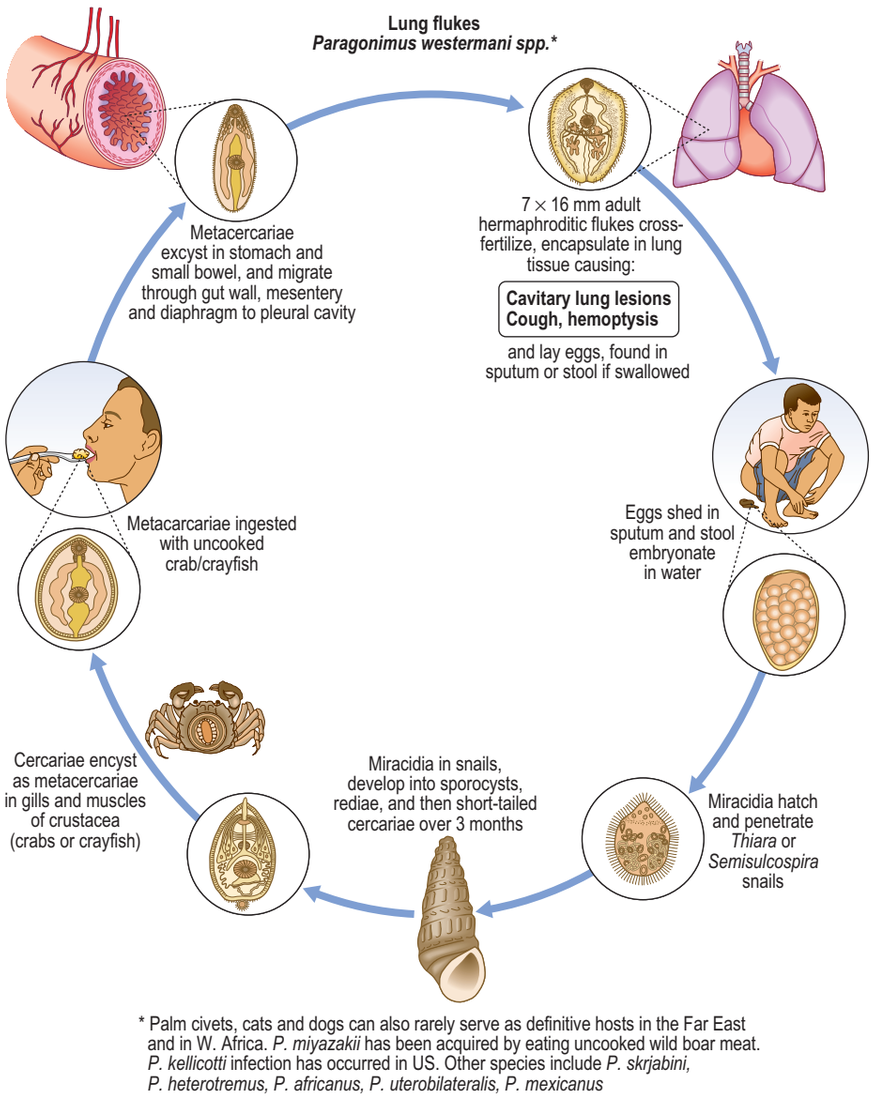

Figure 123.10 Life cycle of *Paragonimus westermani* spp.

(33%).[172,173] Physical findings include ophthalmologic abnormalities (75%), a decline in mental function (70%), hemiparesis (60%), and hemihypoesthesia (45%). Pulmonary paragonimiasis is seen in the majority of cases of CNS disease and, in fact, precedes CNS involvement in two-thirds of patients.

Plain films show calcifications and characteristically aggregated, round or cyst-like "soap bubbles" in more than 40% of patients. Common CT and MRI findings are conglomerated, multiple ring-shaped enhancing lesions with surrounding edema, described as grape clusters.[174] These rings are usually smooth and round, but may at times be irregular in outline. They are usually 1–3 cm in diameter and have contents with a density equal to or slightly greater than that of cerebrospinal fluid (CSF). At times hemorrhages up to 4 cm in diameter are associated with the ringlike structures, or the lesions may be nodular. Calcifications can be punctate, round, cystic, or amorphous and will increase in frequency with the duration of disease.[171,172,174]

Cutaneous Paragonimiasis

Cutaneous presentation, uncommon in *P. westermani* infections, has been reported to occur in 80% of infections due to other species of *Paragonimus* (e.g., *P. skrjabini*, *P. miyazakii*). The cutaneous presentation, which has been called trematode larva migrans, consists of painless and migratory subcutaneous swellings or subcutaneous nodules on the trunk and proximal extremities,[175,176] often accompanied by high-level eosinophilia.

Miscellaneous Sites

Flukes, usually immature, may come to rest in ectopic intra-abdominal sites such as the liver, spleen, peritoneum, intestinal wall, or mesenteric lymph nodes. The clinical picture reflects the site and can include abdominal pain, diarrhea, and even dysentery.

PATHOGENESIS AND IMMUNITY

As with other tissue-dwelling trematodes, infection with *P. westermani* is also associated with eosinophilia and leukocytosis in the early stages of infection, reflecting activation of the immune system. Eosinophil infiltration around the sites of egg deposition is a consistent pathologic feature, as is eosinophilia and an elevated IgE level, indicative of a Th2 cell-regulated response.[177] IgG_4 antibodies predominate, but the role of Th2 lymphocytes in resistance to the parasite remains to be determined.[177] In rodent models, excreted-secreted products of *Paragonimus* appear to regulate the innate and adaptive immune response in the host, by a

number of mechanisms such as attenuating the survival and function of eosinophils, and secreting proinflammatory cytokines and chemokines.[178,179] However, these immune mechanisms have been studied only in rodent and bovine models of paragonimiasis, and their roles in human infections are unconfirmed.

DIAGNOSIS

Pulmonary paragonimiasis must be suspected in persons from known endemic areas when a chronic cough is present; the most important differential diagnoses are tuberculosis, bronchiectasis, and chronic bronchitis. The diagnosis is almost always made by finding the characteristic eggs in sputum, stool, gastric aspirates, or tissue, i.e., by parasitological techniques. Examination of blood-streaked sputum is most likely to yield positive results. Egg detection in sputum may require repeated examinations, and a 24-hour sputum collection can increase the sensitivity.[180] This collection is centrifuged, the sediment dissolved in 3% sodium hydroxide, and then examined for eggs. In children and the elderly, in whom sputum swallowing is more frequent, the examination of stool and gastric aspirate specimens can be more productive.[180] Ziehl–Neelsen stains of specimens for mycobacteria may destroy the fluke eggs, making separate examinations necessary.[166] In patients who have pleural or CNS involvement, it is very uncommon to find eggs in pleural fluid or CSF aspirates.

Immunodiagnosis

The complement fixation test has been a standard test for years. This test is sensitive and becomes negative 6–12 months after cure, making it useful for following therapy.[168,181] Some cross-reactivity with other trematode parasites has been noted, particularly in the chronic phase of paragonimiasis.[182] A skin test using extracts of adult *Paragonimus* is useful for screening in epidemiologic surveys because of its high sensitivity (80–90%), but it remains positive 10–20 years after cure.[183] ELISAs for detection of antibodies to *P. westermani* are both sensitive (92%) and highly specific (>90%), but require longer (4–24 months) to become positive after infection and longer to normalize after cure.[184–187] Crude worm extracts do not provide an acceptably specific ELISA,[188,189] and the most sensitive ELISA to date, using an 8-kDa component of *P. westermani* as the antigen, has been developed by Centers for Disease Control and Prevention (sensitivity, 96%; specificity, 100%).[190] Recently, antigen detection assays have been developed that utilize mixtures of monoclonal antibodies to capture *P. westermani* antigens from serum, with a sensitivity approaching 100% and specificity >95%.[162,191] The utility of these assays in the field remains to be evaluated, but they will likely provide a sensitive measure of active infections in field surveys.

TREATMENT AND PROGNOSIS

Untreated, pulmonary paragonimiasis can resolve in 5–10 years, leaving dysfunction commensurate with the degree of scar tissue produced in the pleura or lungs.[163] Praziquantel is the drug of choice because of minimal side effects and the short course of administration. A regimen of 75 mg/kg/day in three divided doses for 2 days is 90–100% effective.[163,192–194] Symptoms improve within 2–3 days, although radiologic findings may worsen for the first 10 days.[166] Adverse effects are mild and include headache, intestinal symptoms, and transient urticaria. Large pleural effusions may require drainage. Surgical intervention may be required for long-standing effusions (years) or empyemas (months).[163]Triclabendazole, a drug introduced as therapy for fascioliasis, successfully treats pulmonary paragonimiasis at a dosage of 5 mg/kg daily for 3 days or 10 mg/kg bid for 1 day.[195] Bithionol could cure 92% of pulmonary cases at a dosage of 40 mg/kg/day on alternate days for 10–15 doses.[196] Gastrointestinal side effects in 70%, dermatologic side effects in 21%, and the duration of treatment are recognized limitations.

INTESTINAL FLUKES – INTRODUCTION

Little is known about the clinical presentations of 70% of trematode species that inhabit the human intestinal tract, even for those that affect relatively large populations.[12,60,197] The best known are *Fasciolopsis buski*; the Heterophyidae, including *Heterophyes heterophyes* and *Metagonimus yokogawai*; and several *Echinostoma* species. The intestinal flukes are thought to produce no symptoms except when present in very large numbers (a rare occurrence). Few cause serious disease, but community-based and case-control studies have yet to be done. Most of these flukes occur in Asia, but foci of these infections occur in other populations throughout the world. They are usually localized in areas where there are freshwater snail vectors and animal reservoir hosts and occur in people with particular dietary habits.[198]

FASCIOLOPSIS BUSKI

THE AGENT

F. buski, the giant intestinal fluke, was first found, by Busk, in an Indian sailor in London in 1843. The parasite is found in China, Taiwan, Thailand, Laos, Bangladesh, India, Indonesia, Vietnam, Myanmar, and Kampuchea. The worm is elongated, oval, and fleshy, measuring 20–75 × 8–20 × 0.5–3.0 mm. Eggs are large, operculate, and unembryonated when passed, and measure 130–140 × 80–85 μm. The miracidia develop in several weeks, hatch from the eggs, and infect planorbid snail intermediate hosts. Snails of the genera *Segmentina, Hippeutis,* and *Gyraulus* serve as intermediate hosts.[85] After development in the snail, cercariae emerge and encyst as metacercariae on aquatic plants. When the plant is eaten, the attached metacercariae excyst and attach to the small intestinal mucosa. The prepatent period is 3 months, and the worms are known to live for 6 months or more in the human. Pigs and dogs act as reservoirs.

EPIDEMIOLOGY

Several planorbid snails found in muddy ponds and streams, including those found adjacent to slaughterhouses where feces from pigs contaminate the waters, serve as the first intermediate host of *F. buski*. The metacercaria of *F. buski* can attach to most aquatic plants, including water caltrop (*Trapa bicornis*, *T. natans*), water chestnut (*Eliocharis tuberossa*), water bamboo (*Zizania aquatic*), water hyacinth (*Eukhornia crassipes*), water morning glory (*Ipomoea aquatic*), watercress (*Nasturtium officinale*), lotus (*Nymphaea lotus*), and others, that serve as sources of infection, although detached metacercariae can also transmit infection.[1,199] These plants may be cultivated near homes in water contaminated accidentally or fertilized intentionally with human or pig feces. Pigs are a major reservoir host, but there are some areas where humans are infected while pigs are not.[200] Children eating plants during play, especially in rural areas, have the highest prevalence rates.

THE DISEASE

This large fluke attaches to the duodenal and jejunal mucosa and produces focal inflammation, ulceration, and small abscesses at the sites of attachment. However, community-based studies reveal no clinical or biochemical differences between lightly to moderately infected cases and controls.[201] Early symptoms, which begin 30–60 days after exposure, are epigastric pain, mimicking peptic ulcer disease, and diarrhea.[202] Hunger or anorexia, nausea, and vomiting may occur. Rarely, in heavy infections, edema of the face, abdominal wall, and legs, ascites, and severe prostration have been described.[183] The cause of these symptoms is not understood. Large numbers of flukes may cause focal ileus or intermittent obstruction. Eosinophilia is variable but may be marked.[203]

HETEROPHYIDS

There are a large number of small intestinal flukes (less than 2.5 mm long) that have been reported in humans, other mammals, and birds that come from the families Heterophyidae, Plagiorchiidae, Lecithodendridae, Microphallidae, and others. The flukes in the Heterophyidae are the most prevalent and best studied. The importance of these flukes is being increasingly recognized,[204] although their pathogenic potential is as yet unclear.

THE AGENT

Of at least 10 human species of intestinal fluke in the family Heterophyidae, the three most prevalent are *Heterophyes heterophyes*, *H. nocens*, and *Metagonimus yokogawai*. Bilharz described the first, *H. heterophyes*, at the autopsy of a native of Cairo.[50] These are the smallest of the human flukes. They measure 1–2 mm in length, are oval to pear-shaped, and have spiny integuments. The eggs are operculate, ovoid, and yellowish in color, measure 27–30 × 15–17 μm, and are very difficult to speciate. The eggs are embryonated when passed and are ingested by a snail intermediate host. Cercariae from the snail enter freshwater fish, encyst as metacercariae, and, when eaten raw, excyst and complete their development to adult flukes within 1–2 weeks in the small intestine of humans, other mammals, and fish-eating birds. The prepatent period is only 9 days and the parasite may live for a few months to a year in the final host.[12]

EPIDEMIOLOGY

The heterophyids are parasites of fish-eating mammals and birds, and humans acquire the infection by eating raw or incompletely cooked freshwater or brackish water fish. The highest infection rates for *H. heterophyes* have been reported in Egypt, Iran, and Sudan; for *M. yokogawai*, in Korea, China, Taiwan, Indonesia, Russia, and Japan; and for *H. nocens*, in Korea and Japan. *H. heterophyes* infects the gray mullet *Mugil cephalus* in the brackish lagoons of Egypt's Nile delta. Infection rates can reach 65% in children in villages where these fish are traditionally eaten raw. However, reports of these and other heterophyid species in scattered locations around the world (particularly from South and Southeast Asia) are frequent. Distributions of the different Heterophyidae greatly overlap. In Korea, of 19 different intestinal flukes reported in humans, 12 are different heterophyid species.[204,205] Intestinal flukes are common in farmed fish in Vietnam, with dogs, cats, and pigs important reservoir hosts, especially for *Haplorchis* species.[7,200] The overall prevalence of *M. yokogawai* in Japan is low (0.2–0.3%), but in some areas the prevalence is high (51–75%).[206] In Korea, *M. yokogawai* infection rates of 1–2% have been reported for the population as a whole, reaching 29% along some coastal streams.[204] Infection rates of *M. yokogawai* in Taiwan and the Philippines are around 1%.[207]

THE DISEASE

Symptoms begin 9 days following ingestion of the metacercaria, on average, with dyspepsia, and colicky abdominal pain, diarrhea, and eosinophilia.[208,209] A mild focal inflammatory reaction and superficial erosions are produced at the site of attachment.[2] The fluke may penetrate the mucosa, and eggs may embolize from these intramucosal sites via lymphatics to the systemic vascular system. Eggs of three different heterophyid species have been recovered from capillaries of brain, heart, lungs, spleen, and liver, where space-occupying granulomatous lesions induce clinical pathology.[209–212] Myocarditis can follow the occlusion of myocardial vessels by eggs and the resultant granulomatous and fibrotic host reaction. Thickened mitral valves containing ova has been reported.[213]

ECHINOSTOMA SPECIES

THE AGENT

These trematodes are primarily parasites of birds and mammals but are common among certain populations of Asia. Fifteen species have been reported in humans. The parasites are elongate, tapered at both ends, and 5–15 × 1–2 mm in size. The name derives from a collar of spines in two rows surrounding the oral sucker. The anterior integument is also provided with tiny spines. Eggs are operculate, thin-shelled, and vary in size (83–130 × 58–90 μm).[54] The eggs embryonate in freshwater in 14 days, and the miracidia enter the snail host. Cercariae emerge from the snail and encyst in the same snail from which they emerged or in other snails, clams, fish, or tadpoles, which serve as second intermediate hosts. Any of these, if eaten uncooked, infect the human final host.[12,82,213]

EPIDEMIOLOGY

The most common of the 15 reported *Echinostoma* species in humans are *E. ilocanum* in the Philippines and Thailand, and both *E. malayanum* and *E. revolutum* in Thailand.[2,85,214] In northern Luzon in the Philippines, *E. malayanum* infection rates have averaged 10% of surveyed populations, with highs of over 40%.[12,155] In northern Thailand, a variety of echinostomes infect humans, with prevalence rates as high as 50%.[215] These and the other species are found at lower prevalences in Southeast Asia, eastern and South Asia, and also in Egypt and Central and South America.[216] The major source of infection with *E. ilocanum* is the snail *Pila conica*, which is eaten uncooked in parts of the Philippines. Other sources of infections are clams, tadpoles, frogs, and fish, all serving as second intermediate hosts for echinostomes. Rats, dogs, cats, birds, and other fish-eating animals are reservoirs of infection.[12,217]

THE DISEASE

These flukes attach to small intestinal mucosa, producing inflammatory lesions and shallow ulcers at the sites of attachment. A self-infection by ingestion of 113 metacercariae of *Echinochasmus japonicus* resulted, after 10 days, in abdominal pain and diarrhea.[2,12,217] There are no clinical epidemiology studies, but it is generally accepted that symptoms are rare in any but the heaviest infections (approximately 500 flukes), which are uncommon.[54,218] The presentation may include colicky abdominal pain and loose bowel movements and at times diarrhea and eosinophilia.

MISCELLANEOUS INTESTINAL FLUKES

There are many other intestinal flukes within the preceding families – Fasciolidae, Heterophyidae, and Echinostomatidae[12,60] – that have been reported to cause human infections with more limited distributions and are less well studied. Two flukes in two other families, Troglotrematidae and Paramphistomatidae, are worth mentioning.

Nanophyetus salmincola is a small fluke found in eastern Siberia and the northwestern coast of North America. It belongs to the same family, Troglotrematidae, as does *Paragonimus*. Adults are 0.8–2.5 × 0.3–0.5 mm, and eggs are 64–97 × 34–55 μm in size. Fish, such as salmon, are the second intermediate hosts. Intestinal symptoms can occur with heavy infections in a manner similar to that of other intestinal flukes. More unusually, this fluke is a vector for the rickettsial organism *Neorickettsia helminthoeca*, which produces a fatal illness in dogs ("salmon poisoning").[219,220]

Gastrodiscoides hominis is a piriform intestinal fluke that is 8–14 × 5–8 mm in size and produces eggs that measure 150 × 60–70 μm. It is widely distributed from India to the Philippines and north to Kazakhstan. The human colon can be colonized, with resultant mucoid diarrhea. Pigs and rodents appear to be the reservoir.

DIAGNOSIS

Since clinical presentations are nonspecific, infection is often indicated by eosinophilia, a particular dietary history, and the time interval since possible infection; *H. heterophyes* and *M. yokogawai* do not survive in the intestine for more than a year. The diagnosis is made on stool examination or by tissue biopsy or necropsy. Egg identification can be very difficult because many of the intestinal fluke eggs have similar morphology and overlapping sizes. Overlapping "small" fluke eggs include *H. heterophyes* (28–30 × 15–17 μm), *M. yokogawai* (26–28 × 15–17 μm), *Clonorchis sinensis* (28–35 × 12–19 μm), and *O. viverrini* (30 × 12 μm). Overlapping "large" fluke eggs are *Fasciolopsis buski* (130–140 × 80–85 μm), *Echinostoma* spp. (83–130 × 58–90 μm), and *Fasciola hepatica* (130–150 × 60–90 μm). As well, there are many other less common intestinal flukes with focal distribution that produce similarly sized eggs. The eggs of small opisthorchid and heterophyid flukes are difficult to differentiate microscopically and many of the infections are misdiagnosed. Polymerase chain reaction assays targeting ribosomal DNA are being developed to differentiate some species.[221] Examination of stools for expelled adult flukes after treatment with praziquantel (a praziquantel "purge") is often necessary to make a definitive diagnosis. Although praziquantel may damage the integument of the adult fluke, it is still often possible to make a species identification.

TREATMENT AND PROGNOSIS

Although the evidence comes from limited clinical trials, it appears that praziquantel is highly effective against intestinal flukes at 15–25 mg/kg given in a single dose.[12,85,222] The new benzimidazole, triclabendazole, at 5 mg/kg twice daily after a meal at a 6–8-hour interval for 1 day, shows promise in the treatment of intestinal flukes.[223–225] Alternative drugs include for *Fasciolopsis buski*, niclosamide 40 mg/kg/day for 1–2 days (maximum daily dose of 4 g); for *H. heterophyes*, niclosamide 1000 mg in a single dose; and for *Echinostoma* spp., albendazole 400 mg twice daily for 3 days.[54,176,209,226]

Recent studies in rodent models have shown that the artemisinins (e.g., artemether and artesunate) and synthetic peroxides (e.g., synthetic trioxolanes), recognized for their antimalarial properties,[77] and the Chinese anthelminthic drug tribendimidine[78] may be useful against foodborne trematodiases.[176] In one study, a single oral dose of artesunate, artemether, or OZ78 (100–400 mg/kg) resulted in 100% worm burden reductions in a chronic *F. hepatica* infection in the rat model.[227] Artesunate and artemether also showed activities against adult *C. sinensis* flukes in rats, with 99–100% reduction in worm burdens with a single 150 mg/kg oral dose of either drug.[228] In addition, *O. viverrini*-infected hamsters treated with a single oral dose of artesunate and artemether (400 mg/kg) demonstrated 78% and 66% reductions in worm burden, respectively.[228] Trematodes show variable sensitivity to tribendimidine. A single oral dose of tribendimidine (150 mg/kg) administered to rats infected with adult *C. sinensis* flukes resulted in a 99% reduction of worms, and 400 mg/kg caused a 96% reduction in adult *O. viverrini* flukes in hamsters. However, no activity of tribendimidine against *F. hepatica* was observed in the rat model.[229] Scanning electron microscopy indicates that both artemether and tribendimidine disrupt the tegument of adult trematodes.[229] Since treatment options are limited and there is concern about the emergence of resistance, combination chemotherapy may hold promise for the treatment of foodborne trematodiases.[12,225]

PREVENTION AND CONTROL

Public health interventions to prevent these foodborne infections will need to include plans for adequate sanitation, the use of chemical fertilizers, food inspections, and campaigns to disseminate information, education, and communication. The ultimate aim is to change human behavior, specifically to change habits that have been in practice for generations,[230] because the consumption of raw or undercooked freshwater fish and other aquatic products is the key risk factor for acquiring foodborne trematode infections. These habits are variably dependent on attitudes, education, poverty, environmental degradation, food security, and other factors, and control strategies will have to take all these into account. Human vaccines are presently not available for the prevention of foodborne trematodiases, but recent gene discovery efforts may assist in the rational development of vaccines and the next generation of trematocidal drugs.[231] National strategies are necessary to control these parasites. Health education and appropriate regulations, both for water bodies used for pisciculture and aquatic plant crops, can have an impact. Mass treatment programs using praziquantel or triclabendazole may be beneficial but require more experience.[232] Molluscicides for the elimination of the animal host reservoirs do not appear to be realistic over the long term.[1] Irradiation of food may offer an alternative.[233] Preservation by cooking can be difficult in many heavily populated regions where fuel is scarce. On the other hand, some populations prefer to eat raw food, aware of the nutritional value of raw foods.

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