CLINICAL MICROBIOLOGY REVIEWS, July 2009, p. 466–483 Vol. 22, No. 3
0893-8512/09/$08.00+0 doi:10.1128/CMR.00012-09

Food-Borne Trematodiases

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INTRODUCTION

Approximately 6,000 digenean species have been described, but only a few are important human parasites (117). These include liver flukes, lung flukes, and intestinal flukes, collectively known as the food-borne trematodes (54, 92). Infection of humans occurs through the consumption of contaminated freshwater fish, frogs, shellfish, snails, tadpoles, snakes, water plants (e.g., watercress), and other aquatic products eaten raw or insufficiently cooked (92). Hence, fish-borne trematodiases and plant-borne trematodiases are other common names referred to in text books and the peer-reviewed literature (70, 79, 101). The zoonotic nature of food-borne trematodiases—transmission to humans occurs via aquatic products—is an important factor explaining the growing importance of these diseases (68). Of note, not only humans but also other mammals act as definitive hosts; food-borne trematodiases are therefore an important public health and veterinary problem (73). Additionally, pet animals (e.g., cats and dogs) can act as definitive hosts, which should be factored into future appraisals of the true societal impact of food-borne trematodiases (98).

While the public health impact of food-borne trematode infections is driven largely by clinical aspects due to the long-term chronic and debilitating nature of infection and secondary complications such as cholangiocarcinoma, the veterinary impact is due mainly to economic losses of meat and milk products from infected livestock (104). In the current review, only the public health issues affected by food-borne trematodiases are covered, and readers interested in veterinary implications of food-borne trematode infections are referred to another review (104).

Despite the considerable public health impact and the emerging nature of food-borne trematodiases (17, 49, 68), these diseases are among the most neglected of the so-called

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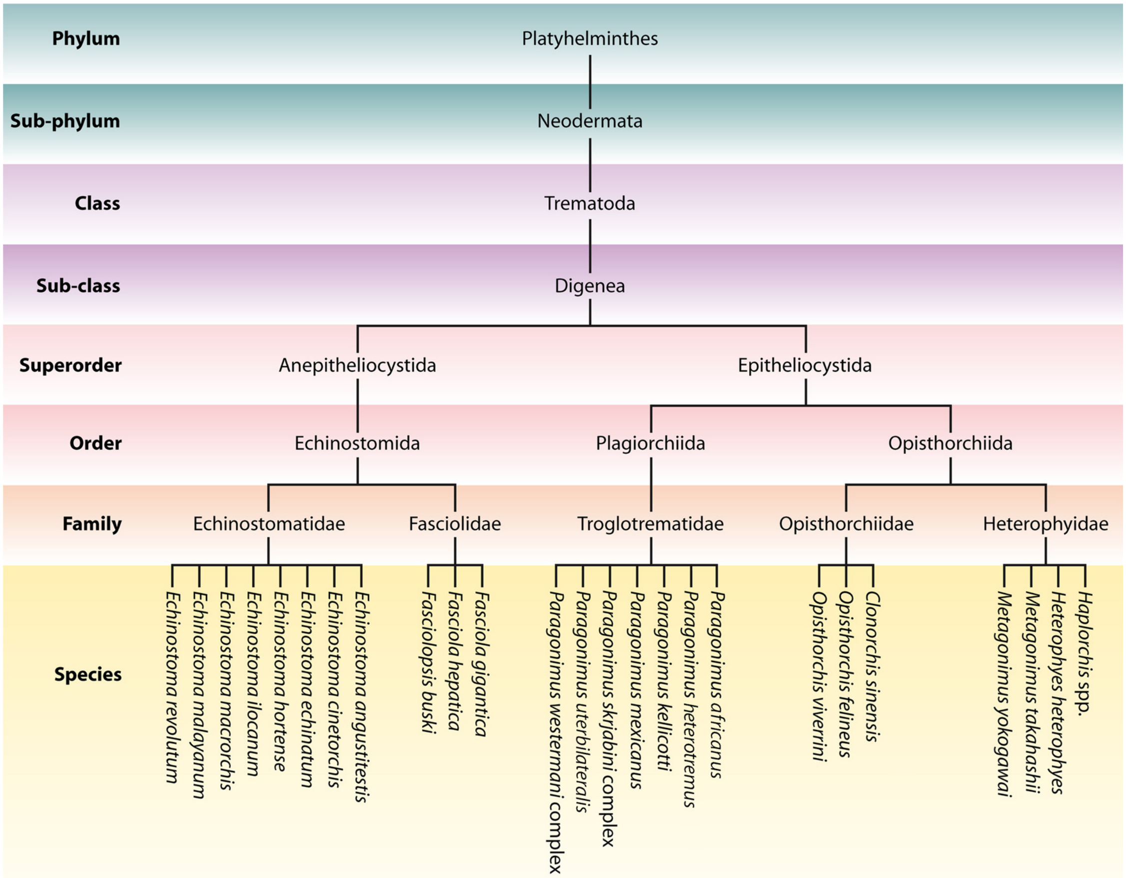

FIG. 1. Taxonomy of food-borne trematodes with emphasis on species parasitizing humans that are covered in the current review. (Based on data from reference 20.)

neglected tropical diseases (25, 42, 98). It should be noted that the neglected tropical diseases are found predominantly in the world's poorest populations in low-income countries, and where these diseases are common, they exacerbate poverty. Compared with, for example, malaria, tuberculosis, and human immunodeficiency virus (HIV)/AIDS, most of the neglected tropical diseases are orphans with regard to research funding and political and financial commitment for prevention and control (43). Still, today, chronic diseases are rarely high on the list of priorities of the ministries of health in countries where these diseases are endemic (96). Given the wide geographic distribution, the large number of people infected, a global burden that has been underestimated, and readily available tools for controlling many of the neglected tropical diseases, they should no longer remain neglected (8, 42, 59, 75). Indeed, hundreds of millions of people are affected by one or several of the neglected tropical diseases, and collectively, these diseases represent the fourth most important group of communicable diseases worldwide, behind lower respiratory infections, HIV/AIDS, and diarrheal diseases (43). Although there is growing international awareness pertaining to the neglected tropical diseases and new political and financial commitments to do something against them (40, 43, 61), with regard to fluke infections, a number of campaigns have been stopped, and the diseases are not on the priority list of the World Health Organization (WHO) and its regional offices (96).

Here, we summarize the taxonomy and key characteristics of food-borne trematodes. The life cycle and contextual determinants are depicted, and estimates of the at-risk population and number of infections, geographic distribution, history, and ecological features of the major food-borne trematodes are reviewed. Current means of diagnosis, treatment, and other control options and clinical manifestations are highlighted. Emphasis is placed on the changing epidemiological pattern and the rapid growth of aquaculture and food distribution networks, as these developments might be associated with an elevated risk of transmission of food-borne trematodiases. Conclusions are drawn, and current research needs are highlighted.

TAXONOMY AND MORPHOLOGICAL CHARACTERISTICS

Figure 1 shows the basic taxonomy of food-borne trematodes, emphasizing species that are infective to humans. Food-borne trematodes are classified into the phylum Platyhelminthes, class Trematoda, and subclass Digenea. They are typically found in major viscera such as the bile ducts, lungs, and gut. The most important species parasitizing humans include liver flukes (*Clonorchis sinensis*, *Fasciola gigantica*, *Fasciola hepatica*, *Opisthorchis felineus*, and *Opisthorchis viverrini*), lung flukes (*Paragonimus* spp.), and intestinal flukes (e.g., *Echinostoma* spp., *Fasciolopsis buski*, and the heterophyids). This list is far from complete. For example, a total of 70 species (14 families and 36 genera) of intestinal flukes have been reported to have been isolated from humans (11).

Adult digeneans are characterized by a dorsoventral, flattened, bilaterally symmetrical body. The average size of the flukes varies according to species. While *C. sinensis* flukes have a size of approximately 10 to 25 by 3 to 5 mm (92), *F. buski* flukes measure 8 to 30 by 20 to 100 mm (70). The body surface of trematodes is covered by a syncytial epithelium, the tegument. The tegument is implicated in nutrient absorption, synthesis, secretion, and osmoregulation and has sensory functions. Moreover, the tegument protects the parasite from the immune system of the host (18, 37, 81). Further typical characteristics of trematodes are the presence of an oral sucker, often a ventral sucker, and a lack of respiratory and circulatory systems (93, 117). The reproductive system is always hermaphroditic (20). With their oral and ventral suckers, the flukes attach to the wall of bile ducts, lung parenchyma, or intestine of the host organism. The individual genera are morphologically diverse. *Echinostoma* spp., for example, are characterized by an elongated body and the presence of a head collar with a crown of spines around the oral sucker (11).

LIFE CYCLE

The life cycle of digeneans is complex, but a common feature is that aquatic snails act as intermediate hosts. There are up to six larval stages with alternations of asexual and sexual reproductive phases in the molluscan and vertebrate hosts (92, 117). The life cycles of the major food-borne trematodes are diverse, with species-specific characteristics. Details of the life cycle of *C. sinensis* were presented in a previous review in this journal (14) and elsewhere (68).

Figure 2 depicts typical life cycles of five different food-borne trematodes, including intestinal, liver, and lung flukes. In brief, eggs are produced by adult worms following sexual reproduction in the final host, which are humans or a range of domestic (or wild) animals. Eggs are released via feces (most food-borne trematodes) or sputum (*Paragonimus* spp.). Of note, *Paragonimus* spp. release their eggs mainly in the sputum, but eggs are also found occasionally in the feces. An adult worm can produce a large number of eggs. For example, an adult *C. sinensis* fluke produces 1,000 to 4,000 eggs/day for at least 6 months (68). An even higher rate of egg production has been reported for *F. buski*; on average, this intestinal fluke produces 16,000 eggs/day (69). Eggs require appropriate environmental conditions for embryonation, such as a specific water temperature range and sufficient moisture coupled with suitable ambient temperature and oxygen tension. Once an egg has hatched, a swimming "sac-like" larva is released, the so-called miracidium. The miracidium, attracted by chemokinesis and chemotaxis, is ingested by snails or penetrates the molluscan intermediate host, which is assisted by a boring action of the larva (93). Eggs of *C. sinensis*, *Opisthorchis* spp., and the heterophyidae are directly ingested by the snail, and miracidia hatch in the gastrointestinal tract of the snail (68).

Various snail species act as a first intermediate host, most of which are trematode species specific. For example, in China, eight snail species have thus far been described to be first intermediate hosts for *C. sinensis* (68). Clearly, *Alocinma longicornis*, *Parafossolarus* spp., and *Bithynia* spp. are the most important first intermediate hosts for this liver fluke (14, 68). The expansion of *F. hepatica* from Europe to other continents was attributed to an adapted susceptibility of the parasite to new snail species and the spread of the main intermediate host snail to other continents. In contrast, the intermediate host snails of *F. gigantica* (*Radix natalensis* and *Radix auricularia*) seem to have a weaker diffusion capacity and, hence, a smaller geographic distribution (69). Young snails were found to be more susceptible to miracidia invasion than older ones, but in general, rates of infection of snails are rather low (44). For example, the prevalence of *O. viverrini* infections in *Bithynia* spp. ranges from 0.07% to 0.63% (92).

Within the snail, asexual reproduction occurs for several weeks. After penetration, the miracidium elongates and sheds its ciliated plates, a stage called the sporocyst. The germinal cells within the sporocysts multiply and produce new germinal masses, which produce daughter sporocysts or rediae. Both of these larval stages produce embryos, which develop into cercariae. Typical features of cercariae include the tail, mouth, gut, suckers, flame cells, and glands. Cercariae leave the snail intermediate host by either active escape or passive extrusion. Released cercariae either encyst on aquatic vegetation such as watercress, water lotus, water caltrop, water chestnut, or water lily (e.g., *F. hepatica* and *F. buski*) or penetrate the skin of a second intermediate host and encyst in the flesh of fish (e.g., *C. sinensis*, *Echinostoma* spp., and *Opisthorchis* spp.), shellfish (e.g., *Paragonimus* spp.), or frogs, snails, and tadpoles (*Echinostoma* spp.) (Table 1). Young fish have been described as being particularly susceptible due to their thin skin and immune status (68). Several dozen fish species, crustaceans, snails, and tadpoles act as second intermediate hosts, which are confined mainly to stagnant or slow-flowing freshwater bodies. For example, in China alone, 132 species of freshwater fish are known to be suitable second intermediate hosts for *C. sinensis* (68). Metacercariae, which are the infective stage, can be found in various parts of the fish body, with the highest density being reported for fish muscle (44).

The time required for metacercariae to become infective lasts from 1 h to several months. Humans and animal hosts become infected when eating raw, pickled, or insufficiently cooked aquatic products harboring metacercariae or when drinking contaminated water (68, 118). After ingestion of the metacercariae, gastric juices help effect the excystation of the metacercariae and release of a juvenile worm, which migrates to the target organ. Infection with *Paragonimus westermani* (and, occasionally, *Paragonimus skrjabini* and *Paragonimus miyazakii*) might also occur through the consumption of undercooked meat of wild boars (contaminated with immature lung flukes), which act as a paratenic host (6). Adult *C. sinensis* and *O. viverrini* flukes have been reported to live up to 25 years in the human host (46, 68). The life span of *F. hepatica* in humans has been estimated to be 9 to 13 years (27). Several animal hosts play a role in the transmission of food-borne

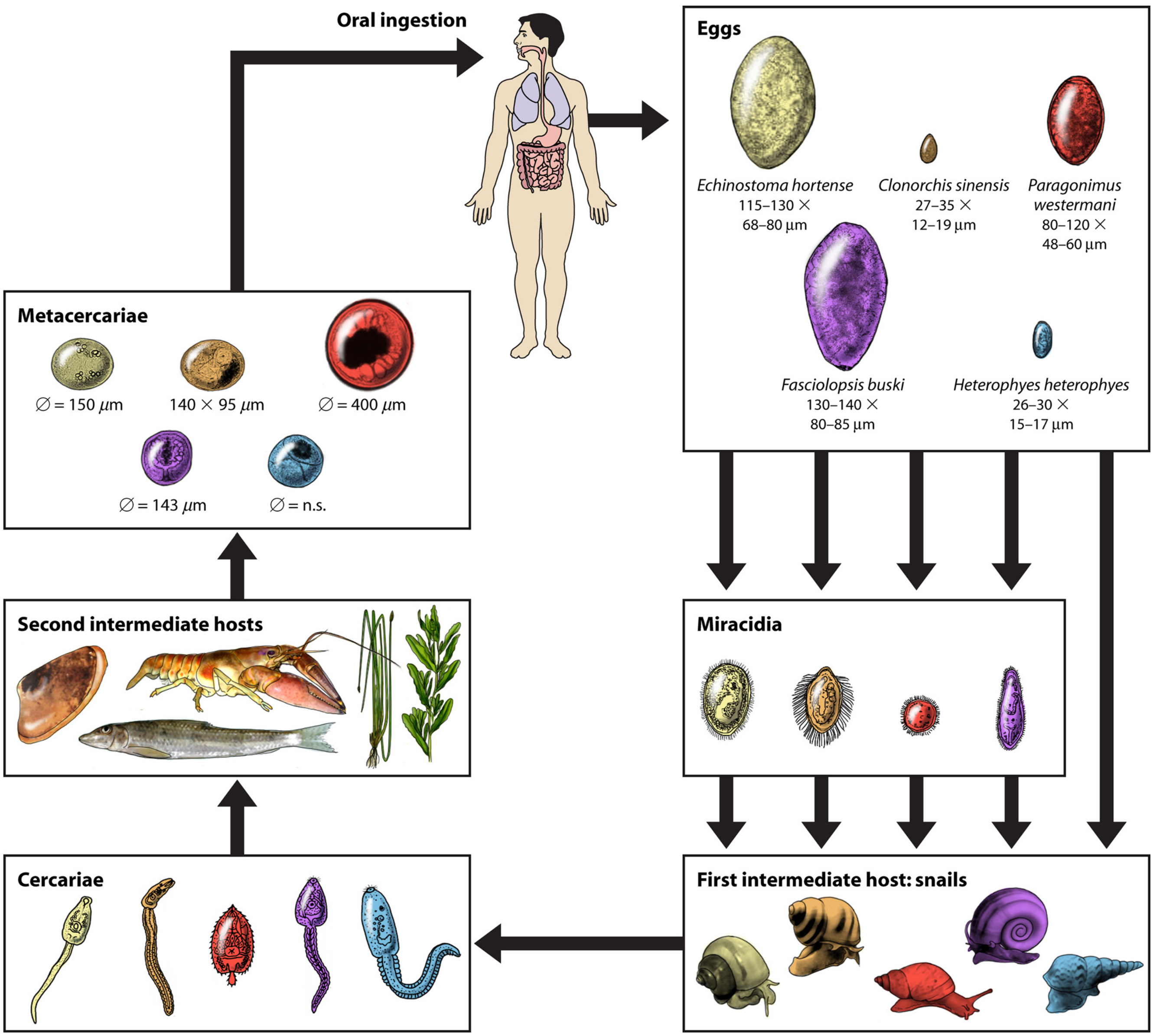

FIG. 2. Life cycles of five different food-borne trematodes including intestinal flukes (*Echinostoma hortense*, *Fasciolopsis buski*, and *Heterophyes heterophyes*), a liver fluke (*Clonorchis sinensis*), and a lung fluke (*Paragonimus westermani*). Sizes of eggs and metacercariae are based on data from references 4 and 23.

trematodiases. For example, myriad definitive hosts have been reported for *F. hepatica*, such as sheep, cattle, pig, donkeys, and, as recently discovered, a number of wild animals including beaver, deer, elk, and hare (69).

AT-RISK POPULATION, NUMBER OF INFECTIONS, AND GLOBAL BURDEN ESTIMATES

In the mid-1990s, when there were approximately 5,713 million inhabitants worldwide (107), it was estimated that 750 million people were at risk of food-borne trematodiases (118), hence, over 10%. More recent estimates for at-risk populations for clonorchiasis, paragonimiasis, fascioliasis, and opisthorchiasis are 601 million, 293 million, 91 million, and 80 million, respectively, as summarized in Table 1 (49). At present, it is not known how many people are at risk of intestinal fluke infections.

The global estimate for the number of people infected with *C. sinensis* is 35 million, almost half of whom (15 million) are Chinese (68). More than 20 million people are infected with *Paragonimus* spp. For *O. viverrini*, it is estimated that 10 million people are infected, with 8 million infections in Thailand and 2 million infections in the Lao People's Democratic Republic (PDR) (3). Estimates for *Fasciola* species infections range between 2.4 million and 17 million (54). Approximately 1.2 million people are infected with *O. felineus* (91). Finally, an estimated 40 to 50 million people are infected with one or several species of intestinal flukes (11) (Table 1).

TABLE 1. Infection sources, at-risk population, numbers of infections, DALY estimates, clinical manifestations, and current treatments of choice of major food-borne trematodes and their underlying diseases[g]

Food-borne trematode	Species	Infection source(s) (second intermediate host)[a]	At-risk population (10^6)[b]	No. of infections (10^6)[b]	DALY estimate[c]	Clinical manifestation of underlying disease[a]	Treatment (dose)[d]
Liver fluke	*Clonorchis sinensis*	Small freshwater fish	601.0	35.0	NK	Acute stage consisting of fever, diarrhea, loss of appetite, rash, edema, night blindness, enlargement of liver, swollen abdomen; chronic stage consisting of biliary obstruction, intrahepatic stone formation, cholangitis, cholecystitis, cholelithiasis, pancreatitis, hepatitis, biliary and liver abscesses, risk of cholangiocarcinoma	Praziquantel (3 × 25 mg/kg for 2 days or single dose of 40 mg/kg)
	Opisthorchis felineus	Small freshwater fish	79.8[e]	1.2	NK	Acute stage consisting of fever, abdominal pain, nausea, emesis; chronic stage consisting of obstruction of biliary tract, inflammation, fibrosis of biliary tract, liver abscess, pancreatitis, suppurative cholangitis	
	Opisthorchis viverrini	Small freshwater fish		10.0	NK	Acute stage consisting of flatulence, fatigue, dyspepsia, abdominal pain, anorexia, mild hepatomegaly; chronic stage consisting of obstructive jaundice, cirrhosis, cholangitis, cholecystitis, bile peritonitis, risk of cholangiocarcinoma	
	Fasciola hepatica, *Fasciola gigantica*	Freshwater vegetables, infected water, infected raw liver	91.1	2.4–17.0	NK	Acute stage consisting of dyspepsia, fever, right upper quadrant pain, anorexia, hepatomegaly, splenomegaly, ascites, urticaria, respiratory symptoms, jaundice; chronic stage consisting of biliary cholic, intermittent jaundice, epigastric pain, nausea, fatty food intolerance, cholangitis, acute pancreatitis, cholecystitis	Triclabendazole (single dose of 10 mg/kg, or in the event of treatment failures, the dosage can be increased to 20 mg/kg given in 2 split doses within 12–24 h)
Lung fluke	*Paragonimus* spp.	Freshwater crabs, crayfish, wild boar meat	292.8	20.7	NK	Acute stage consisting of cough, fever, bloody sputum, loss of appetite, chest pain, headache; chronic stage consisting of chronic, productive cough with brownish sputum, chest pain, night sweats, ectopic paragonimiasis	Praziquantel (3 × 25 mg/kg for 2 days)
Intestinal fluke	*Fasciolopsis buski*	Freshwater plants	NK	1.3[f]	NK	Acute stage consisting of diarrhea, constipation, headache, flatulence, poor appetite, vomiting, abdominal pain, fever; chronic stage consisting of edema, anemia, anorexia, vomiting, gastric pain, pallor, malnutrition, abdominal pain, nausea, bitemporal headache	Praziquantel (single dose of 25 mg/kg)
	Echinostoma spp.	Freshwater fish, frogs, mussels, snails, snakes, tadpoles	NK		NK	Abdominal pain, diarrhea, fatigue	
	Heterophyidae	Freshwater fish	NK		NK	Abdominal pain, diarrhea, fatigue	

[a] Based on data from references 78 and 118.
[b] Based on data from references 48 and 49.
[c] Based on data from reference 41.
[d] Based on data from references 48 and 118.
[e] Including both *O. felineus* and *O. viverrini*.
[f] Including *F. buski*, *Echinostoma* spp., and Heterophyidae.
[g] NK, not known.

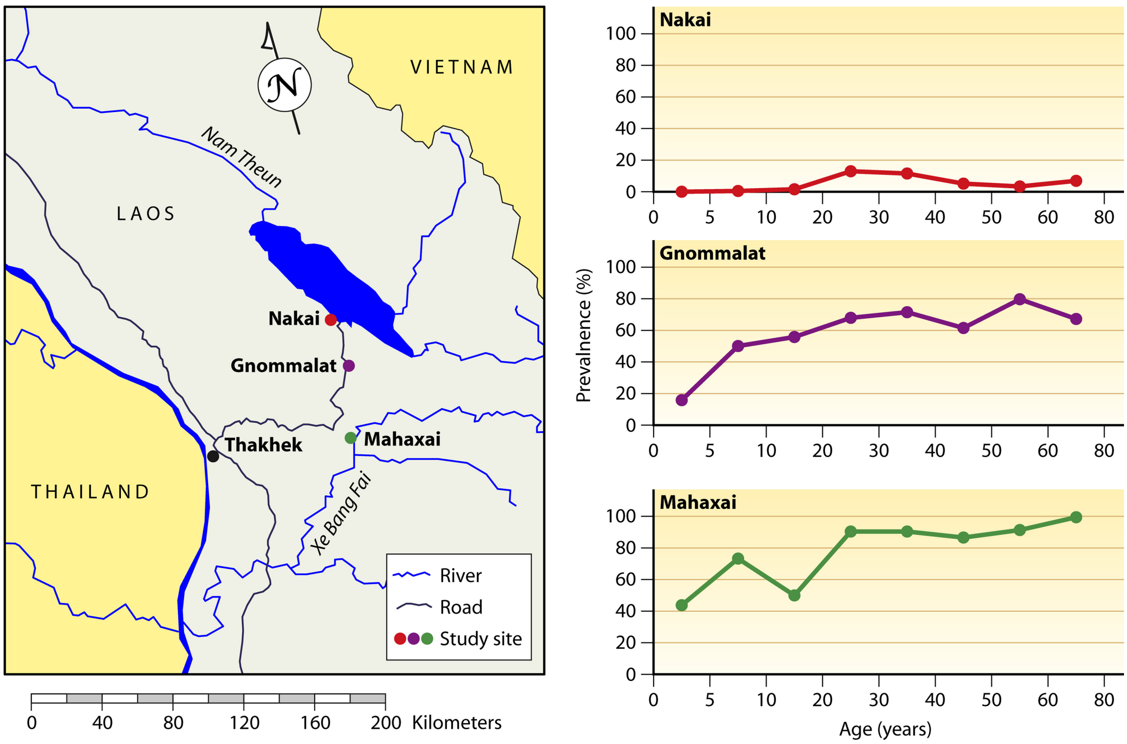

FIG. 3. Age prevalence curves for *Opisthorchis viverrini* at three different locations in the Nam Theun 2 hydroelectric project area in central Lao PDR in the late 1990s.

From the mid-1990s until 2004, the WHO provided global burden estimates for many different diseases in the annex tables of their annual world health reports. The burden is a measure of the number of healthy life years lost due to a disease or injury; hence, it combines premature death and the number of years lived with disability. To compare the burden across diseases and risk factors, the so-called disability-adjusted life year (DALY) measure was introduced (76). Interestingly, no burden estimates were given for food-borne trematodiases (see, for example, reference 119). This lack of a global burden estimate for food-borne trematodiases has been discussed in recent articles pertaining to neglected tropical diseases (40, 43, 109). It is encouraging that new efforts are under way to remedy this issue, as experts have been called upon to review the literature in a systematic manner to obtain prevalence, incidence, morbidity, and mortality data for over 150 risk factors and diseases (77). Food-borne trematodiases are now part of this endeavor. The ultimate outcome of this renewed effort is to come forward with new or revised burden estimates for diseases, injuries, and risk factors, expressed in DALYs. This research will also allow researchers to scrutinize the previous estimate of the annual mortality rate for food-borne trematodiases, which has been reported to be 10,000 (22).

GEOGRAPHIC DISTRIBUTION

The transmission of food-borne trematodiases is restricted to areas where the first and second intermediate hosts coexist (which requires suitable climatic and environmental factors) and where humans have the habit of eating raw, pickled, or undercooked fish and other aquatic products (49, 95). These contextual determinants explain why the distribution of food-borne trematodiases is focal. Figure 3 underscores this issue; while *O. viverrini* is highly prevalent among inhabitants of the Xe Bang Fai downstream area in central Lao PDR, with all age groups affected, only very low prevalences were found for the Nakai plateau a few dozen kilometers apart. It will be interesting to carefully monitor the occurrence of *O. viverrini* and other food-borne trematode infections in this setting of the Lao PDR, as there is a large water resource project being implemented, the Nam Theun 2 hydroelectric project (26).

C. sinensis is endemic in China, the Republic of Korea, Taiwan, and Vietnam. *O. viverrini* is prevalent in Cambodia, the Lao PDR, Thailand, and Vietnam, whereas *O. felineus* is endemic to the former Soviet Union, Kazakhstan, and the Ukraine (49). At present, *F. hepatica* is endemic on all continents but is of particular public health importance in the Andean countries, Cuba, the Islamic Republic of Iran, Egypt, and western Europe (e.g., France, Portugal, and Spain) (70). Infections with *F. gigantica* are restricted to Africa and Asia (70). *Paragonimus* infections occur mainly in tropical and subtropical areas of East and South Asia and sub-Saharan Africa. They are also found in the Americas, ranging from southern Canada to Peru (6). With regard to intestinal flukes, infections with *Echinostoma* spp. in China, India, Indonesia, Japan, Malaysia, Russia, the Republic of Korea, the Philippines, and Thailand have been reported (11). *F. buski* is endemic to Bangladesh, China, India, Indonesia, the Lao PDR, Malaysia, Taiwan, Thailand, and Vietnam (11). *Heterophyes heterophyes* infections in Egypt, Greece, the Islamic Republic of Iran, Italy, Japan, the Republic of Korea, Sudan, Tunisia, and Turkey have been reported (11). The most commonly found intestinal fluke infection in the Far East (China, the Republic of Korea, and Taiwan) is *Metagonimus yokogawai.* It also occurs in the Balkan states, Israel, Russia, Siberia, and Spain (11).

HISTORICAL ACCOUNT

Paragonimus spp.

The first report of an adult *Paragonimus* fluke obtained from humans dates back to 1879, during an autopsy carried out in a hospital in Taiwan (formerly Formosa). A few years earlier, *Paragonimus* flukes were recovered from animals including otter, tiger, and mongoose. A first attempt to determine the life cycle of *Paragonimus* was undertaken by the British physician and tropical disease specialist Patrick Manson, working in China at the time, who obtained miracidia and correctly speculated that snails are involved in the transmission of the infection. Half a century later, in Shinchiku, Taiwan, in 1915, the Japanese physician Koan Nakagawa discovered that crabs act as a second intermediate host. Two years later, Nakagawa eventually succeeded in infecting the first intermediate host, the snail *Melania libertine*, with *Paragonimus* miracidia. Elucidation of the entire *Paragonimus* life cycle was completed using experimental animals in 1934 (19, 36).

Clonorchis sinensis

An early documentation of human clonorchiasis dates back some 2,000 years to the Western Han dynasties. For example, *C. sinensis* eggs were detected in fecal remains from an ancient corpse exhumed in Hubei, China (68). The liver fluke *C. sinensis* was first reported in India in 1874. During an autopsy of a 20-year-old Chinese person, “small bodies” escaped from the bile ducts. Thirteen years later, the same parasite was discovered in cats and dogs in Japan. Following the publication of the life cycle and intermediate hosts of several food-borne trematodes, new research was launched to identify intermediate hosts of *C. sinensis*. It was the second intermediate host that was first discovered by the Japanese zoologist Harujiro Kobayashi while working in South Korea in 1911. Kobayashi fed kittens and cats with two different kinds of fish (i.e., *Pseudorasbora parva* and *Leucogobia guntheri*), and all kittens and cats died from a *C. sinensis* infection. The first intermediate host snail (i.e., *Bithynia striatula* var. *japonica*) was identified toward the end of the 1910s by another Japanese researcher (19, 36).

Opisthorchis felineus and *Opisthorchis viverrini*

Infections of humans by *O. felineus* and *O. viverrini* were first recorded in 1892 and 1911, respectively. The full elucidation of the life cycle of *O. felineus* was accomplished in Germany in 1934, following earlier successful studies of the second intermediate host. The life cycle of *O. viverrini* was only fully elucidated in 1965 (19, 36).

Fasciola spp.

Fascioliasis must have been common in prehistoric times; *Fasciola* eggs have been documented by many paleoparasitological studies of different European settings (69). For example, *Fasciola* eggs were discovered recently when 20 human coprolites from Arbon Bleiche, the Neolithic lakeside settlement at Lake Constance in Germany (3384 to 3370 BC) were analyzed (63). It is not surprising that *F. hepatica* was the first food-borne trematode to be described, which is explained by the large burden which this liver fluke caused in domestic sheep. Reports date back to 1379, and the first detailed descriptions of *F. hepatica* were published in 1523 (36, 83). The presence of *F. hepatica* flukes in humans was first documented in 1760, during an autopsy of a female in Berlin, Germany (36). Between 1880 and 1883, two researchers, Algernon Thomas from England and Rudolf Leuckart from Germany, discovered at the same time that *Lymnea* snails act as the first intermediate host (36, 83). In 1893, it was finally revealed that domestic herbivorous animals acquire this liver fluke infection by swallowing the encysted stage of the parasites (metacercariae) on grass and vegetables, and hence, the life cycle was fully elucidated (36, 83).

Intestinal Flukes

In London, English surgeon George Busk described *F. buski* in 1843 following an autopsy of an Indian sailor (16). Subsequently, detailed studies pertaining to the biology and the morphology of this intestinal fluke were carried out. Nakagawa, who was already instrumental in discovering that crabs are the second intermediate host of paragonimiasis and who successfully infected the first intermediate host snail with *Paragonimus* metacarcariae, fully elucidated the life cycle of *F. buski* in 1920 (36). For a detailed review of the discovery and description of a number of the Echinostomatidae, an important group of intestinal flukes, the reader is referred to works by Chai (10, 11). For example, *Echinostoma revolutum*, the oldest echinostome species in the literature, was discovered by Froelich in 1802 in Germany (10, 11).

CLINICAL MANIFESTATIONS

Food-borne trematodes and other parasitic worms do not replicate in the human host, which is different from, for example, infections with the protozoan parasites *Plasmodium* spp. (causing malaria) (66). Previous studies have shown that morbidity due to food-borne trematodiases and other trematode-borne diseases (such as schistosomiasis) is associated with the number of worms, and infection intensity (usually expressed by the number of parasite eggs per gram of feces) is often utilized as a proxy measure for morbidity (91). Depending on the worm burden, which is indirectly assessed by infection intensity, inflammatory lesions and damage of tissues and target organs can occur. Chronic inflammation and infection with *C. sinensis* and *O. viverrini* were suggested to contribute to serious secondary complications such as cholangiocarcinoma (96). The clinical manifestations of acute and chronic infections are summarized in Table 1.

Clonorchis sinensis

Light infections with *C. sinensis* (<100 flukes) are in general asymptomatic or have few clinical signs such as diarrhea and abdominal pain. Infections with a moderate parasite load (101 to 1,000 flukes) might cause fever, diarrhea, loss of appetite, rash, edema, night blindness, swollen abdomen, and enlargement of the liver at the onset of the disease (91). Patients with a very high worm burden (up to 25,000 flukes) might also present with acute pain in the right upper quadrant (68). Often, the acute symptoms subside after a few weeks and are followed by chronic complications.

In the chronic stages, the liver might eventually become malfunctioning. Biliary obstruction, intrahepatic stone formation, cholangitis, cholecystitis, cholelithiasis, biliary and liver abscesses, pancreatitis, and hepatitis are further severe symptoms. An increased risk of developing cholangiocarcinoma, a malignant tumor that arises from any portion of the bile ducts, is the most important clinical manifestation (58, 85, 91, 92). *C. sinensis* has been classified by the International Agency for Research on Cancer (IARC) as a probable carcinogen (group 2A) (58). Although the actual mechanism of carcinogenesis is not known, irritation of the fluke might be involved (14). The tumor is rare in patients younger than 40 years of age and usually occurs in patients aged 60 years and older (14). Further details regarding the etiology, diagnosis, and management of cholangiocarcinoma and clonorchiasis were described in a recent review (14).

Opisthorchis viverrini

Infections with *O. viverrini* are often free of symptoms, particularly when of light intensity. Flatulence, fatigue, dyspepsia, right upper quadrant abdominal pain, anorexia, and mild hepatomegaly occur in approximately 5 to 10% of infections (91). Severe infestations, which are rare, might cause obstructive jaundice, cirrhosis, cholangitis, acalculous cholecystitis, or bile peritonitis. Cholangiocarcinoma is the most serious complication of infections with *O. viverrini*. Studies carried out in the northeastern part of Thailand found a positive correlation between the endemicity of opisthorchiasis and the frequency of cholangiocarcinoma (97). Indeed, the highest incidence of cholangiocarcinoma has been reported for areas where *O. viverrini* is highly endemic. In Sakol Nakhon (upper Northeast Thailand), the highest national mortality rate of liver and bile duct cancer, a mortality rate of 61.4 per 100,000 people, has been reported (97). A similar association between opisthorchiasis and bile duct cancer is expected for the Lao PDR, where the prevalence of *O. viverrini* is high on a national scale (84), with pockets of very high prevalences and infection intensities in the central and southern parts of the country (88, 89). However, there is a paucity of high-quality data, which is partially explained by missing diagnostic and medical equipment, such as ultrasonography and trained medical staff, and challenges in the species-specific parasitological diagnosis of food-borne trematode infections. Hence, further in-depth clinical and epidemiological investigations are warranted for the Lao PDR (88, 89, 126).

The liver fluke *O. viverrini* is classified by the IARC as definitely carcinogenic (class 1) (71). Cholangiocarcinoma is a malignant tumor starting in the biliary epithelium of the intrahepatic biliary tree and might invade the sinusoids of the liver parenchyma (91). Although the etiology is not entirely known, many factors are likely to be involved in carcinogenesis, including mechanical and chemical irritation of the tissue by the flukes and immune responses (91, 97). Recent gene discovery studies provide a basis for subsequent molecular studies of cholangiocarcinogenesis (62). There are no early symptoms of cholangiocarcinoma. Once the bile ducts are obstructed, jaundice, ascending cholangitis, and pain are common (39). Cholangiocarcinoma is a tumor with extremely poor prognosis: bile duct surgery is difficult, and many patients suffer a painful death (39, 96, 97).

Opisthorchis felineus

In contrast to infections with *C. sinensis* and *O. viverrini*, many patients infected with *O. felineus* suffer from fever and hepatitis-like symptoms in the acute stage of infection (91). Right upper quadrant abdominal pain, nausea, and emesis have been reported (106). Chronic symptoms include obstruction of the biliary tract, inflammation and fibrosis of the biliary tract, liver abscesses, pancreatitis, and suppurative cholangitis (58, 106).

Fasciola hepatica and *Fasciola gigantica*

Dyspepsia, fever, right upper quadrant pain, anorexia, hepatomegaly, splenomegaly, ascites, urticaria, respiratory symptoms, and jaundice might be seen in the acute stage of a *Fasciola* infection (58). Once the parasites have entered the biliary tree in the chronic stages of fascioliasis, biliary colic, intermittent jaundice, epigastric pain, nausea, fatty food intolerance, cholangitis, acute pancreatitis, and cholecystitis may be seen (58, 70).

Intestinal Flukes

Clinical symptoms of patients suffering an infection with intestinal flukes might be associated with the parasite load (69). Light infections with intestinal flukes such as *F. buski* are often characterized by mild symptoms such as diarrhea, constipation, abdominal pain, dizziness, and headache. Moderate and particularly heavy infections cause severe abdominal pain or colic, diarrhea, vomiting, fever, nausea, and allergic reactions such as edema of the face, abdominal wall, and lower extremities (70). A heavy *F. buski* infection can be fatal, as the flukes cause extensive intestinal erosions, ulceration, hemorrhage, abscesses, and inflammation (69). Abdominal pain, diarrhea, and fatigue are typical symptoms of *Echinostoma* infections. Unless patients are heavily infected, clinical symptoms due to infections with species of heterophyidae are usually mild and transient (11).

Paragonimus spp.

Various symptoms have been associated with lung fluke infections depending on the infection intensity, species involved, stage of infection, and individual idiosyncrasies (6). In the early stages of pulmonary infections, symptoms include cough, fever, bloody sputum, loss of appetite, chest pain, and headache (6). A chronic, productive cough with brownish sputum, chest pain, and night sweats occur once the infection is established (92). Occasionally, adult worms migrate into ectopic locations such as the skin, liver, eye, abdominal organs, or brain. Cerebral paragonimiasis may lead to eosinophilic meningitis, which presents with headache, visual impairment, and convulsions (6, 92).

PATTERNS OF INFECTION, REINFECTION, AND COINFECTION

An important epidemiological feature of helminth infection is overdispersion. This means that among infected individuals, most harbor only a few worms, whereas a few individuals have high worm burdens. Those people with the largest numbers of worms are usually at the highest risk of disease, and they contaminate the environment disproportionately (1, 9). The typical pattern of overdispersion is well documented for both *O. viverrini* and *C. sinensis*. The distribution of infection intensity in a community is therefore characterized by most people having only light infections, whereas a few individuals are heavily infected (91). For example, a purging study of 246 Thai individuals found that 81% of *O. viverrini* flukes were expelled by 25 patients, hence, just 10% of the population surveyed (92). It has been speculated that the overdispersion of helminths is the result of environmental exposure (host behavior showing a strong age dependency), individual susceptibility (genetic and immunogenetic factors), and social factors (9, 43). While low prevalences of *O. viverrini* and *C. sinensis* in young children have been found, the prevalence increases with age and often reaches a plateau in the late teenage years and early adulthood. A study in Thailand found that slightly more males than females were affected by these two liver flukes, which suggests that males consume more raw or undercooked fish than females (91).

With regard to the liver fluke *Fasciola* spp., epidemiological studies of the Andean countries showed that infections were more prevalent and of higher intensity among children, although adults were also infected. An important aspect of such an age pattern is that there might be partial protection in acquiring an infection with age, giving rise to the possibility of developing a vaccine (73). Interestingly, higher prevalences and infection intensities were observed for females, most likely pointing to differential exposures (70).

Regarding intestinal fluke infections, school-aged children were found to be at the highest risk of infection and infection intensity, as documented previously for *F. buski* (32, 70). Similar observations have been made for the lung fluke *Paragonimus* spp.: most infections occur among school-aged children. However, in Japan, a change in the age distribution of paragonimiasis was observed, with the disease now being seen most prominently in middle-aged men (6).

Reinfection occurs rapidly after treatment, particularly in communities where a high incidence has been reported. For example, a study in Chooabot, Thailand, showed that in a community where 97.4% of inhabitants were infected with *O. viverrini*, the prevalence was again very high 1 year posttreatment, i.e., 94% (92).

Individuals are commonly infected with multiple species of trematodes as the areas of endemicity of the various parasites often overlap. In a purging study carried out in Thailand with 431 participants, seven different species of food-borne trematodes were discovered (82). In a more recent purging study conducted in the Lao PDR, with 97 patients aged ≥15 years suffering from hepatobiliary or intestinal symptoms, seven trematode species were observed. The liver fluke *O. viverrini* was recovered from all but two individuals (98%), whereas *Haplorchis taichui* (78%) was the most prevalent among the six different intestinal fluke species diagnosed. More than 80% of the patients investigated were coinfected with *O. viverrini* and one or multiple species of intestinal flukes (89).

New research is needed to investigate the largely neglected issue of multiple-species trematode infections. Although this will pose considerable challenges for diagnosis, it will deepen our understanding of how polytrematode infections affect human health and well-being, particularly from a clinical, pathological, and therapeutic point of view. It was previously reported that individuals that are coinfected with multiple helminths are at an elevated risk of morbidity (43). Indeed, multiple-species low-intensity nematode infections result in clinically significant morbidity, such as anemia (28). It is therefore conceivable that concurrent trematode infections, even at low intensities, negatively impact human health.

DIAGNOSIS

The importance of accurate diagnosis cannot be overemphasized for adequate patient management and for monitoring of community-based helminth control programs. An important underlying reason why food-borne trematodiases and other helminthic diseases are often neglected is related to a number of challenges and dilemmas in helminth diagnosis (5, 100). Moreover, there is no "one-size-fits-all" diagnostic technique, and there is a need to adapt the diagnostic approach to the stage of a control program (5).

The three main approaches that are currently available are direct parasitological diagnosis (detection of parasite eggs in stool, sputum, or other biofluids), immunodiagnosis (indirect diagnosis), and tests based on molecular biology approaches. Complementary tools for the diagnosis of the hepatobiliary parasitic infections are ultrasound, computer tomography, magnetic resonance imaging, and tissue harmonic imaging (58, 65, 68). These imaging devices are able to reveal the pathology of the bile ducts. Ultrasound, for example, can detect biliary stones, dilatation, and fibrosis due to liver fluke infections, while computer tomography and magnetic resonance imaging lend themselves to determinations of the lumen diameter of bile ducts, fibrosis, calcification, and epithelial hyperplasia. Tissue harmonic imaging is useful for the observation of bile duct wall trauma and stones (68). Finally, novel diagnostic assays might be developed as new research is under way for biomarker discovery in host animals experimentally infected with food-borne trematodes. Indeed, biomarker recovery might be harnessed for the rational development of not only novel diagnostics but also drugs and vaccines (86, 87, 111, 112).

Parasitological Diagnosis

Detection of eggs in feces (intestinal flukes, liver flukes, and lung flukes) and sputum (lung flukes) is the most commonly used approach for the diagnosis of food-borne trematode infections in epidemiological surveys and for the monitoring of control interventions. Occasionally, eggs are also recovered from other biofluids such as bile content, duodenal content, and gastric washing samples (92). Several coprological diagnostic methods are available, such as direct fecal smears, Kato-Katz thick smears, sedimentation techniques, Stoll's dilution egg count method, or the formalin-ethyl-acetate technique (91, 92, 118). It is commonly assumed that flotation techniques with salt or sugar solutions, which are widely and effectively used for the detection of nematode and cestode eggs (74), are less appropriate for the detection of trematode eggs such as those of *O. viverrini* (38). However, excellent results have been reported with the recently developed FLOTAC technique, a multivalent fecal egg count technique (21), for *F. hepatica* in sheep (56). Studies are under way in different epidemiological settings to further validate the FLOTAC technique for human food-borne trematode diagnosis.

The most widely used techniques are the Kato-Katz and formalin-ethyl-acetate techniques, because these two methods have a reasonably high sensitivity at the level of moderate and heavy infections and allow the quantification of infection intensity (92). It should be noted, however, that trematode species-specific diagnosis on the basis of egg morphology is difficult. For example, the sizes and shapes of *C. sinensis*, *O. viverrini*, and *O. felineus* eggs are similar (44). Egg morphologies are also similar for *F. hepatica*, *F. buski*, and echinostomes (92). Hence, differential diagnosis poses problems. In addition, very low numbers of eggs at low levels of infection or due to low egg-laying capacities of flukes may easily be missed. For example, numbers of echinostome eggs in stool samples vary greatly due to species-dependent differences in egg-laying capacities; while many eggs are seen in *Echinostoma hortense* infections, levels of egg release for *Echinochasmus japonicum* are low (11).

Diagnosis of heterophyids is a particular challenge since the egg-laying capacity of heterophyids is limited, and there is a problem in differentiating eggs from those of *Clonorchis* spp. and *Opisthorchis* spp. (92). Not only does accurate and species-specific diagnosis require highly qualified laboratory technicians and appropriate equipment, but adherence to external quality assurance schemes and participation in multicentric testing should also be considered for enhancing and sustaining diagnostic performance (110). However, a definite diagnosis requires the examination of adult worms, which can be obtained following expulsion chemotherapy (44, 89).

In settings characterized by low infection intensities, which are gaining importance in the era of "preventive chemotherapy" targeting helminthic diseases (60, 120), examinations of multiple stool samples and the use of a combination of diagnostic tests can further enhance the sensitivity of helminth diagnosis in general (60, 99) and food-borne trematode diagnosis in particular (67). This issue is of particular relevance for the diagnosis of *Paragonimus* spp., since eggs are not found in every sputum sample, necessitating multiple samples and additional diagnostic tools (6).

Immunodiagnosis

Several types of tests aimed at the detection of specific antibodies during (or after) an infection are available, such as intradermal tests, indirect hemagglutination assays, indirect fluorescent antibody tests, and tests based on the indirect enzyme-linked immunosorbent assay (6, 91). These tests use different extracts from parasites, such as adult extracts, secretory substances, or egg antigens, and have the advantage of being applicable during both the acute and the chronic stages of the disease (92). For example, intradermal tests make use of small amounts of diluted antigen, which is injected into the skin. A specific, immunological reaction manifests itself as swelling and a flare reaction, which generally become evident within a day. False-negative results are rare, but the test can remain positive for several years after the successful removal of an infection with anthelmintic drugs (6). Due to its low specificity, the test is not recommended any longer for the diagnosis of *C. sinensis* (14). Cross-reactivity is another important issue to be considered. Several enzyme-linked immunosorbent assays have been developed in the past years for lung, liver, and intestinal fluke infections and have been improved considerably in terms of sensitivity and specificity, and their costs have been reduced. Research in this field is ongoing, as there is still no consensus on the ideal test system or specific antigen. Once these data have been assembled, it is hoped that immunodiagnostic tests will be further developed for commercial use.

Molecular Diagnosis

In the past few years, various PCR-based methods have been developed and improved to detect egg trematode DNA in stool samples or metacercariae in the second intermediate host, which today offer high sensitivity and specificity, even at low infection intensities, compared to those of direct parasitological and indirect immunological diagnostic tools (67). However, PCR diagnosis is unlikely to become a tool for routine diagnosis in resource-constrained settings, as investments and the costs involved are high, but the approach remains useful as a research tool (6, 67, 72, 91, 105).

Novel Approaches for Biomarker Discovery

Research is currently under way to assess the scope and limits of a metabolic profiling strategy for biomarker discovery that may give rise to novel diagnostic and prognostic assays for a host of helminth infections. Metabolic profiling uses a combination of analytical tools such as high-resolution nuclear magnetic resonance spectroscopy and mass spectrometry in combination with multivariate statistical analysis to quantitatively measure dynamic biochemical responses of living organisms to physiological or pathological stimuli (80). A metabolic profiling strategy has been successfully employed for biomarker discovery of the two blood flukes *Schistosoma mansoni* and *Schistosoma japonicum* (114, 115). Recently, the metabolic fingerprint of an *E. caproni* infection in different biofluids obtained from mice has been characterized (87). Changes in the metabolic profiles of blood plasma, fecal water, and urine samples from mice were already apparent 1 day after experimental infection with 35 metacercariae. For example, concentrations of plasma choline, acetate, formate, lactate, and urinary creatine were decreased, while the concentration of plasma glucose was upregulated (87). A study that examined the urine of *E. caproni*-infected mice by high-performance thin-layer chromatography–densitometry revealed qualitative and quantitative differences in concentrations of alanine and taurine (112).

In another recent study, serum biomarkers in sheep consequential to an *F. hepatica* infection were recovered and validated over the course of the first 12 weeks of infection by using surface-enhanced laser desorption ionization–time of flight mass spectrometry. Among other biomarkers, transferrin and apoliprotein A-IV were significantly upregulated at 9 weeks postinfection (86).

While these early results hold promise for the further development of a metabolic profiling strategy and other approaches for biomarker discovery, it will take years until new diagnostic assays become available. Sensitivity and specificity are important issues to be considered (5), as are overall cost and applicability in resource-constrained settings where food-borne trematode infections are most highly endemic.

EPIDEMIOLOGICAL PATTERNS

Nutrition-related behavior and formal and informal distribution networks of aquatic products that are contaminated with metacercariae govern the distribution of infections with food-borne trematodes. While contamination of human-made freshwater environments (inland fish ponds) with food-borne trematode metacercariae is quite common, human infections occur mainly in areas characterized by traditional food dishes in specific high-risk ethnic groups (126). Raw, pickled, or undercooked fish and other aquatic products are prepared in various ways. Such dishes might have been rooted in traditions for hundreds of years and hence have high cultural, ethnic, and nutritional significance, making it exceedingly difficult to change food habits (91). Examples of typical traditional preparations include raw crab meat spiced with soy sauce (ke-jang) in the Republic of Korea, raw grass carp dishes in China, and fresh uncooked small- or medium-sized fish, moderately or extensively fermented (lab pla, koi pla, pla som, and pla ra), in Thailand (91) and in the Lao PDR (45).

Interestingly, while food-borne trematodiases diminished or disappeared in some countries, in other settings, food-borne trematodiases are emerging (32, 49). In China, for example, the number of people infected with *C. sinensis* has almost tripled over the past decade, reaching a level of 15 million cases in the new millennium (54, 68). Population growth, a changing network of food distributions, pollution, and poverty, among other factors, contribute to changing patterns in the epidemiology of food-borne trematode infections. Human-made ecological transformations, including dam constructions and implementation of irrigation schemes to maintain food security as irrigation systems and aquaculture (described below), influence the spread of these diseases. For example, it was demonstrated that fascioliasis emerged after irrigation systems had been built in Egypt and Peru (70). The construction of roads, dams, power plants, and factories negatively influences the presence of aquatic animals (6). A lack of improved sanitation due to declining socioeconomic conditions as well as the discharge of human excreta directly into canals contaminate water bodies, leading to infections of snails, fish, crustaceans, or aquatic plants, and, hence, result in an increased availability of contaminated aquatic flora and fauna that is subsequently used for human consumption (48). Additionally, in many resource-poor countries, appropriate food inspections are lacking, in the face of expanding distribution networks. On the other hand, social and economic advances, combined with health education campaigns and mass drug administration, have reduced prevalences of food-borne trematode infections in several countries (48). For example, in Japan, prevalences of paragonimiasis, which had been high in children, decreased enormously in the 1960s following education programs. Interestingly, the disease resurged in the 1990s, now common mainly in middle-aged men, due to the consumption of undercooked meat of wild boar (6). Population dynamics of intermediate host snails are governed by different environmental factors, i.e., quality, current, and temperature of freshwater bodies. Hence, pollution might influence the growth and reproduction of food-borne trematodes, resulting in lower parasite diversities and intensities at polluted sites. For example, snails exposed to metals had lower trematode infection intensities, and fewer species were found (64). In Henan province, China, low prevalences of paragonimiasis were found for two villages, where gold mining had contaminated streams and killed crabs, the second intermediate host of paragonimiasis (6). Pesticide use in rice paddies and farms has been described to cause the death of the first and second intermediate aquatic hosts in China and South Korea (6).

Climate change also influences the distribution of food-borne trematodiases. For example, the incidence of human fascioliasis increased in a 2-year period following the El Niño Southern Oscillation in Ecuador (34). Air temperature and rainfall are crucial climatic factors in the transmission of fascioliasis, as they affect the free-living stages of the parasite, the incidence of vegetation, and mammals (definitive host) and govern the population dynamics of the intermediate host snails (33, 70). Finally, food-borne trematodiases are being more frequently diagnosed in developed countries due to increasing travel patterns and consumption of exotic foods (47).

FOOD-BORNE TREMATODIASES AND WASTE-FED ACQUACULTURE

Fish is rich in minerals, proteins, and essential fatty acids and, hence, is highly nutritious. The global per-capita fish consumption rate has almost doubled over the past 40 years; in 2003, it was estimated at 16.5 kg per capita (30). Today, aquaculture (inland freshwater fish production) already contributes one-third to the global aquatic animal supply (29). To meet the projected demand and to maintain food security, aquaculture is steadily expanding in terms of both total production and the range of species farmed. At an average annual growth rate of 8.8%, aquaculture is expanding more rapidly than any other food-producing sector. From a capacity of less than 1 million tons 50 years ago, the production of freshwater fish and aquatic plants has grown to almost 60 million tons in 2004 (29). On the other hand, capture fisheries at a global scale are leveling off, as most of the important marine species are fully exploited or even overexploited (29).

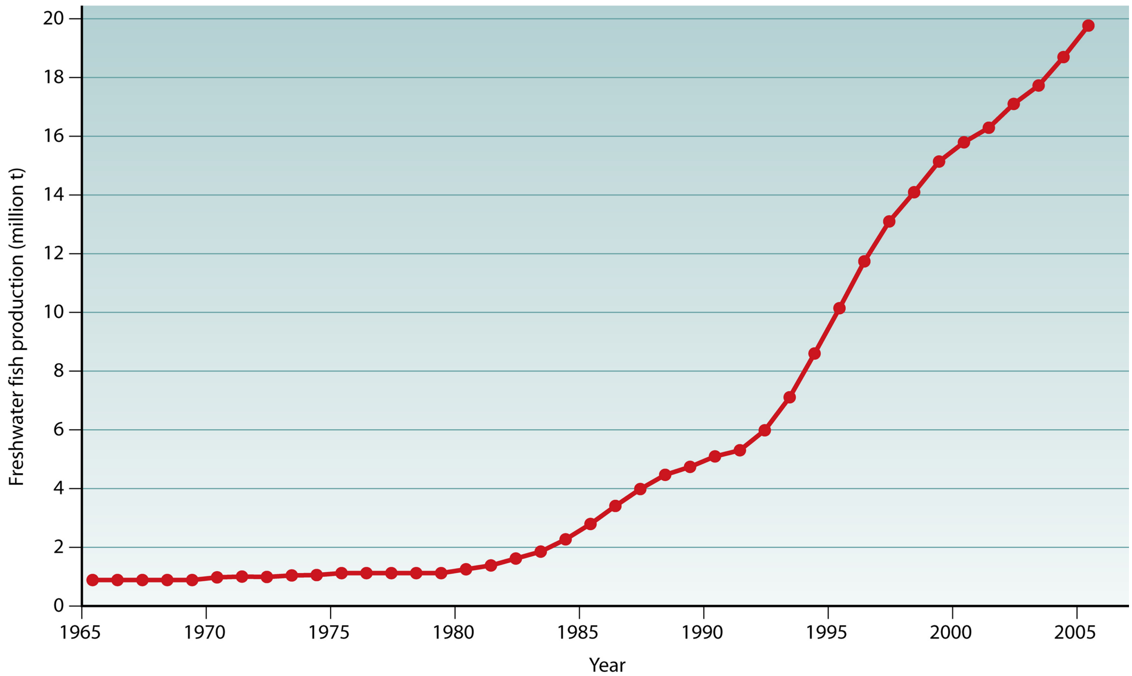

FIG. 4. Evolution of inland freshwater fish production in China from 1965 to 2005. (Based on data from http://www.fao.org/figis/servlet/TabLandArea?tb_ds=Aquaculture&tb_mode=TABLE&tb_act=SELECT&tb_grp=COUNTRY.)

Aquaculture production varies from one region to another. In Asia, particularly in China, where food-borne trematodes are highly endemic, aquaculture plays an important role. Figure 4 shows the exponential growth of aquaculture freshwater fish production in China over the past 40+ years. Indeed, freshwater fish cultivation in China has grown by more than a factor of 25, from 0.7 million tons in 1963 to more than 19 million tons in 2005. The rate of production of inland freshwater crustaceans in China was only 10,626 tons in 1950. In 2005, 1.9 million tons were farmed. Not only China but also other Asian countries where food-borne trematodes are endemic experienced an enormous growth of freshwater fish production. In the Lao PDR, aquaculture fish production increased from 16 tons in 1963 to 78,000 tons in 2005. For additional country statistics, the reader is referred to the Food and Agricultural Organization (FAO) website (http://www.fao.org/figis/servlet/TabLandArea?tb_ds=Aquaculture&tb_mode=TABLE&tb_act=SELECT&tb_grp=COUNTRY). Of note, these are official summary statistics. Since small businesses, rice field fisheries, and recreational fishing are not included, it is conceivable that the "true" extent of inland freshwater fish production might be considerably greater (15). For example, in Luang Prabang, Lao PDR, 83% of the households are engaged in small-scale fishing, but their catches are not included in national statistics (15). The discrepancy between officially reported catches and estimates based upon independent scientifically based surveys was found to vary by a factor 4.2 up to 21.4 (15).

Of potential concern for food-borne trematodiases is not only the expansion of habitat area and increase in intermediate hosts but also that wastewater and excreta use, driven by water scarcity and a lack of availability of nutrients, is more often considered to be an ideal method of combining water and nutrient recycling (121). Hence, these developments should be examined closely in countries where food-borne trematodes are endemic, as the number of infections might increase due to a rise in numbers of infected freshwater products.

In Vietnam, several in-depth studies have analyzed intermediate hosts for the presence of metacercariae under different environmental conditions, such as cultured fish or waste-fed ponds. In traditional and household fish ponds, 30 to 50% of the fish were found to be infected with food-borne trematodes (13, 79, 121). Interestingly, though, a study comparing cultured and wild fish found a higher prevalence of liver and intestinal trematode metacercariae in wild fish (30.6%) than in cultured Tra catfish (2.6%) (102). Fish analyzed from wastewater-fed ponds in the periurban areas of Hanoi and Nam Dinh showed prevalences of metacercariae of intestinal flukes of around 5%. The infection rates were slightly lower in the fall (2 to 2.5%), most likely due to ecological factors (79). Finally, a seasonal investigation analyzing mono- and polycultured fish and an integrated vegetable-aquaculture-animal husbandry system found the highest prevalence of intestinal trematode metacercariae (6.6%) in carp raised in polyculture (101). In conclusion, suitable conditions for the transmission of food-borne trematodes occur in different ecosystems (be they waste-fed, traditional, or wild aquaculture systems) and depend on environmental and climatic conditions, physical characteristics of wastewater, the presence of reservoir hosts, and the contamination of water bodies from human defecation and animal feces, among others.

PREVENTION, TREATMENT, AND CONTROL

Public health interventions to prevent infections with food-borne trematodes and to reduce the prevalence and intensity of infections, and, hence, morbidity and mortality, include chemotherapy, improved access to adequate sanitation, and the use of chemical fertilizers, food inspections, and information, education, and communication (IEC) campaigns. The ultimate aim is to change human behavior, because the consumption of raw or undercooked freshwater fish and other aquatic products is the key risk factor for acquiring a food-borne trematode infection (118). Vaccines are presently not available for the prevention of food-borne trematodiases, but recent gene discovery efforts may assist in the rational development of vaccines and the next generation of trematocidal drugs (62).

Chemotherapy: Current Drugs

In areas where food-borne trematodiases are highly endemic, the current emphasis rests on chemotherapy-based morbidity control. However, the arsenal consists of only two drugs: praziquantel and triclabendazole (48, 54). Praziquantel was synthesized by Merck in the mid-1970s (90) and was further developed by Bayer in the late 1970s/early 1980s (2). Praziquantel has long been the drug of choice for schistosomiasis, with tens of millions of treatment courses administered in Africa, China, and elsewhere (12, 24, 31, 108). Praziquantel exhibits a broad spectrum of activity against trematodes and has an excellent safety profile, and hence, it became the drug of choice for clonorchiasis, opisthorchiasis, paragonimiasis, and intestinal fluke infections (48). Although the exact mechanism of action of praziquantel on trematodes remains to be elucidated, it has been postulated that a disruption of Ca^{2+} homeostasis occurs as praziquantel induces a rapid contraction of trematodes (35). For intestinal fluke infections, a single oral dose of praziquantel of 25 mg/kg body weight is recommended by the WHO. For the treatment of liver and lung fluke infections, 25 mg/kg praziquantel three times a day for two consecutive days is recommended, a treatment schedule which is often used in hospitals and outpatient facilities (118). In mass drug administration programs, a single 40-mg/kg oral dose of praziquantel is commonly applied for the treatment of clonorchiasis, opisthorchiasis, and paragonimiasis (Table 1) (118). All treatment schedules with praziquantel were well tolerated, with only few adverse events that were minor and transient, including abdominal pain, dizziness, headache, nausea, and urticaria (48). Thus far, praziquantel resistance is of no clinical concern, although a low cure rate (29%) was documented for patients infected with *C. sinensis* who were treated with three daily 25-mg/kg doses of praziquantel in Vietnam (103). Given the growing rate of praziquantel administration in the era of "preventive chemotherapy" (120), there is growing concern that resistance to praziquantel might eventually develop (24).

Triclabendazole, a benzimidazole derivative, is used for the treatment of fascioliasis and holds promise for treating *Paragonimus* infections. The drug was developed as a veterinary product and is still not widely available for the treatment of humans suffering from fascioliasis. Currently, triclabendazole is registered in only four countries: Ecuador, Egypt, France, and Venezuela (50). A single postprandial dose of 10 mg/kg triclabendazole is recommended, which can be repeated after 12 to 24 h for patients suffering from heavy infections. A double dose is also recommended in the event of treatment failures with a 10-mg/kg dose (Table 1). Abdominal pain, biliary colic, fever, nausea, pruritus, vomiting, weakness, and liver enlargement following treatment have been reported. So far, treatment failures have not been reported for humans; however, there are concerns that triclabendazole resistance might emerge, as it is common in veterinary medicine (7, 50). Bithionol, a bacteriostatic agent which has been used since the early 1960s for the treatment of fascioliasis and paragonimiasis, is still applied for fascioliasis in cases where triclabendazole is not available (54). Long treatment schedules of 10 to 15 days are required (54).

Combination Chemotherapy and Future Drugs?

Preliminary studies of rodent models have shown that the artemisinins (e.g., artemether and artesunate) and synthetic peroxides (e.g., synthetic trioxolanes [OZs]), best known for their antimalarial properties (113, 116), and the Chinese anthelmintic drug tribendimidine (122) might be further developed for use against food-borne trematodiases (54). For example, a single oral dose of either artesunate, artemether, or OZ78 (100 to 400 mg/kg) resulted in 100% worm burden reductions in a chronic *F. hepatica* infection in the rat model (52, 51). Moreover, artesunate and artemether showed promising activities against adult *C. sinensis* flukes harbored in rats: worm burden reductions of 99 to 100% were observed with a single 150-mg/kg oral dose of either drug (53). Finally, studies carried out using *O. viverrini*-infected hamsters showed that a single 400-mg/kg oral dose of artesunate and artemether resulted in worm burden reductions of 78% and 66%, respectively (53). With regard to tribendimidine, a single 150-mg/kg oral dose administered to rats infected with adult *C. sinensis* flukes yielded a 99.1% reduction of worms, whereas at a dose of 400 mg/kg tribendimidine reduced adult *O. viverrini* flukes in hamsters by 95.7%. On the other hand, tribendimidine showed no activity against *F. hepatica* in the rat model (55).

In Fig. 5, we show how artemether and tribendimidine disrupt the tegument of adult trematodes, as revealed by scanning electron microscopy. Tegumental alterations observed among adult *F. hepatica* (Fig. 5B), adult *C. sinensis* (Fig. 5D), and adult *O. viverrini* (Fig. 5F) flukes are shown after the administration of a single oral dose of artemether (Fig. 5B and D) and tribendimidine (Fig. 5F). For comparison, control specimens recovered from animals without drug treatment are shown in Fig. 5A, C, and E. Prominent morphological features observed following the treatment of rodents with artemisinins included sloughing, blebbing, and roughening of the tegument (Fig. 5B and D). A closure of the oral sucker was seen for *O. viverrini* flukes treated with tribendimidine (Fig. 5F).

Combination chemotherapy, which is, for example, widely used for the treatment of tuberculosis (124), might also hold promise for the treatment of food-borne trematodiases, as treatment options are very limited, and drug resistance is a growing threat in the era of "preventive chemotherapy." Studies are ongoing in our laboratories and the National Institute of Parasitic Diseases in Shanghai, China, to study the effect of combined doses of praziquantel or triclabendazole with artemether, artesunate, OZ78, and tribendimidine in selected trematode-rodent models. For example, combination chemotherapy with praziquantel plus artemether, artesunate, OZ78,

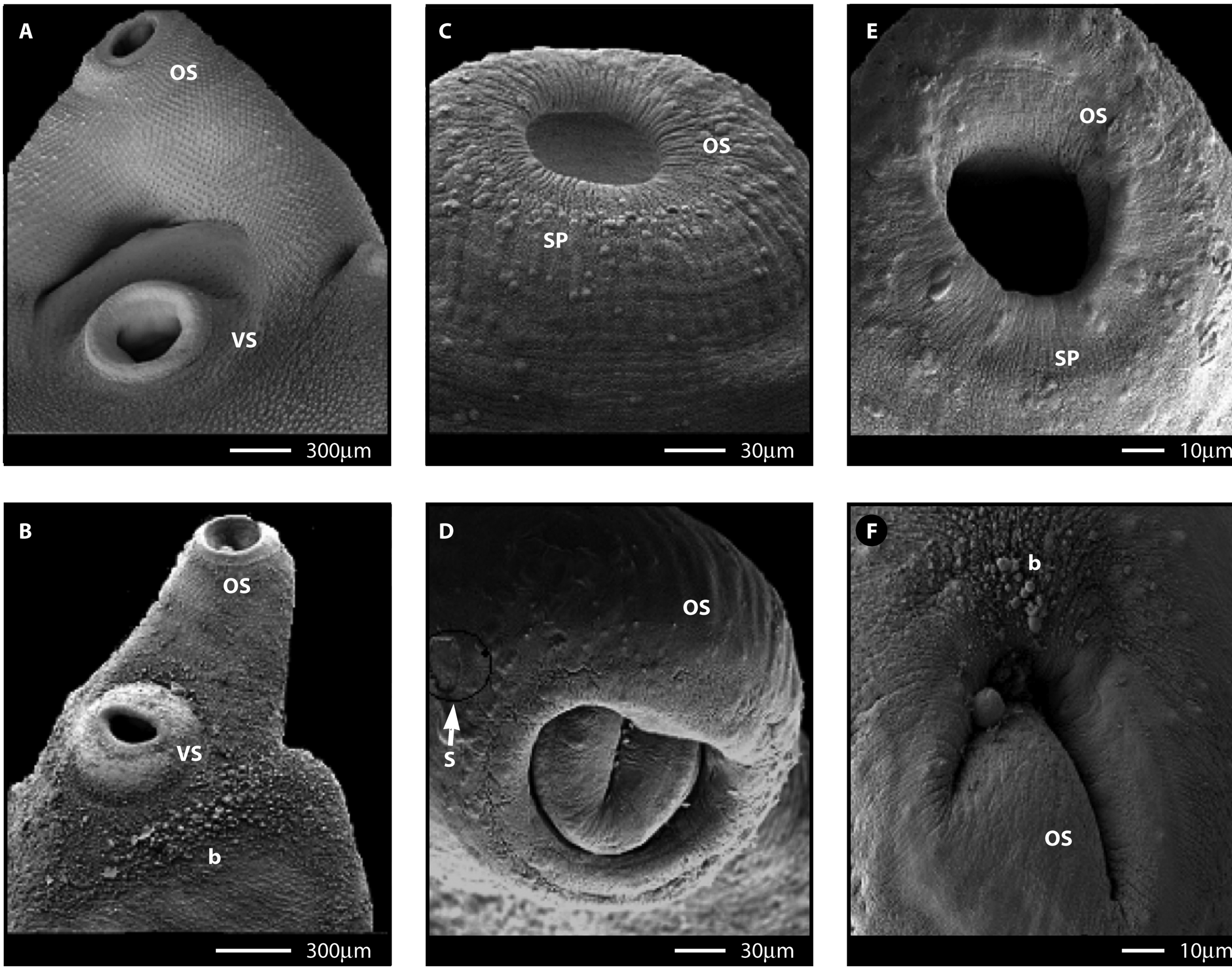

FIG. 5. Scanning electron microscope observations of the anterior part of an untreated *Fasciola hepatica* depicting the oral sucker (OS) and ventral sucker (VS) (A), a *Fasciola* fluke revealing blebbing (b) near the oral sucker and ventral sucker after administration of a single oral dose of 200 mg/kg artemether (B), the oral sucker region of a *Clonorchis sinensis* fluke with sensory papillae (SP) visible (C), sloughing (s) seen on the oral sucker of an artemether-treated *C. sinensis* fluke (D), oral sucker region of an *Opisthorchis viverrini* fluke with sensory papillae (E), and blebbing and closure of oral sucker observed on an *O. viverrini* fluke treated with a single oral dose of 400 mg/kg tribendimidine (F).

and tribendimidine showed either synergistic or antagonistic effects depending on the doses administered to *C. sinensis*-infected rats (123; J. Keiser et al., unpublished observations).

Integrated Control

Reinfection can occur rapidly following treatment when there are no supportive control measures in place, such as locally adopted IEC, stressing the importance of thoroughly cooking all aquatic products and boiling water prior to consumption. A comparative study was carried out in two villages in Thailand. In the first village, an integrated-control approach consisting of chemotherapy, sanitation improvement, and health education was implemented, while the other village focused on treatment only. The prevalence of *O. viverrini* 1 year after the control program commenced was significantly lower in the village using integrated control than in the village where treatment alone was implemented (36.8% versus 54.8%). Moreover, monthly reinfection rates in the integrated-control village were less than half of those in the treatment-only village (2.0% versus 5.0%) (94).

In Zhejiang province, China, the prevalence of *Paragonimus* spp. was reduced from an initial level of 31.1% to 0.05% some 30 years later, and this decline was due mainly to health education (6). In addition, hygiene levels need to be improved, clean water needs to be provided, and adequate sanitation facilities must be installed and properly used. For example, recent research conducted in the Lao PDR found a significant association between opisthorchiasis and lack of sanitation (88). In several countries where fluke infections are endemic, toilets are built in close proximity to fish ponds so that feces, also food-borne trematode egg-contaminated ones, are directed into pond water (68, 125). Hence, there is a need to eliminate these water toilets and construct more adequate sanitation facilities. It is important that implementing a setting-specific IEC and improving access to sanitation are challenging public health tasks, as they require a deep understanding of cultural beliefs, practices, and agricultural traditions and are costly.

Finally, food control is necessary to protect customers. For example, guidelines by the Food and Drug Administration (FDA) recommend freezing at −20°C or below for 7 days or at −35°C or below for 15 h to retailers who provide fish intended for raw consumption (29). Subjecting aquatic products to cold temperatures is an acceptable method of killing metacercariae. Countries where fish inspection services currently do not have the resources to practice effective control should be financially supported. In addition, the application of hazard analysis and critical control point, which is a systematic preventive approach to food safety and includes hazard analysis, identification of critical control points, preventive measures, critical limits, monitoring, recording, and validation, should be encouraged. This approach has been successfully applied to the prevention and control of *O. viverrini* in farming carp. For example, water supply, fish feed, and pond conditions were carefully monitored, and control measures were taken accordingly for each hazard (57).

CONCLUSION AND RESEARCH NEEDS

Food-borne trematodes must have accompanied men and women since prehistoric times, and today, 1/10 of the world population are at risk for infection. These infections are neglected yet are emerging in many parts of the world, which contributes to the considerable growth in the number of infected people, who are already counted in the millions. Nonetheless, considerable progress has been made in recent years toward an improved diagnosis and a better understanding of the epidemiology, pathology, pathogenesis, prevention, treatment, and control of the major food-borne trematodiases, as reviewed here. These advances should facilitate, for the first time ever, a reasonably accurate estimate of the global burden due to food-borne trematodiases. In our view, estimating the global burden of neglected tropical diseases, including food-borne trematodiases, is of pressing necessity, and hence, we applaud the new initiative under way since late 2007, which should deliver these estimates by mid-2010.

Given the root cause of food-borne trematodiasis transmission via the consumption of raw or undercooked freshwater fish and other aquatic products, concerted efforts should be made to enhance setting-specific IEC materials. In our opinion, these efforts should go hand-in-hand with other preventive and curative interventions. From a fundamental research point of view, discovery and development of new diagnostic tools, vaccines, and new trematocidal drugs are urgently needed. There are currently only two drugs available, and there is concern about resistance development. Piggybacking on drug development against other tropical diseases should be further pursued, which is justified based on promising results with the artemisinins, OZs, and tribendimidine. From an operational point of view, it should be determined how improved access to clean water, adequate sanitation and sewage treatment, and enhanced food safety measures have an impact on food-borne trematodiases. While chemotherapy-based morbidity control should serve as the backbone of control programs in areas where food-borne trematodiases are highly endemic, integrated control approaches and intersectoral collaboration between public health and veterinary medicine warrant more attention, including considerations of feasibility, efficacy, and cost-effectiveness.

ACKNOWLEDGMENTS

We are both indebted to the Swiss National Science Foundation for personal career development grants (project no. PPOOA-114941 to J.K. and PPOOB-102883 and PPOOB-119129 to J.U.).

We thank our colleague Robert Bergquist for comments and suggestions on the diagnostics section, Theresia Manneck for her assistance with Fig. 2, and Patrick Lane for outstanding work in further enhancing the figures.

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