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REVIEW

Current status of food-borne trematode infections

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Abstract Food-borne trematodiases constitute an important group of the most neglected tropical diseases, not only in terms of research funding, but also in the public media. The Trematoda class contains a great number of species that infect humans and are recognized as the causative agents of disease. The biological cycle, geographical distribution, and epidemiology of most of these trematode species have been well characterized. Traditionally, these infections were limited, for the most part, in populations living in low-income countries, particularly in Southeast Asia, and were associated with poverty. However, the geographical limits and the population at risk are currently expanding and changing in relation to factors such as growing international markets, improved transportation systems, and demographic changes. The diagnosis of these diseases is based on parasitological techniques and only a limited number of drugs are currently available for treatment, most of which are unspecific. Therefore, in-depth studies are urgently needed in order to clarify the current epidemiology of these helminth infections and to identify new and specific targets for both effective diagnosis and treatment. In this review, we describe the biology, medical and epidemiological features, and current treatment and diagnostic tools of the main groups of flukes and the corresponding diseases.

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Introduction

Humans suffer from numerous parasitic food-borne parasitic zoonoses, many of which are caused by trematodes (phylum Platyhelminthes: Digenea). In fact, more than 100 species of food-borne trematodes are known to infect humans, including liver flukes, lung flukes, and intestinal flukes. Food-borne trematode infections are one major group of the so-called neglected tropical diseases worldwide, with more than 40 million people infected and more than 10% of the world's population at risk [1–4]. In the past, these infections were limited, for the most part, in populations living in low-income countries, particularly in Southeast Asia, and were associated with poverty. However, the geographical limits and the population at risk are currently expanding and changing in relation to factors such as growing international markets, improved transportation systems, and demographic changes.

Despite the considerable public health impact and the emerging nature of the food-borne trematodiases, they are orphans with regard to research funding and presence in the press media [5–7]. In this sense, it should be noted that, although there is an increasing interest on neglected tropical diseases, with regard to food-borne trematodiases, a number of control campaigns have been stopped and these diseases are not included in the priority list of the World Health Organization (WHO). Moreover, the difficulties of diagnosis, the complexities of human cultural behaviors, and poor knowledge of the potential economic consequences of these infections impede research on this topic, especially in developed countries.

The list of potential food-borne trematodes that might be discussed in a review is quite large. In the present review, we summarize the key characteristics of the major liver (clonorchiasis, opisthorchiasis, and fascioliasis), lung (paragonimiasis), and intestinal (diplostomiasis, echinostomiasis, fasciolopsiasis, gymnophalloidiasis, and heterophyiasis) food-borne trematodiases. We highlight the life cycles, epidemiology and impact, clinical aspects, pathology, and new methods of diagnosis and experimental treatment of these infections.

Taxonomy and general morphology

Food-borne trematodes are classified into the phylum Platyhelminthes, class Trematoda, and subclass Digenea. The systematics of these flukes are being investigated and debated thoroughly; the digenetic trematodes constitute the largest group of Platyhelminthes. Table 1 shows the taxonomy of the main species covered in this review.

Adult digeneans are characterized by a dorsoventral, flattened, bilaterally symmetrical body. Usually, there are two suckers; an anterior oral sucker surrounding the mouth and a ventral sucker, sometimes termed the acetabulum, on the ventral surface. The oral sucker surrounds the mouth, while the ventral sucker is a blind muscular organ with no connection to any internal structure. The tegument, a syncytial epithelium, is implicated in nutrient absorption, synthesis, secretion, and osmoregulation, and has sensory functions [8]. Most Digenea are hermaphroditic. The female reproductive system consists of an ovary, oviduct, sometimes a seminal receptacle (in which sperm from the copulatory partner is stored), ootype (where the egg is formed) surrounded by Mehlis' gland, yolk gland (vitellarium) which opens into the ootype or near it, and the uterus, in which eggs mature. The uterus opens through a common gonopore together with the male system. The male system consists of one testis, or several testes, and sperm ducts which unite and widen terminally to form a seminal vesicle often enclosed in a cirrus pouch. The average size of the flukes varies according to species.

General biology

Within their definitive hosts, food-borne trematodes can be found in practically every organ (liver, lungs, blood system, and the alimentary tract and its ducts), and have indirect and complex life cycles involving a number of diverse larval types (see Fig. 1).

Usually, there are seven developmental stages (i.e., adult, egg, miracidium, sporocyst, redia, cercaria, and metacercaria) with alternations of asexual and sexual reproductive phases in the molluscan and definitive host. The life cycle of the major food-borne trematodes include two or three different hosts: a vertebrate definitive host, including humans; an invertebrate first intermediate host (a mollusk); and, frequently, a second

Table 1 Taxonomy up to the generic level of the trematodes causing major food-borne diseases

Phylum Platyhelminthes	
Subphylum Neodermata	
Class Trematoda	
Subclass Digenea	
Order Echinostomida (Echinostomatida)	
Family Cathaemasiidae	
	Genus: *Cathaemasia*
Family Echinostomatidae	
	Genera: *Acanthoparyphium*, *Artyfechinostomum*, *Echinochasmus*, *Echinoparyphium, Episthmium*, *Euparyphium, Himashtla*, *Hypoderaeum* and *Isthmiophora*
Family Fasciolidae	
	Genera: *Fasciola*, *Fascioloides* and *Fasciolopsis*
Family Gastrodiscidae	
	Genus: *Gastrodiscoides*
Family Paramphistomidae	
	Genus: *Fischoederius*
Family Psilostomidae	
	Genus: *Psilorchis*
Order Diplostomida	
Family Diplostomidae	
	Genera: *Fibricola* and *Neodiplostomum*
Family Strigeidae	
	Genus: *Cotylurus*
Order Opisthorchiida	
Family Heterophyidae	
	Genera: *Centrocestus*, *Haplorchis*, *Heterophyes*, *Heterophyopsis*, *Metagonimus*, *Phagicola*, *Procerovum*, *Pygidiopsis*, *Stellantchasmus* and *Stictodora*
Family Opisthorchiidae	
	Genera: *Clonorchis* and *Opisthorchis*
Order Plagiorchiida	
Family Gymnophallidae	
	Genus: *Gymnophalloides*
Family Lecithodendriidae	
	Genera: *Phaneropsolus* and *Prosthodendrium*
Family Microphallidae	
	Genus: *Spelotrema*
Family Paragonimidae	
	Genus: *Paragonimus*
Family Plagiorchiidae	
	Genus: *Plagiorchis*

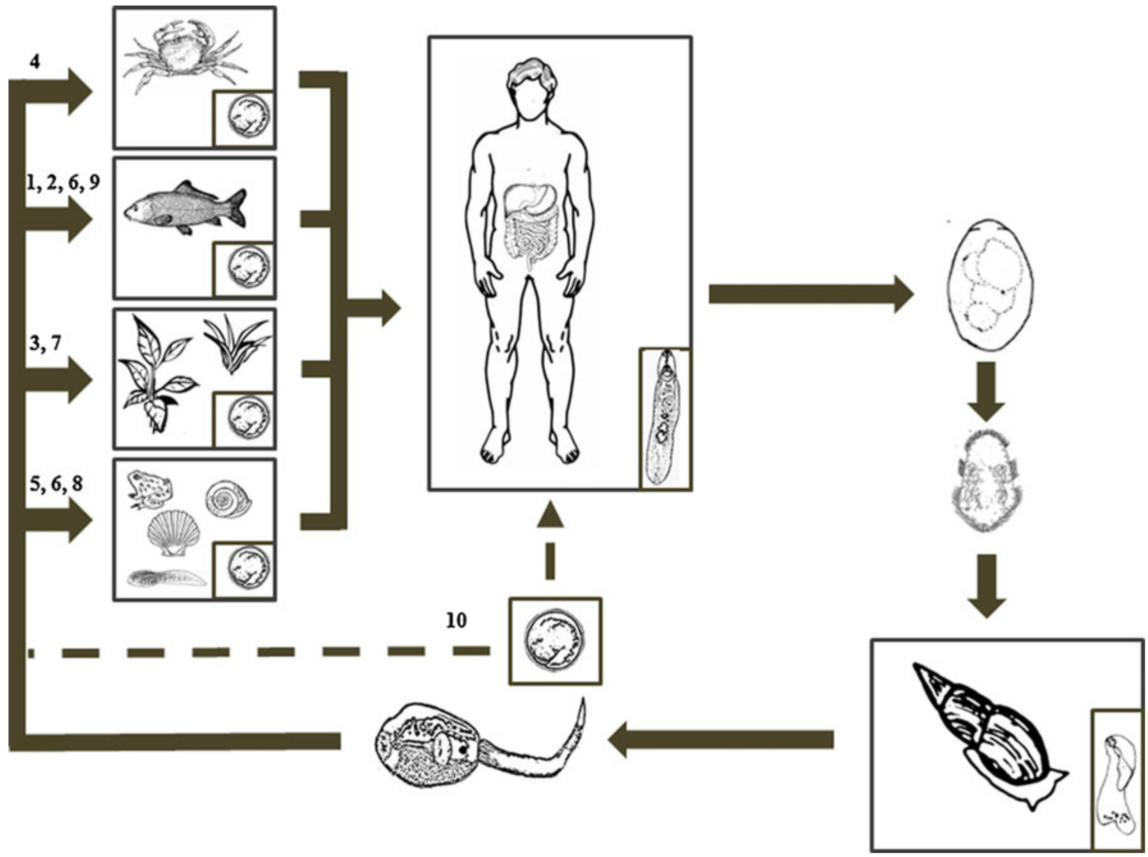

Fig. 1 Schematic representation of the life cycle patterns of the causative species of major food-borne trematodes with emphasis on the source of transmission to humans: 1. *Clonorchis sinensis*; 2. *Opisthorchis* spp.; 3. *Fasciola* spp.; 4. *Paragonimus* spp.; 5. *Neodiplostomum seoulense*; 6. Echinostomatidae; 7. *Fasciolopsis buski*; 8. *Gymnophalloides seoi*; 9. Heterophyidae; 10. some trematodes, including *Fasciola* spp. and Echinostomatidae, can infect humans by the ingestion of water contaminated with metacercariae

intermediate host carrying the encysted metacercarial stage. Eggs are produced by adult worms following sexual reproduction in the final host, which are humans or wild and domestic animals. Eggs are released via feces (most of the human food-borne trematodes) or sputum (*Paragonimus* spp.). The eggs of some digenetic trematodes are fully developed when laid, whereas others require some time for embryonation. Eggs require appropriate environmental conditions for embryonation. The egg releases a swimming ciliated larva, the miracidium, which actively penetrates the snail intermediate host or is ingested by the host. In some cases, the eggs are directly ingested by the intermediate host and the miracidia hatch in the gastrointestinal tract of the snail. Various snail species act as the first intermediate host, most of which are trematode species-specific.

Asexual reproduction occurs for several weeks in the snail first intermediate host. Miracidia develop into sporocysts, but, in some cases, the miracidia directly give rise to redia. The germinal cells within the sporocysts produce new germinal masses which produce daughter sporocysts or rediae. The development of sporocysts and rediae follows different patterns depending on the digenean species [4]. Finally, these larval stages produce cercariae. The free-swimming cercariae escape from the host and either come in contact with a compatible second intermediate host in which they penetrate and encyst (e.g., *Clonorchis sinensis*, *Echinostoma* spp., or *Opisthorchis* spp.) or encyst on aquatic vegetation, such as watercress, water lotus, water caltrop, water chestnut, or water lily (e.g., *Fasciola hepatica* or *Fasciolopsis buski*) (Fig. 1). Numerous invertebrates and poikilothermal vertebrates serve as the second intermediate host. Several fish species, crustaceans, snails, and tadpoles have been reported to act as the second intermediate host. Human and animal definitive hosts become infected when eating raw, pickled, or insufficiently cooked second intermediate hosts harboring metacercariae, aquatic vegetation, or even drinking contaminated water [9, 10]. After ingestion, metacercariae excysts in the gastrointestinal tract release a juvenile worm which migrates to the target organ. Infection with *Paragonimus* spp. might also occur through the consumption of undercooked meat of wild boar, which act as a paratenic host [11]. The survival of the adult worms in the definitive host may vary from days to several years [2].

Global impact of food-borne trematodes

The transmission of food-borne trematodes is restricted to areas where the first and second intermediate hosts coexist and where humans have the habit of eating raw, pickled, or undercooked fish and other aquatic products. This determines the focal distribution of the food-borne trematode infections [1, 3].

Despite their importance, the global burden of the food-borne trematode infections has remained unknown since recent years. In fact, these infections were disregarded in the estimations of the WHO from the mid-1990s to 2004 [12, 13]. In recent years, several studies in relation to neglected diseases have been reporting interesting data on this issue.

The number of infected people varies depending on the trematode species (Table 2). The global estimate for the number of people infected with *C. sinensis* is 35 million [9, 14]. More than 20 million people are infected with *Paragonimus* spp. [11]. For *O. viverrini*, it is estimated that 10 million people are infected [15]. Estimates for *Fasciola* spp. range from 2.4 to 17 million people [2]. Approximately 1.2 million people are infected with *O. felineus* [16]. An estimated 40 to 50 million people are infected with species of intestinal flukes [17]. However, these estimations only constitute a small part of the problem since the worldwide population at risk is considerably higher (Table 2).

Clonorchiasis and opisthorchiasis

The fish-borne liver flukes, *Opisthorchis viverrini*, *O. felineus*, and *Clonorchis sinensis*, have close morphological and biological characteristics. Both genera belong to the family Opisthorchiidae and the differentiation of the species is based on several characteristics of the adult worms, such as the size, shape and position of the testes, and the arrangement of the vitelline glands [16].

The life cycle of *Opisthorchis* spp. and *C. sinensis* is shown in Fig. 1. These species follow a three-host life cycle. Embryonated eggs are released and ingested by *Bithynia* spp. and hatch. Sporocysts give rise to redia and, in turn, to cercaria. The free-living cercariae may penetrate and develop in several species of freshwater fishes of the family Cyprinidae where they encyst. Humans become infected after the consumption of raw or undercooked fish harboring infective metacercariae. Adult worms inhabit the intrahepatic bile duct, but they can also be found in the common bile duct, cystic duct, and even in the gallbladder.

These liver flukes are endemic in Asia and Eastern Europe [3, 16]. *C. sinensis* is widespread in the People's Republic of China (PR China), Korea, and North Vietnam, while *O. viverrini* is endemic in Southeast Asia, including Thailand, Laos People's Democratic Republic (PDR), Cambodia, and Central Vietnam [3]. *O. felineus* is found in Russia and, possibly, Eastern Europe [16]. In addition to these endemic areas, the migration of people has expanded the parasite distribution. Furthermore, there are some reports in other areas but only some of them are thought to be locally acquired [17, 18].

Most chronic human opisthorchiasis and clonorchiasis cases show few specific signs or symptoms, except an increased frequency of palpable liver [19]. Among patients of clonorchiasis with a very high worm burden (up to 25,000 flukes), acute pain in the right upper quadrant may

Table 2 Habitat, infection sources, number and infections, population at risk, and treatment of choice of major food-borne trematodes and their underlying diseases

Species	Habitat	Source of infection	Number of infections (10^6)[a]	At-risk population (10^6)[a]	Treatment (dose)
Clonorchis sinensis	Liver	Freshwater fish	601	601	Praziquantel (3×25 mg/kg for 2 days or single dose of 40 mg/kg)
Opisthorchis spp.	Liver	Freshwater fish	11.2	79.8	Praziquantel (3×25 mg/kg for 2 days or single dose of 40 mg/kg)
Paragonimus spp.	Lung	Freshwater crabs, crayfish, wild boar meat	20.7	292.8	Praziquantel (3×25 mg/kg for 2 days)
Echinostomatidae	Intestine	Freshwater fish, frogs, mussels, snails, tadpoles	Not known	Not known	Praziquantel (single dose of 25 mg/kg)
Gymnophalloides seoi	Intestine	Oysters	Not known	Not known	Praziquantel (single dose of 10 mg/kg)
Heterophyidae	Intestine	Freshwater fish	Not known	Not known	Praziquantel (single dose of 25 mg/kg)
Fasciola spp.	Liver	Freshwater vegetables, contaminated water	2.4-17	2.4-17	Triclabendazole (single dose of 10 mg/kg or 20 mg/kg in two split doses within 12-24 h)

[a]Based on data from references [1, 2]

also appear [9]. Severe opisthorchiasis may cause obstructive jaundice, cirrhosis, cholangitis, acalculous cholecystitis, or bile peritonitis [1].

Cholangiocarcinoma is the most serious complication of infections with *O viverrini* and *C. sinensis*. *O. viverrini* is classified by the International Agency for Research on Cancer (IARC) as definitely carcinogenic (class 1) and *C. sinensis* as a probable carcinogen (class 2A) [20–22]. Cholangiocarcinoma is a malignant tumor starting in the epithelium of the intrahepatic biliary tree and might invade the sinusoids of the liver parenchyma [16]. The prognosis of this tumor is extremely poor [23]. Although the etiology is not entirely known, many factors are likely to be involved in carcinogenesis involving mechanical and chemical irritation of the tissue by the fluke and immune responses [16, 21, 23].

Patients infected with *O. felineus* suffer from fever to hepatitis-like symptoms in the acute stage of the infection. Chronic symptoms include obstruction, inflammation, and fibrosis of the biliary tract, liver abscesses, pancreatitis, and suppurative cholangitis [23].

Fascioliasis

F. hepatica and *F. gigantica* are the causative agents of liver fluke disease (fascioliasis) in domestic animals and humans. Both species follow a two-host life cycle. The eggs, released by the adults residing in the bile ducts of the mammalian host, are carried into the intestine and are passed in the feces. Embryonation and hatching occurs in freshwater. The free-swimming miracidia find and penetrate the molluscan intermediate host: *F. hepatica* typically infects *Galba truncatula* in Europe and parts of Asia, whereas *F. gigantica* can infect a wide range of snail species, including *Lymnaea natalensis* in Africa and *L. rubiginosa* in Asia [24]. Within the digestive gland of the infected snail, the parasite undergoes a series of developmental stages (sporocyst–rediae–cercaria). The free-swimming cercariae adhere to and encyst, as metacercariae, on vegetation. Following ingestion of the contaminated vegetation, the parasite excysts in the small intestine and juvenile worms penetrate through the gut wall and enter the peritoneal cavity. After 10–12 weeks of migration, the parasites enter the bile ducts, where they mature [25, 26].

Fascioliasis is now recognized as an important emerging zoonotic disease of humans [27]. The contamination sources of human infection are the ingestion of wild and cultivated freshwater and terrestrial plants (common and wild watercress, dandelion, leaves, lamb's lettuce, spearmint, algae, kjosco, totora) [28]. Drinking beverages made from local plants and drinking untreated water directly or in dishes and soups and washing kitchen utensils or other objects with contaminated water may be several sources of human infection due to the presence of free-floating metacercarial cysts [28, 29]. The majority of reported human cases of fascioliasis are due to infections with *F. hepatica*, though some reports indicate a rise in human infections with *F. gigantica* [30].

The highest prevalence of human fascioliasis is found in the Altiplan region of Northern Bolivia [31]. Hyperendemic human fascioliasis has also been reported in the Nile Delta region between Cairo and Alexandria and several provinces of Northern Iran [32, 33]. In Europe, human fluke infections occur more sporadically, though outbreaks of the disease in France, Portugal, and Spain have been reported [30]. Sporadic cases have also been reported in the USA [18].

Two different phases can be distinguished in the fascioliasis: acute and chronic fascioliasis [26, 34]. Acute fascioliasis, corresponding with the migratory stages of the life cycle, is characterized by fever, abdominal pain, hepatomegaly, and other gastrointestinal symptoms resulting from the destruction of liver tissues by the migratory flukes. Chronic fascioliasis, corresponding with the presence of the adult worms in the bile ducts, is often subclinical or show symptoms indistinguishable from other hepatic diseases, such as cholangitis, cholecystitis, and cholelithiasis [26]. Few human deaths have been reported in relation to fascioliasis [26, 35].

Paragonimiasis

Paragonimiasis is the disease caused by lung flukes of the genus *Paragonimus*. There are about 15 species of *Paragonimus* known to infect humans. *P. westermani* is the most common worldwide, while *P. heterotremus* is the etiological agent of human paragonimiasis in PR China, Laos PDR, Vietnam, and Thailand [3, 11]. Other species of *Paragonimus* are reported to infect humans in other locations such as Asia, West Africa, and America [11].

Humans become infected after the ingestion of raw or undercooked freshwater crustaceans, such as crabs, shrimp, or crayfishes. The metacercariae excyst in the small intestine and penetrate through the intestinal wall into the abdominal cavity, prior to migration through the subperitoneal tissues, the muscle, the liver, the diaphragm, and, finally, enters the lung, where maturation occurs. Adult flukes lay eggs, which are coughed up and ejected by spitting with the sputum or swallowed and passed in the feces. After hatching, miracidia invade freshwater snails, mainly of the genus *Semisulcospira*, and a series of developmental stages (sporocyst–rediae–cercaria) occur within the snail and, finally, the cercariae emerge. Crustacea probably acquire the infection by consuming cercariae or eating infected snails containing the fully developed cercariae (Fig. 1).

About 20 million people are infected with lung flukes and an estimated 293 million people are at risk of infection [1]. Human paragonimiasis occurs in three endemic focal areas: Asia (PR China, Japan, Korea, Laos PDR, Philippines, Vietnam, Taiwan, and Thailand), South and Central America (Ecuador, Peru, Costa Rica, and Columbia), and Africa (Cameroon, Gambia, and Nigeria) [3, 36, 37]. Endemic areas can be identified as the people who eat raw, pickled, and semi-cooked freshwater species of crabs, shrimps, and crayfishes. The sources of infection in the endemic areas have been recently reviewed by Sripa et al. [3].

The main pathological signs induced by *Paragonimus* spp. are due to the mechanical damage caused by the migration of the worm from the gut to the lungs and the toxins and other mediators released by the migratory parasites. Furthermore, ectopic migrations to aberrant sites including the brain and subcutaneous sites at the extremities may occur [11]. The flukes in the lung cause hemorrhage, inflammatory reaction and necrosis of lung parenchyma, and fibrotic encapsulation. There are several signs that allow to characterize the acute and chronic paragonimiasis. In pulmonary paragonimiasis, the most noticeable symptom is a chronic cough with brown and blood-streaked pneumonia-like sputum. Moreover, hemoptysis is commonly induced by heavy work. When symptoms include only a chronic cough, the paragonimiasis can be confused with a chronic bronchitis or bronchial asthma [3]. In extrapulmonary paragonimiasis, the symptoms vary in relation to the location of the fluke, including cerebral and abdominal paragonimiasis [3, 11].

Intestinal food-borne trematodiases

The intestinal food-borne trematodes category constitutes a large assemblage of species that induce parasitic zoonoses. Collectively, these parasites have a major impact on the health and economy in developing countries of the tropics and subtropics in Asia, Africa, Europe, and the Americas [3]. A total of 70 species (14 families and 36 genera) of intestinal flukes have been isolated from humans [17]. Herein, we summarize the main characteristics of diplostomiasis, echinostomiasis, fasciolopsiasis, gymnophalloidiasis, and heterophyiasis that can be considered as the major intestinal food-borne trematodes.

Diplostomiasis

The family Diplostomidae contains digeneans from numerous orders of birds and mammals, but at the intestinal level, only *Neodiplostomum seoulense* and *Fibricola cratera* parasitize humans. In general, species of the Diplostomidae have a three-host life cycle. Fork-tailed cercariae are produced in sporocysts in the gastropod first intermediate host. The cercariae emerge from the snails and penetrate and form metacercariae in fishes, amphibians, molluscs, and annelids [38]. Definitive hosts become infected by the ingestion of the second intermediate host or the paratenic host harboring metacercariae. Eggs typically hatch and penetrate the first intermediate host [39].

Human infections with *N. seoulense* have been reviewed by Chai and Lee [39] and Fried et al. [40]. A total of 28 human cases have been reported in the Republic of Korea, but none in other countries [38]. This species was first implicated when an infected human was found to be suffering from severe enteritis with abdominal pain, fever, diarrhea, fullness, and anorexia. The patient had a history of eating raw snakes, which appears to be the most important food source for human infections [41]. The estimation of the total number of human cases in the Republic of Korea is about 1,000 [39]. More anecdotal is the human infection with *F. cratera*, a trematode species indigenous to North America [18].

Echinostomiasis

The family Echinostomatidae contains a rather heterogeneous group of cosmopolitan and hermaphroditic digeneans that parasitize, as adults, numerous vertebrate hosts of all classes [42]. Adult echinostomatids are predominantly found in birds, but also parasitize mammals and, occasionally, reptiles and fishes. The main distinguishing feature of the Echinostomatidae is the presence of a circumoral collar armed with one or two ventrally interrupted crowns of spines.

Members of the Echinostomatidae follow a three-host life cycle. The first intermediate hosts are aquatic snails in which a sporocyst, two generations of rediae, and cercariae develop. Emerged cercariae infect the second intermediate host, which may be several species of snails, clams, frogs, and even fishes. The definitive host becomes infected after ingestion of the second intermediate host harboring the encysted metacercariae [42–44].

The distribution of echinostomes is ubiquitous. The current incidence of human echinostomiasis is difficult to determine with any accuracy because of the unavailability of epidemiological surveys and most of the data rely on historic surveys and occasional case reports. The distribution of human echinostomiasis is strongly determined by the dietary habits. Humans become infected when they eat raw or inadequately cooked food, especially fish, snakes, amphibians, clams, and snails, containing encysted echinostome metacercariae [42]. Moreover, it has been postulated that humans can also be infected by drinking untreated water containing echinostome cercariae, which could become encysted when exposed to the human gastric juice [45]. Infections are, thus, most prevalent in areas where traditional cultural practices encourage the ingestion of raw or undercooked fish, frogs, snakes, or snails and bivalves or drinking tainted water. Hence, the disease distribution is highly focal, though occasional cases can also occur [1].

Although echinostomiasis occurs worldwide, most human infections are reported from foci in East and Southeast Asia.

Echinostomiasis is relatively rare, yet, the foci of transmission remain endemic owing to the local dietary preferences. Most of these endemic foci are localized in Cambodia, China, India, Indonesia, Korea, Malaysia, Philippines, Russia, Taiwan, and Thailand [46–48]. Moreover, occasional cases have also been reported in other countries.

The number and identity of the echinostome species causing human echinostomiasis is uncertain in relation to the absence of systematic surveys and occasional case reports. Moreover, the problematical taxonomy of the group complicates further the specific diagnosis of the worms found in humans [46]. Haseeb and Eveland [47] listed a total of 21 species infecting humans belonging to eight genera of Echinostomatidae (*Artyfechinostomum*, *Echinochasmus*, *Echinoparyphium*, *Echinostoma*, *Episthmium*, *Himasthla*, *Hypoderaeum*, and *Paryphostomum*). Chai [46] listed 20 species belonging to nine genera (*Acanthoparyphium*, *Artyfechinostomum*, *Echinochasmus*, *Echinoparyphium*, *Echinostoma*, *Episthmium*, *Himasthla*, *Hypoderaeum*, and *Isthmiophora*) that have been found parasitizing humans.

Major clinical symptoms due to echinostome infection may include abdominal pain, diarrhea, easy fatigue, and loss of body weight [44, 49–51]. The symptoms in echinostomiasis seem to be more severe than those observed in other intestinal trematode infections [46]. Human morbidity is due to the prolonged latent phase, symptomatic presentations, and similarity of symptoms with other intestinal helminth infections [50, 52]. Clinical symptoms depend on the parasite load [39]. Heavy infections are associated with eosinophilia, abdominal pain, watery diarrhea, anemia, edema, and anorexia [53]. Pathological damage includes catarrhal inflammation, erosion, and even ulceration [50].

Fasciolopsiasis

This helminth infection is caused by an intestinal fluke, *Fasciolopsis buski*, belonging to the family Fasciolidae. This trematode species shares a similar morphology and life cycle to *Fasciola* spp. (Fig. 1). The final host range of *F. buski* is limited and many mammals are refractory to infection. Humans become infected through the consumption of viable metacercaria on the seed pods, bulbs, stems, or roots of water plants. Metacercariae excyst in the duodenum and the juvenile worms attach to the duodenal and jejunal wall, where they develop into adult worms. Eggs are large and operculate. If the eggs reach freshwater sources, embryonation occurs over a period of 3–7 weeks and, then, miracidia hatch and penetrate snail intermediate hosts of the family Planorbidae (especially in the genera *Segmentina*, *Hippeutis*, and *Gyraulus*). After transformation and asexual multiplication as sporocysts, rediae, and cercariae, free-swimming cercariae attach and encyst on the seed pods of any freshwater plant surface [3].

F. buski infection is largely confined to Asian countries, namely, southern PR China, India, Bangladesh, Thailand, Malaysia, Borneo, Sumatra, and Myanmar, and may reach high prevalences [3, 17]. Human infections are associated with the consumption of freshwater vegetables. Vegetables in endemic foci are confined to low-lying land where susceptible snail hosts abound. The highest prevalences occur in areas with the cultivation or year-round availability of water caltrops and other aquatic vegetation and where people enjoy eating water chestnuts. Community-based prevalences in endemic areas generally reach 20% and children are often more frequently and heavily infected than adults, since they usually eat water plants during play [2, 3].

In general, symptoms of fasciolopsiasis are absent or mild, but may include chronic diarrhea, abdominal colic, hunger pains, flatulence, vomiting, eosinophilia, and fever. The abdominal pain may be due to duodenal ulcer caused by mechanical damage. Patients may pass stools containing large amounts of undigested material. Some deaths have been reported in long-standing heavy infection with intestinal obstruction [3, 17].

Gymnophalloidiasis

The family Gymnophallidae consists of a small group of digeneans occurring in the intestine, gall bladder, and bursa Fabricii of birds and also in the intestine of mammals. Although the number of genera included within this family varies greatly [54], a recent revision of the family accepts a total of five valid genera (*Gymnophalloides*, *Parvatrema*, *Gymnophallus*, *Pseudogymnophallus*, and *Bartolius*) [55]. A typical gymnophallid life cycle involves bivalves as the first intermediate host, and bivalves, polychaetes, gastropods, or brachiopods as the second intermediate hosts. The definitive host becomes infected after ingestion of the second intermediate host harboring the metacercariae [54].

Within the Gymnophallidae, studies on the pathology and immunology of the infection are available for only one species, *Gymnophalloides seoi*. This is a minute intestinal fluke that has been reported from humans in Korea [39, 55]. The first intermediate host of *G. seoi* is unknown, but a second intermediate host is the oyster *Crassostrea gigas*. Humans, the oystercatcher (*Haematopus ostralegus*), and wading birds are natural definitive hosts [54].

Human infections with *G. seoi* have only been recorded in Korea [54, 56]. *G. seoi* was first discovered in a woman who suffered from acute pancreatitis and gastrointestinal discomfort [56]. *G. seoi* is highly prevalent among villagers in the southwestern coastal islands of Korea, where half of the population was infected [57]. They became infected by consuming raw oysters [54].

Heterophyiasis

The family Heterophyidae contains small egg-shaped trematodes with infective metacercariae that are usually encysted in fish second intermediate host. The definitive host becomes infected by eating raw or poorly cooked fish harboring metacercariae. Heterophyids show little specificity toward the definitive host and numerous fish-eating mammals, including humans, can be infected. The adult worms live between the villi of the anterior region of the small intestine and release fully embryonated eggs into water. The eggs are then ingested often by littorine snails (particularly *Littorina littorea* and *L. scutulata*), and hatch within the snail's intestine.

Although there are a great number of genera within the Heterophyidae, most of the studies in relation to human infections of these infections are focused on *Metagonimus yokogawai*. This species parasitizes humans in Asia [17, 39], and its life cycle can be maintained easily in the laboratory in various experimental hosts, thus, facilitating studies on heterophyids.

Human infections by heterophyids have been often reported. Chai and Lee [39] listed 12 species of heterophyids that parasitize humans in Korea belonging to the genera, including: *Metagonimus*, *Heterophyes*, *Stictodora*, *Heterophyopsis*, *Pygidiopsis*, *Stellantchasmus*, and *Centrocestus*. Moreover, members of the genera *Haplorchis*, *Phagicola*, and *Procerovum* have also been implicated in human heterophyiasis [40]. The most prevalent species in humans are *M. yokogawai* and *H. heterophyes*, which are distributed mainly in Asia, Africa, and Eastern Europe [39, 40].

Humans become infected by eating raw, pickled, or poorly cooked fish. Low-grade infections are of no clinical consequence, but cases with heavy infections are associated with diarrhea, mucus-rich feces, abdominal pain, dyspepsia, anorexia, nausea, and vomiting [39, 40, 58]. Anaphylactic reactions have also been reported [58]. Occasionally, worm eggs may enter the circulatory system though the crypts of Lieberkühn, causing emboli which may be fatal, depending on the affected tissue [58].

Diagnosis

The diagnosis of food-borne trematode infections can be accomplished by several approaches and have been recently reviewed [59].

Demonstration of eggs in feces, bile, duodenal fluids, or sputum (in the case of *Paragonimus* spp.) is the gold standard diagnosis. Parasitological examination of fecal samples provides routine diagnosis and is widely used. The most frequently employed methods to detect eggs are Kato–Katz thick smear, Stoll's dilution, and the formalin ethyl acetate concentration technique [59]. However, the similarity of the eggs of trematodes sometimes makes the specific diagnosis difficult.

Apart from traditional methods, several immunological tests have been developed for several trematode species. In *Opisthorchis* and *Clonorchis* infections, good results have been obtained using individual antigens detecting isotype-specific antibodies [60]. Fecal antigen detection by enzyme-linked immunosorbent assay (ELISA) also shows promise [61–63]. Recently, Huang et al. [64] developed a polymerase chain reaction (PCR) assay with a detection limit of a single egg of *C. sinensis* in human feces. Real-time PCR has also been developed [65]. Furthermore, Arimatsu et al. [66] developed a method based on loop-mediated isothermal amplification (LAMP), allowing the detection of 10^{-3} ng of *O. viverrini* DNA in 1 μl of human feces.

Several ELISAs for the detection of antibodies against *F. hepatica* have been developed [67–69]. An accurate serological test using recombinant cathepsin L has been developed and can be applied to blood samples placed onto filter paper. This method has been validated in various endemic regions [69].

A recombinant antigen of *P. westermani* eggs has been tested as an ELISA antigen offering high levels of sensitivity and specificity [70]. Immunoblotting assay for the detection of IgG4 to excretory/secretory products of *P. heterotremus* provides a sensitive and specific method for diagnosis [71]. Recently, Doanh et al. [72] developed a PCR method based on the amplification of the ITS-2 region. Using this method, a single parasite egg of *Paragonimus* is detected in human sputum. A method of LAMP for the detection of adults of *P. westermani* has been developed [73]. This technique provides approximately 100 times greater sensitivity than conventional PCR.

Lee et al. [74] used an ELISA method to detect metagonimiasis in humans with metacercarial crude antigens. Of the 11 metagonimiasis sera, 10 became positive to the analysis. However, cross-reactivity with other trematodiases such as fascioliasis, schistosomiasis, and paragonimiasis was detected. A Western blot study showed also a high degree of cross-reactivity [75]. Ditrich et al. [75] developed indirect ELISA and Western blot methods to detect *H. taichui* in humans using cytoplasmic and membranous antigens from adult worms. ELISA analysis showed that cytoplasmic antigens were more sensitive, but cross-reactions between both species were found.

Analytical methods of foods

Methods for the detection of food-borne trematodes have expanded from traditional microscopic methods to include such molecular techniques as PCR. Although the identification of worms remains as the gold standard method for the detection of trematodes in food, molecular and immunological tools are being progressively incorporated.

The detection and identification of tissue-encysted metacercariae rely heavily on morphological characterization by visual inspection and the use of methods ranging from direct tissue examination to mechanical or enzymatic tissue disruption. The examination of metacercariae in the second intermediate host, mainly in fishes, is commonly performed by two methods, i.e., muscle compression and pepsin-HCl artificial digestion [76]. In the compression technique, a sample of flesh from different parts of fish (e.g., head, gill, muscle, fin, scale, intestine, other viscera) or other host is compressed between two glass slides and examined under the stereomicroscope with 20- to 100-fold magnification. The artificial digestion method in more complicated, but a detailed description of the method can be found in the work by Sohn [76]. Unfortunately, there are no developed methods for the detection of metacercariae in plants and the developed methods are focused on tissue-encysted metacercariae.

For the specific (or generic) identification of the metacercariae, they should be observed in detail under a light microscope and, if possible, identified based on characteristic features and dimensions. As the characteristic features, the shape of the cysts, presence and size of suckers and spines, and shape and contents of the excretory bladder are important [76]. If the morphological features are not obvious and difficult to see, they should be encysted. When the cyst wall is very thin, the metacercariae can be easily released with slight pressure. However, if the cyst is thick, artificial digestion is recommended. There are several methods available for the digestion of the metacercarial cysts and, basically, they involve metacercarial incubation in trypsin or bile at 37°C [77, 78]. A key for the identification of the metacercariae can found by Sohn [76].

The application of molecular methods for the detection of metacercariae in foods is still very limited. However, in recent years, a number of species-specific PCR-based methods are being developed that are capable of detecting and differentiating trematode species from intermediate hosts. These methods are rapid and provide increased discriminatory power and the ability to analyze small amounts of sample. Moreover, they negate morphological investigation of the intermediate host for the presence of metacercariae. For example, a tandem repeated DNA sequence has been described and used to detect metacercariae of *E. caproni* from snail tissues. This methodology could be used in other intermediate hosts such as fishes [78]. Moreover, some of the molecular methods described above for human diagnosis can be alternatively used in food samples [63, 73, 79, 80].

Current treatment

Praziquantel is the drug of choice for the food-borne trematodiasis, except for fascioliasis. Praziquantel exhibits a broad spectrum activity against trematodes and has an excellent safety profile [81]. All treatment schedules with praziquantel are well tolerated, with only a few adverse events, including abdominal pain, dizziness, headache, nausea, and urticaria [81]. The doses employed for the different trematode infections are shown in Table 2. There are no reports of food-borne trematodes resistant to praziquantel. However, a low cure rate has been found in patients with clonorchiasis in a small study in Vietnam [82].

Triclabendazole, a benzimidazole derivative, is used for the treatment of fascioliasis and holds promise for the treatment of paragonimiasis. Currently, triclabendazole is registered for human use only in Ecuador, Egypt, France, and Venezuela, and the doses employed are shown in Table 2. Abdominal pain, biliary colic, fever, nausea, vomiting, weakness, and liver enlargement have been reported as adverse reactions. There are some concerns that triclabendazole resistance might emerge, since it has been reported in veterinary medicine [81]. Bithionol also can be used against fascioliasis when triclabendazole is not available. However, long treatment schedules of 10 to 15 days are required [81].

Experimental drugs in development

The limited number of available drugs for food-borne trematodiases, together with the concerns in relation to the development of resistance against these drugs, makes necessary the search for alternative drugs. In this context, preliminary studies have shown that a number of compounds might be further developed for the treatment of food-borne trematodiases.

Compound alpha is a novel flukicide that has been shown to have a high level of activity against all stages of *F. hepatica*. It is a derivative of triclabendazole and has a similar range of activity against *F. hepatica* [82–88]. It kills flukes from 3 days to 12 weeks, fulfilling the criterion of efficacy against both acute and chronic fascioliasis [88]. As occurs with other anthelmintic molecules of the benzimidazole class, compound alpha is likely to act as a β-tubulin antagonist. It interferes with the assembly of microtubules, which have an essential role in the movement of subcellular components and metabolites within the cytoplasm, as well as the spindle formation during cell division. Apart from structural changes, compound alpha affects the reproductive structures and spermiogenesis of *F. hepatica* [89–91]. Although compound alpha has shown a high level of efficacy against isolates of *F. hepatica* susceptible to triclabendazole, it was not effective against resistant isolates, which raises some concerns on its utility [88, 91].

Artemisinin, the active constituent of the herb *Artemisia annua*, is a sesquiterpene lactone that contains an unusual peroxide bridge. Artemisinin and its derivatives have been extensively used as anti-malaric drugs. In recent years, the activity of artemisinin and its derivatives, including artemether, artesunate, and dihydroartemisinin, against food-borne trematodes has been investigated in vitro and in vivo. Although the exact mechanism of action of the artemisinins is not well known, their mode of action appears to be related to the peroxide bridge that undergoes reductive activation by heme released by the *Plasmodium*, which leads to carbon-centered free radicals or carbocations [92]. In trematodes, it has been shown that artemether disrupts the tegument [2]. Treatment with these drugs resulted in significant reductions of the worm burden of *C. sinensis* in rats [93, 94]. Artemether and artesunate have also been screened in hamsters, showing significant reductions of worm burden [93]. Complete healing of *E. caproni* infections was achieved in mice using a single oral dose of 200 mg/Kg [95]. Recently, artesunate has been successfully studied against heterophyids in experimentally infected mice [96]. With regard to *F. hepatica*, artesunate and artemether showed a high degree of activity in chronic infections in rats. A worm burden reduction of 100% was achieved using a single oral dose of 200–400 mg/Kg. Lesser activity of these drugs was shown against juvenile worms [95]. Interestingly, single oral doses of 200 mg/Kg of artemether completely cured infections with isolates of *F. hepatica* resistant to triclabendazole [97]. Artesunate and artemether, given by the intramuscular route, yielded high egg and worm burden reductions in *F. hepatica*-infected sheep [98]. Although there are promising results obtained from animal models, the data on human infections is somewhat confused. A study on 100 patients from Vietnam showed that artesunate may be useful for the treatment of acute fascioliasis. Treated patients developed a lower frequency of abdominal pain than those treated with triclabendazole [99]. In contrast, a recent study that assessed the efficacy and safety of oral artemether in patients with chronic fascioliasis showed that cure rates were lower than those obtained with triclabendazole [100].

Due to the problems that artemisinins have in relation to their bioavailability, preparation, and pharmacokinetics, many synthetic peroxide analogs have been prepared and investigated. One of them, ozonide OZ78, has shown to be an effective flukicide in the rodent model. Complete *F. hepatica* worm burden reduction was achieved in acute and chronic infections in rats [101]. Moreover, OZ78 also cured rats infected with isolates of *F. hepatica* resistant to triclabendazole [97]. However, a recent study showed that single 50 mg/Kg oral and subcutaneous doses of OZ78 lacked activity against *F. hepatica* infection in sheep [102].

Several studies have shown that the Chinese anthelminthic drug tribendimidine might be a useful flukicide. This drug has been shown to be effective in the treatment of *C. sinensis* and *O. viverrini* in rats and hamsters. In contrast, it does not show activity against *F. hepatica* in rats [94, 103]. Soliman and Taha [104] have studied recently the efficacy in vitro of the rhodanine derivative (Ro-354) against adults of *F. gigantica*. One hour post-incubation, significant tegumental damage was observed.

The increasing interest on medicinal plants as new sources of antiparasitic drugs has led to the study of several extracts as flukicides. For example, it has been shown that several flavonoids have a direct effect on trematodes [105]. Recently, Ferreira et al. [106] showed that ethanolic extracts of *Artemisia annua*, *A. absinthium*, and *Asimina triloba* kill *E. caproni* and *F. hepatica*, probably in relation to their elevated content in artemisinins and acetogenins.

Concluding remarks

Although food-borne trematodiases have been traditionally considered as minor diseases confined to low-income countries, they are currently re-emerging worldwide, including in developed regions. Although, in the past, the importance of these diseases has been often underestimated, in recent years, the transcendence has been highlighted. Considerable progress has been made toward improved diagnoses and a better understanding of the epidemiology, pathology, control, and treatment of the major food-borne diseases. However, the number of infected persons is counted in the millions and 10% of the world's population is considered to live at risk for infection. Current control strategies based on the use of chemotherapy to reduce morbidity, interrupting transmission, and reducing risky human behavior are reasonable and logical. However, several factors such as deeply embedded cultural habits or the strong link of these diseases with poverty, together with the increasing international markets or demographic changes, make the effective control of food-borne trematodiases difficult. In this context, further research on the food-borne trematodiases using new technologies is needed.

The emergent nature of the food-borne trematodiases and the new risks of transmission introduced by the "globalized world" have expanded the distribution of the food-borne trematodiases, which makes it necessary for new epidemiological approaches. New maps of risk using geographical information systems and remote sensing should be undertaken for most of the food-borne trematodiases. Furthermore, PCR-based approaches may also be useful for future epidemiological surveys. For example, a recent study using this methodology has allowed the identification of a new *C. sinensis* endemic community in central Thailand that was overlooked using traditional methodologies [107].

Molecular biology tools should help greatly in several aspects, including diagnosis, the identification of new biomarkers, and the control and knowledge of host–parasite interactions. Genomics and proteomics may provide new opportunities for the development of more rapid and sensitive diagnostic tools, facilitating field studies and also vaccines. For example, recent studies have identified several antigenic proteins of *C. sinensis*, including tetraspanins, cathepsins, 14-3-3 proteins, cyclophilins, or carboxylases, that are promising candidates for diagnosis or vaccines [108–113]. Although significant progress is being achieved in the studies of genomics and molecular genetics of flukes such as *C. sinensis* [114–116], the understanding of the functions of the genes is limited by the absence of whole genome sequences. Increase of the available sequence information from diverse trematodes is needed and this will facilitate the identification of new candidates for diagnosis and vaccines. Molecular approaches, together with basic biochemical and physiological studies, may also be useful to gain further insight into the host–parasite interactions in food-borne trematodiases. Functional genomics may be helpful for a better understanding of processes such as the mechanisms of parasite invasion, pathogenesis, secreted proteins of these parasites with the host, and, indeed, the molecular basis of the carcinogenesis induced by some of these parasites. In this context, the recent massive flux of research in sequencing techniques and application of functional genomics studies can be essential for the control of the food-borne trematodiases. These advances should facilitate a reasonably accurate estimate of the global burden due to food-borne trematodiases and the implementation of adequate control measures.

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References

1. Keiser J, Utzinger J (2005) Emerging foodborne trematodiasis. Emerg Infect Dis 11:1507–1514
2. Keiser J, Utzinger J (2009) Food-borne trematodiases. Clin Microbiol Rev 22:466–483
3. Sripa B, Kaewkes S, Intapan PM, Maleewong W, Brindley PJ (2010) Food-borne trematodiases in Southeast Asia: epidemiology, pathology, clinical manifestation and control. Adv Parasitol 72:305–350
4. Toledo R, Bernal MD, Marcilla A (2011) Proteomics of foodborne trematodes. J Proteomics 74:1485–1503
5. Balasegaram M, Balasegaram S, Malvy D, Millet P (2008) Neglected diseases in the news: a content analysis of recent international media coverage focussing on leishmaniasis and trypanosomiasis. PLoS Negl Trop Dis 2:e234
6. Hotez PJ, Fenwick A, Savioli L, Molyneux DH (2009) Rescuing the bottom billion through control of neglected tropical diseases. Lancet 373:1570–1575
7. Moran M, Guzman J, Ropars AL, McDonald A, Jameson N, Omune B, Ryan S, Wu L (2009) Neglected disease research and development: how much are we really spending? PLoS Med 6:e30
8. Halton DW (2004) Microscopy and the helminth parasite. Micron 35:361–390
9. Lun ZR, Gasser RB, Lai DH, Li AX, Zhu XQ, Yu XB, Fang YY (2005) Clonorchiasis: a key *foodborne* zoonosis in China. Lancet Infect Dis 5:31–41
10. World Health Organization (WHO) (1995) Control of foodborne trematode infections. Report of a WHO Study Group. World Health Organ Tech Rep Ser 849:1–157
11. Blair D, Agatsuma T, Wang W (2007) Paragonimiasis. In: Murrell KD, Fried B (eds) Food-borne parasitic zoonoses: fish and plant-borne parasites. World class parasites, vol 11. Springer, New York, pp 117–150
12. Murray CJ, Lopez AD (1996) Evidence-based health policy—lessons from the Global Burden of Disease Study. Science 274:740–743
13. World Health Organization (WHO) (2004) The world health report 2004—changing history. WHO, Geneva, Switzerland
14. Hong ST, Fang Y (2012) *Clonorchis sinensis* and clonorchiasis, an update. Parasitol Int 61:17–24
15. Andrews RH, Sithithaworn P, Petney TN (2008) *Opisthorchis viverrini*: an underestimated parasite in world health. Trends Parasitol 24:497–501
16. Sithithaworn P, Yongvanit P, Tesana S, Pairojkul C (2007) Liver flukes. In: Murrell KD, Fried B (eds) Food-borne parasitic zoonoses: fish and plant-borne parasites. World class parasites, vol 11. Springer, New York, pp 3–52
17. Chai JY (2007) Intestinal flukes. In: Murrell KD, Fried B (eds) Food-borne parasitic zoonoses: fish and plant-borne parasites. World class parasites, vol 11. Springer, New York, pp 53–115
18. Fried B, Abruzzi A (2010) Food-borne trematode infections of humans in the United States of America. Parasitol Res 106:1263–1280
19. Rim HJ (2005) Clonorchiasis: an update. J Helminthol 79:269–281
20. International Agency for Research on Cancer (IARC) (1994) IARC monographs on the evaluation of carcinogenic risks to humans: schistosomes, liver flukes and *Helicobacter pylori*. IARC Monographs 61:9–175
21. Choi BI, Han JK, Hong ST, Lee KH (2004) Clonorchiasis and cholangiocarcinoma: etiologic relationship and imaging diagnosis. Clin Microbiol Rev 17:540–552
22. Mayer DA, Fried B (2007) The role of helminth infections in carcinogenesis. Adv Parasitol 65:239–296
23. Sripa B, Pairojkul C (2008) Cholangiocarcinoma: lessons from Thailand. Curr Opin Gastroenterol 24:349–356
24. Malone JB (1997) The landscape epidemiology of fasciolosis: geographic determinants of disease risk. In: Boray JC (ed) Immunology, pathobiology and control of fasciolosis. MSD AGVET, Rahway, NJ, pp 65–81
25. Andrews SJ (1999) The life cycle of *Fasciola hepatica*. In: Dalton JP (ed) Fasciolosis. CABI Publishing, Oxford, pp 1–29
26. Robinson MW, Dalton JP (2009) Zoonotic helminth infections with particular emphasis on fasciolosis and other trematodiases. Philos Trans R Soc Lond B Biol Sci 364:2763–2776
27. Mas-Coma S, Valero MA, Bargues MD (2009) Fasciola, lymnaeids and human fascioliasis, with a global overview on disease transmission, epidemiology, evolutionary genetics, molecular epidemiology and control. Adv Parasitol 69:41–146

28. Mas-Coma S (2004) Human fascioliasis. In: Cotruvo JA, Dufour A, Rees G, Bartram J, Carr R, Cliver DO, Craun GF, Fayer R, Gannon VPJ (eds) Waterborne zoonoses: identification, causes and control. World Health Organization. IWA Publishing, London, UK, pp 305–322
29. Esteban JG, González C, Bargues MD, Angles R, Sánchez C, Náquira C, Mas-Coma S (2002) High fascioliasis infection in children linked to a man-made irrigation zone in Peru. Trop Med Int Health 7:339–348
30. Mas-Coma S, Bargues MD, Valero MA (2005) Fascioliasis and other plant-borne trematode zoonoses. Int J Parasitol 35:1255–1278
31. Esteban JG, Flores A, Angles R, Mas-Coma S (1999) High endemicity of human fascioliasis between Lake Titicaca and La Paz valley, Bolivia. Trans R Soc Trop Med Hyg 93:151–156
32. Esteban JG, González C, Curtale F, Muñoz-Antoli C, Valero MA, Bargues MD, el-Sayed M, el-Wakeel AA, Abdel-Wahab Y, Montresor A, Engels D, Savioli L, Mas-Coma S (2003) Hyperendemic fascioliasis associated with schistosomiasis in villages in the Nile Delta of Egypt. Am J Trop Med Hyg 69:429–437
33. Rokni MB, Massoud J, O'Neill SM, Parkinson M, Dalton JP (2002) Diagnosis of human fasciolosis in the Gilan province of Northern Iran: application of cathepsin L-ELISA. Diagn Microbiol Infect Dis 44:175–179
34. Garcia HH, Moro PL, Schantz PM (2007) Zoonotic helminth infections of humans: echinococcosis, cysticercosis and fascioliasis. Curr Opin Infect Dis 20:489–494
35. Chen MG, Mott KE (1990) Progress in assessment of morbidity due to *Fasciola hepatica* infection: a review of recent literature. Trop Dis Bull 87:R1–R38
36. Aka NA, Adoubryn K, Rondelaud D, Dreyfuss G (2008) Human paragonimiasis in Africa. Ann Afr Med 7:153–162
37. Procop GW (2009) North American paragonimiasis (caused by *Paragonimus kellicotti*) in the context of global paragonimiasis. Clin Microbiol Rev 22:415–446
38. Cribb TH, Bray RA, Olson PD, Littlewood DT (2003) Life cycle evolution in the digenea: a new perspective from phylogeny. Adv Parasitol 54:197–254
39. Chai JY, Lee SH (2002) Food-borne intestinal trematode infections in the Republic of Korea. Parasitol Int 51:129–154
40. Fried B, Graczyk TK, Tamang L (2004) Food-borne intestinal trematodiases in humans. Parasitol Res 93:159–170
41. Hong ST, Chai JY, Lee SH (1986) Ten human cases of *Fibricola seoulensis* infection and mixed one with *Stellantchasmus* and *Metagonimus*. Korean J Parasitol 24:95–97
42. Esteban JG, Muñoz-Antolí C (2009) Echinostomes: systematics and life cycles. In: Fried B, Toledo R (eds) The biology of echinostomes. From the molecule to the community. Springer, New York, pp 1–34
43. Huffman JE, Fried B (1990) *Echinostoma* and echinostomiasis. Adv Parasitol 29:215–269
44. Toledo R, Esteban JG, Fried B (2009) Recent advances in the biology of echinostomes. Adv Parasitol 69:147–204
45. Graczyk TK, Fried B (2007) Human waterborne trematode and protozoan infections. Adv Parasitol 64:111–160
46. Chai JY (2009) Echinostomes in humans. In: Fried B, Toledo R (eds) The biology of echinostomes. From the molecule to the community. Springer, New York, pp 147–183
47. Haseeb MA, Eveland LK (2000) Human echinostomiasis: mechanisms of pathogenesis and host resistance. In: Fried B, Graczyk TK (eds) Echinostomes as experimental models for biological research. Kluwer Academic Publishers, Dordrecht, the Netherlands, pp 83–98
48. Sohn WM, Kim HJ, Yong TS, Eom KS, Jeong HG, Kim JK, Kang AR, Kim MR, Park JM, Ji SH, Sinuon M, Socheat D, Chai JY (2011) *Echinostoma ilocanum* infection in Oddar Meanchey Province, Cambodia. Korean J Parasitol 49:187–190
49. Toledo R (2009) Immunology and pathology of echinostome infections in the definitive host. In: Fried B, Toledo R (eds) The biology of echinostomes. From the molecule to the community. Springer, New York, pp 185–206
50. Toledo R, Esteban JG, Fried B (2006) Immunology and pathology of intestinal trematodes in their definitive hosts. Adv Parasitol 63:285–365
51. Toledo R, Monteagudo C, Espert A, Fried B, Esteban JG, Marcilla A (2006) *Echinostoma caproni*: intestinal pathology in the golden hamster, a highly compatible host, and the Wistar rat, a less compatible host. Exp Parasitol 112:164–171
52. Graczyk TK, Fried B (1998) Echinostomiasis: a common but forgotten food-borne disease. Am J Trop Med Hyg 58:501–504
53. Chattopadyay UK, Das MS, Pal D, Das S, Mukherjee A (1990) A case of echinostomiasis in a tribal community in Bengal. Ann Trop Med Parasitol 84:193
54. Lee SH, Chai JY (2001) A review of *Gymnophalloides seoi* (Digenea: Gymnophallidae) and human infections in the Republic of Korea. Korean J Parasitol 39:85–118
55. Scholz T (2002) Family Gymnophallidae Odhner, 1905. In: Gibson DI, Jones A, Bray RA (eds) Keys to the Trematoda, vol 1. CABI Publishing, Wallingford, pp 345–351
56. Chai JY, Choi MH, Yu JR, Lee SH (2003) *Gymnophalloides seoi*: a new human intestinal trematode. Trends Parasitol 19:109–112
57. Chai JY, Lee GC, Park YK, Han ET, Seo M, Kim J, Guk SM, Shin EH, Choi MH, Lee SH (2000) Persistent endemicity of *Gymnophalloides seoi* infection in a southwestern coastal village of Korea with special reference to its egg laying capacity in the human host. Korean J Parasitol 38:51–57
58. Marty AM, Andersen EM (2000) Fasciolopsiasis and other intestinal trematodiasis. In: Meyers WM, Neafie RC, Marty AM, Wear DJ (eds) Pathology of infectious diseases, vol I helminthiases. Washington DC: Armed Forces Institute of Pathology (AFIP) and American Registry of Pathology, pp 93–105
59. Johansen MV, Sithithaworn P, Bergquist R, Utzinger J (2010) Towards improved diagnosis of zoonotic trematode infections in Southeast Asia. Adv Parasitol 73:171–195
60. Wongratanacheewin S, Pumidonming W, Sermswan RW, Pipitgool V, Maleewong W (2002) Detection of *Opisthorchis viverrini* in human stool specimens by PCR. J Clin Microbiol 40:3879–3880
61. Chaicumpa W, Ruangkunaporn Y, Kalambaheti T, Limavongpranee S, Kitikoon V, Khusmith S, Pungpak S, Chongsa-nguan M, Sornmani S (1991) Specific monoclonal antibodies to *Opisthorchis viverrini*. Int J Parasitol 21:969–974
62. Sirisinha S, Chawengkirttikul R, Haswell-Elkins MR, Elkins DB, Kaewkes S, Sithithaworn P (1995) Evaluation of a monoclonal antibody-based enzyme linked immunosorbent assay for the diagnosis of *Opisthorchis viverrini* infection in an endemic area. Am J Trop Med Hyg 52:521–524
63. Mazidur Rahman SM, Choi MH, Bae YM, Hong ST (2012) Coproantigen capture ELISA for detection of *Clonorchis sinensis* infection in experimentally infected rats. Parasitol Int 61:203–207
64. Huang SY, Tang JD, Song HQ, Fu BQ, Xu MJ, Hu XC, Zhang H, Weng YB, Lin RQ, Zhu XQ (2012) A specific PCR assay for the diagnosis of *Clonorchis sinensis* infection in humans, cats and fishes. Parasitol Int 61:187–190
65. Kim EM, Verweij JJ, Jalili A, Van Lieshout L, Choi MH, Bae YM, Lim MK, Hong ST (2009) Detection of *Clonorchis sinensis* in stool samples using real-time PCR. Ann Trop Med Parasitol 103:513–518
66. Arimatsu Y, Kaewkes S, Laha T, Hong SJ, Sripa B (2012) Rapid detection of *Opisthorchis viverrini* copro-DNA using loop-mediated isothermal amplification (LAMP). Parasitol Int 61:178–182
67. Espinoza JR, Maco V, Marcos L, Saez S, Neyra V, Terashima A, Samalvides F, Gotuzzo E, Chavarry E, Huaman MC, Bargues

MD, Valero MA, Mas-Coma S (2007) Evaluation of Fas2-ELISA for the serological detection of *Fasciola hepatica* infection in humans. Am J Trop Med Hyg 76:977–982

68. Marcilla A, De la Rubia JE, Sotillo J, Bernal D, Carmona C, Villavicencio Z, Acosta D, Tort J, Bornay FJ, Esteban JG, Toledo R (2008) Leucine aminopeptidase is an immunodominant antigen of *Fasciola hepatica* excretory and secretory products in human infections. Clin Vaccine Immunol 15:95–100
69. O'Neill SM, Parkinson M, Strauss W, Angles R, Dalton JP (1998) Immunodiagnosis of *Fasciola hepatica* infection (fascioliasis) in a human population in the Bolivian Altiplano using purified cathepsin L cysteine proteinase. Am J Trop Med Hyg 58:417–423
70. Lee JS, Lee J, Kim SH, Yong TS (2007) Molecular cloning and characterization of a major egg antigen in *Paragonimus westermani* and its use in ELISA for the immunodiagnosis of paragonimiasis. Parasitol Res 100:677–681
71. Maleewong W, Intapan PM, Wongkham C, Wongsaroj T, Kowsuwan T, Pumidonming W, Pongsaskulchoti P, Kitikoon V (2003) Detection of *Opisthorchis viverrini* in experimentally infected bithynid snails and cyprinoid fishes by a PCR-based method. Parasitology 126:63–67
72. Doanh PN, Dung do T, Thach DT, Horii Y, Shinohara A, Nawa Y (2011) Human paragonimiasis in Viet Nam: epidemiological survey and identification of the responsible species by DNA sequencing of eggs in patients' sputum. Parasitol Int 60:534–537
73. Chen MX, Ai L, Zhang RL, Xia JJ, Wang K, Chen SH, Zhang YN, Xu MJ, Li X, Zhu XQ, Chen JX (2011) Sensitive and rapid detection of *Paragonimus westermani* infection in humans and animals by loop-mediated isothermal amplification (LAMP). Parasitol Res 108:1193–1198
74. Lee SC, Chung YB, Kong Y, Kang SY, Cho SY (1993) Antigenic protein fractions of *Metagonimus yokogawai* reacting with patient sera. Korean J Parasitol 31:43–48
75. Ditrich O, Kopacek P, Giboda M, Gutvirth J, Scholz T (1991) Serological differentiation of human small fluke infections using *Opisthorchis viverrini* and *Haplorchis taichui* antigens. Southeast Asian J Trop Med Public Health 22:174–178
76. Sohn WM (2009) Fish-borne zoonotic trematode metacercariae in the Republic of Korea. Korean J Parasitol 47:S103–S113
77. Fried B (1994) Metacercarial excystment of trematodes. Adv Parasitol 33:91–144
78. Fried B, Peoples RC (2009) Maintenance, cultivation and excystation of echinostomes: 2000–2007. In: Fried B, Toledo R (eds) The biology of echinostomes. From the molecule to the community. Springer, New York, pp 111–128
79. Hertel J, Haberl B, Hamburger J, Haas W (2003) Description of a tandem repeated DNA sequence of *Echinostoma caproni* and methods for its detection in snail and plankton samples. Parasitology 126:443–449
80. Cai XQ, Yu HQ, Bai JS, Tang JD, Hu XC, Chen DH, Zhang RL, Chen MX, Ai L, Zhu XQ (2012) Development of a TaqMan based real-time PCR assay for detection of *Clonorchis sinensis* DNA in human stool samples and fishes. Parasitol Int 61:183–186
81. Keiser J, Utzinger J (2004) Chemotherapy for major food-borne trematodes: a review. Expert Opin Pharmacother 5:1711–1726
82. Tinga N, De N, Vien HV, Chau L, Toan ND, Kager PA, Vries PJ (1999) Little effect of praziquantel or artemisinin on clonorchiasis in Northern Vietnam. A pilot study. Trop Med Int Health 4:814–818
83. Ibarra VF, García SE, Fernández RM, Vera MY, Castillo BR, Hernández CA (1997) Eficacia de dos compuestos de síntesis química in vitro e in vivo en ovinos. Vet Méx 28:291–296
84. Ibarra VF, Vera MY, Hernández CA, Castillo BR (1997) Eficacia fasciolicida del compuesto "alfa" contra estadios juveniles y adultos en ovinos. Vet Méx 28:297–301
85. Rivera FN, Ibarra VF, Olazarán JS, Vera MY, Castillo BR, Hernández CA (2002) Efficacy of 5-2-chloro-methylthio-6-(1-naftiloxi)-*ih*-benzimidazole against different stages of *Fasciola hepatica* in Pelibuey sheep. Vet Méx 33:55–61
86. Vera MY, Ibarra VF, Quiroz RH, Hernández CA, Castillo R (2003) Field trial on the efficacy of an experimental fasciolicide compared with some commercial compounds in naturally infected cattle. Parasitol Res 91:1–4
87. Vera MY, Ibarra VF, Liébano HE, Quiroz RH, Castillo BR, Hernández CA, Ochoa GP (2004) Efficacy of an experimental fasciolicide against immature and mature *Fasciola hepatica* in artificially infected calves. Parasitol Res 92:211–214
88. McConville M, Brennan GP, Flanagan A, Edgar HW, Hanna REB, McCoy M, Gordon AW, Castillo R, Hernández-Campos A, Fairweather I (2009) An evaluation of the efficacy of compound alpha and triclabendazole against two isolates of *Fasciola hepatica*. Vet Parasitol 162:75–88
89. McConville M, Hanna REB, Brennan GP, McCoy M, Edgar HWJ, McConnell S, Castillo R, Hernández-Campos A, Fairweather I (2010) *Fasciola hepatica*: disruption of spermatogenesis by the fasciolicide compound alpha. Parasitol Res 106:311–323
90. McConville M, Brennan GP, McCoy M, Castillo R, Hernández-Campos A, Ibarra F, Fairweather I (2006) Adult triclabendazole-resistant *Fasciola hepatica*: surface and subsurface tegumental responses to in vitro treatment with the sulphoxide metabolite of the experimental fasciolicide compound alpha. Parasitology 133:195–208
91. Hanna REB, Edgar HWJ, McConnell S, Toner E, McConville M, Brennan GP, Devine C, Flanagan A, Halferty L, Meaney M, Shaw L, Moffett D, McCoy M, Fairweather I (2010) *Fasciola hepatica*: histological changes in the reproductive structures of triclabendazole (TCBZ)-sensitive and TCBZ-resistant flukes after treatment *in vivo* with TCBZ and the related benzimidazole derivative, Compound Alpha. Vet Parasitol 168:240–254
92. Keiser J, Utzinger J (2007) Food-borne trematodiasis: current chemotherapy and advances with artemisinins and synthetic trioxolanes. Trends Parasitol 23:555–562
93. Keiser J, Xiao SH, Xue J, Chan ZS, Odermatt P, Tesana S, Tanner M, Utzinger J (2006) Effect of artesunate and artemether against *Clonorchis sinensis* and *Opisthorchis viverrini* in rodent models. Int J Antimicrob Agents 28:370–373
94. Xiao SH, Xue J, Tanner M, Zhang YN, Keiser J, Utzinger J, Quiang HQ (2008) Artemether, artesunate, praziquantel and tribendimidine administered singly at different dosages against *Clonorchis sinensis*: a comparative in vivo study. Acta Trop 106:54–59
95. Keiser J, Brun R, Fried B, Utzinger J (2006) Trematocidal activity of praziquantel and artemisinin derivatives: in vitro and in vivo investigations with adult *Echinostoma caproni*. Antimicrob Agents Chemother 50:803–805
96. Fathy FM (2011) Anthelmintic effect of artesunate in experimental heterophyid infection. J Egypt Soc Parasitol 41:469–483
97. Keiser J, Utzinger J, Vennerstrom JL, Dong Y, Brennan G, Fairweather I (2007) Activity of artemether and OZ78 against triclabendazole-resistant *Fasciola hepatica*. Trans R Soc Trop Med Hyg 101:1219–1222
98. Keiser J, Rinaldi L, Veneziano V, Mezzino L, Tanner M, Utzinger J, Cringoli G (2008) Efficacy and safety of artemether against a natural *Fasciola hepatica* infection in sheep. Parasitol Res 103:517–522
99. Hien TT, Truong NT, Minh NH, Dat HD, Dung NT, Hue NT, Dung TK, Tuan PQ, Campbell JI, Farrar JJ, Day JN (2008) A randomized controlled pilot study of artesunate versus triclabendazole for human fascioliasis in central Vietnam. Am J Trop Med Hyg 73:388–392
100. Keiser J, Sayed H, el-Ghanam M, Sabry H, Anani S, el-Wakeel A, Hatz C, Utzinger J, el-Din SS, el-Maadawy W, Botros S

(2011) Efficacy and safety of artemether in the treatment of chronic fascioliasis in Egypt: exploratory phase-2 trials. PLoS Negl Trop Dis 5:e1285
101. Keiser J, Utzinger J, Tanner M, Dong Y, Vennerstrom JL (2006) The synthetic peroxide OZ78 is effective against *Echinostoma caproni* and *Fasciola hepatica*. J Antimicrob Chemother 58:1193–1197
102. Keiser J, Kirchhofer C, Haschke M, Huwyler J, Dong Y, Vennerstrom JL, Vanhoff K, Kaminsky R, Malikides N (2010) Efficacy, safety and pharmacokinetics of 1,2,4-trioxolane OZ78 against an experimental infection with *Fasciola hepatica* in sheep. Vet Parasitol 173:228–235
103. Keiser J, Shu-Hua X, Chollet J, Tanner M, Utzinger J (2007) Evaluation of the in vivo activity of tribendimidine against *Schistosoma mansoni*, *Fasciola hepatica*, *Clonorchis sinensis*, and *Opisthorchis viverrini*. Antimicrob Agents Chemother 51:1096–1098
104. Soliman MI, Taha HA (2011) Tegumental alterations of *Fasciola gigantica* due to in vitro treatment with Ro-354. Trop Biomed 28:283–292
105. Kerboeuf D, Riou M, Guégnard F (2008) Flavonoids and related compounds in parasitic disease control. Mini Rev Med Chem 8:116–128
106. Ferreira JFS, Peaden P, Keiser J (2011) In vitro trematocidal effects of crude alcoholic extracts of *Artemisia annua*, *A. absinthium*, *Asimina triloba*, and *Fumaria officinalis*. Trematocidal plant alcoholic extracts. Parasitol Res 109:1585–1592
107. Traub RJ, Macaranas J, Mungthin M, Leelayoova S, Cribb T, Murrell KD, Thompson RC (2009) A new PCR-based approach indicates the range of *Clonorchis sinensis* now extends to Central Thailand. PLoS Negl Trop Dis 3:e367
108. Kim TY, Chung EJ, Sohn WM, Hong SH, Yong TS (2011) Molecular characterization of *Clonorchis sinensis* tetraspanin 2 extracellular loop 2. Parasitol Res (in press)
109. Chen W, Wang X, Li X, Lv X, Zhou C, Deng C, Lei H, Men J, Fan Y, Liang C, Yu X (2011) Molecular characterization of cathepsin B from *Clonorchis sinensis* excretory/secretory products and assessment of its potential for serodiagnosis of clonorchiasis. Parasit Vectors 4:149
110. Wang X, Chen W, Li X, Zhou C, Deng C, Lv X, Fan Y, Men J, Liang C, Yu X (2011) Identification and molecular characterization of a novel signaling molecule 14-3-3 epsilon in *Clonorchis sinensis* excretory/secretory products. Parasitol Res (in press)
111. Lee MR, Kim YJ, Kim DW, Yoo WG, Cho SH, Hwang KY, Ju JW, Lee WJ (2011) The identification of antigenic proteins: 14-3-3 protein and propionyl-CoA carboxylase in *Clonorchis sinensis*. Mol Biochem Parasitol (in press)
112. Wu W, Chen J, Zeng S, Zhang Z, Gan W, Yu X, Hu X (2011) Molecular cloning, expression, and characterization of cyclophilin A from *Clonorchis sinensis*. Parasitol Res 109:345–351
113. Zhou Z, Xia H, Hu X, Huang Y, Li Y, Li L, Ma C, Chen X, Hu F, Xu J, Lu F, Wu Z, Yu X (2008) Oral administration of a *Bacillus subtilis* spore-based vaccine expressing *Clonorchis sinensis* tegumental protein 22.3 kDa confers protection against *Clonorchis sinensis*. Vaccine 26:1817–1825
114. Huang SY, Zhao GH, Fu BQ, Xu MJ, Wang CR, Wu SM, Zou FC, Zhu XQ (2012) Genomics and molecular genetics of *Clonorchis sinensis*: current status and perspectives. Parasitol Int 61:71–76
115. Wang X, Chen W, Huang Y, Sun J, Men J, Liu H, Luo F, Guo L, Lv X, Deng C, Zhou C, Fan Y, Li X, Huang L, Hu Y, Liang C, Hu X, Xu J, Yu X (2011) The draft genome of the carcinogenic human liver fluke *Clonorchis sinensis*. Genome Biol 12:R107
116. Yoo WG, Kim DW, Ju JW, Cho PY, Kim TI, Cho SH, Choi SH, Park HS, Kim TS, Hong SJ (2011) Developmental transcriptomic features of the carcinogenic liver fluke, *Clonorchis sinensis*. PLoS Negl Trop Dis 5:e1208