Intestinal Trematode Infections

7

Rafael Toledo, Maria Alvárez-Izquierdo,
Carla Muñoz-Antoli, and J. Guillermo Esteban

7.1 Introduction

It is estimated that more than one billion people are at risk of infection with food-borne trematodes and about 56 million people were infected in 2005 (Fürst et al. 2012a). According to the target organ in the definitive host, those trematodes are classified as liver, lung, and intestinal flukes. Intestinal trematodes are the largest group and about seven million people are infected worldwide (Fürst et al. 2012a). About 76 of species belonging to 14 families have been recorded infecting humans. Infection commonly occurs when humans eat raw or undercooked foods that contain the infective metacercariae. A variety of ailments are involved in the transmission of intestinal flukes (Table 7.1) and the eating habits are essential to determine the distribution of these parasitic diseases. High incidence of intestinal trematodiasis is strongly associated with populations living near freshwater bodies and the practice of eating raw or undercooked aquatic products. Thus, intestinal trematode infections are commonly considered as tropical diseases with severe endemic foci in Asia, where it is estimated that more than six million people are infected (Fürst et al. 2012a). 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, changes in eating habits in Western countries and demographic changes.

Despite the considerable public health impact and the emerging nature of intestinal trematodiases, these diseases are among the most neglected of the so-called neglected tropical diseases, and they are found predominantly in the world's poorest populations in low-income countries and, where these diseases are common, they exacerbate poverty. This makes necessary additional efforts to gain a better knowledge of these diseases to facilitate their control. In this chapter, we describe the biology, medical and epidemiological features, and current treatment and diagnostic tools of the main groups of intestinal flukes and the corresponding diseases.

R. Toledo (✉) · M. Alvárez-Izquierdo
C. Muñoz-Antoli · J. G. Esteban
Área de Parasitología, Departamento de Farmacia y Tecnología Farmacéutica y Parasitología, Facultad de Farmacia, Universidad de Valencia, Valencia, Spain
e-mail: Rafael.Toledo@uv.es; alizma@alumni.uv.es; Carla.Munoz@uv.es; Jguillermo.Esteban@uv.es

7.2 Family Brachylaimidae

7.2.1 Background

The family Brachylaimidae contains numerous species of terrestrial trematodes that infect mammals, birds, and reptiles (Gibson and Bray 1994).

R. Toledo, B. Fried (eds.), *Digenetic Trematodes*, Advances in Experimental Medicine and Biology 1154, https://doi.org/10.1007/978-3-030-18616-6_7

Table 7.1 Families of trematodes involved in intestinal human infections with data on the number of species involved, sources of infection, and geographical distribution

Family	Number of species cited in humans	Source of infection	Geographical distribution of human cases[a]
Brachylaimidae	1	Terrestrial snails	Oceania
Cathaemaciidae	1	Not known	Asia
Diplostomidae	2[b]	Snakes, frogs, tadpoles	Asia
Echinostomatidae	20	Freshwater fish, frogs, mussels, snails, tadpoles	Africa, Asia, Europe
Fasciolidae	1	Aquatic vegetables, contaminated water	Asia
Gastrodiscidae	1	Aquatic vegetables, crustaceans, molluscs, amphibians	Africa, Asia, Europe
Gymnophallidae	1	Oysters	Asia
Heterophyidae	26	Freshwater fishes	Africa, America, Asia, Europe
Lecithodendriidae	3	Dragonflies	Asia
Microphallidae	2	Shrimps, crabs	Asia
Nanophyetidae	1	Salmonid fishes	America, Europe
Paramphistomidae	2	Aquatic plants	Africa, Asia
Plagiorchiidae	4	Insect larvae	Asia
Strigeidae	1	Not known	Asia

[a]Imported cases are not considered
[b]One of them (*Fibricola cratera*) only in experimental infections

Brachylaima is the most representative genus within this family. This is, however, a very problematic genus that includes many poorly known species, for which the description of the adult stage is the only information available. This problem is compounded by the morphological similarity of many of the adult worms. Species of *Brachylaima* follow a three-host terrestrial life cycle (Yamaguti 1975). The first and second intermediate hosts of brachylaimids are either the same or two different species of terrestrial snail species. The definitive host can be either a mammal or a bird.

7.2.2 Species Reported in Humans and Geographical Distribution

Humans only have been reported in Australia as an incidental definitive host (three cases) for one species in the family, *Brachylaima cribbi* (Fig. 7.1). The first human infections with this trematode were described in two young children (21 months old) in rural South Australia in whom brachylaimid eggs were seen repeatedly in stools, but no adult worms were recovered. Both infants had been seen eating raw snails (Butcher et al. 1996). Thereafter, a 78-year-old woman presented with an 18-month history of intermittent diarrhea was found to be infected with a brachylaimid infection. Examination of her stools revealed the presence of brachylaimid eggs. She lived in a rural area of South Australia and ate raw vegetables which had been contaminated with helicid snails. After treatment with praziquantel a degenerate adult *Brachylaima* species was recovered in her stools (Butcher et al. 1998). The parasite was identified as *B. cribbi* (Butcher and Grove 2001).

Infections in humans usually become chronic and can persist as long as 18 months (Butcher et al. 1996, 1998). Clinical symptoms depend on the parasite load and heavy infections are associated with diarrhea with offensive stools several times a day, abdominal pain, low-grade fever, and fatigue (Butcher et al. 1996, 1998). There are no further studies on the pathology of infections with brachylaimids.

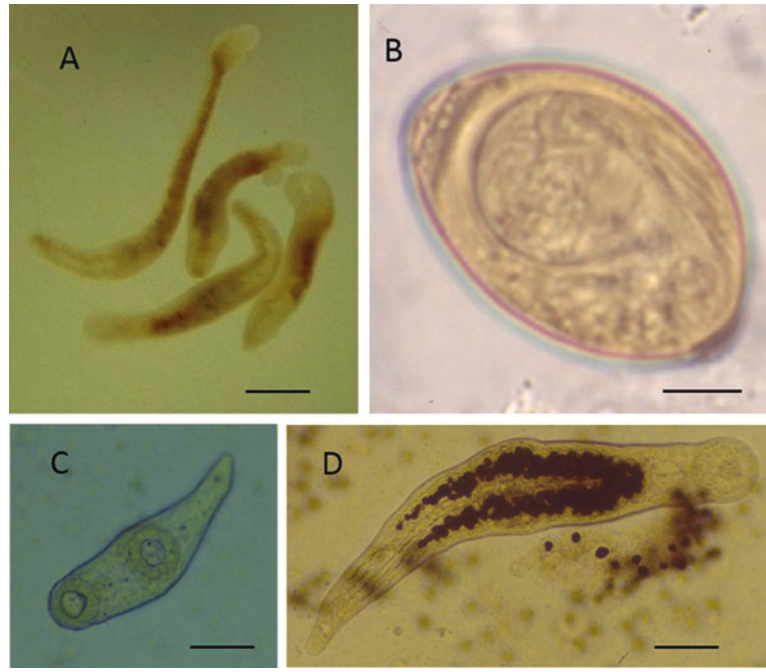

Fig. 7.1 Unstained specimens of *Brachylaima cribbi*: (**a**) adults (Scale bar: 1 mm); (**b**) fertile egg (Scale bar: 5 µm); (**c**) cercaria (Scale bar: 25 µm); and (**d**) metacercaria (Scale bar: 500 µm). Photomicrographs courtesy of Andrew R. Butcher

7.2.3 Host–Parasite Relationships and Immunology

The complexity of the life cycles of *Brachylaima* spp. may explain the scarcity of the data on host–parasite relationships in infections with brachylaimids. However, the use of different strains of mice as experimental hosts has allowed for studies on several aspects of the development of these flukes and the immunology of the infections. These studies have been focused on *B. cribbi*. However, there was variability in susceptibility in relation to gender and maturity of mice (Butcher et al. 2002). Mature female CB57BL/6J mice were significantly more resistant to *B. cribbi* infection that older mature females and adolescent females. These differences in susceptibility were attributed to physiological factors. Butcher et al. (2002) suggested that sex hormones provided a significant level of protection to *B. cribbi*.

B. cribbi evokes significant antibody responses as determined by indirect ELISA. Butcher et al. (2003) showed that humoral and/or cellular mediated immunity are important in mediating resistance and influencing the fertility of adult worms. The course of the infection in immunocompetent CB57BL/6J and immunodeficient NOD SCID mice re-infected with *B. cribbi* was assessed. In the case of CB57BL/6J mice, there were significant differences in the mean fecal eggs per gram of feces and worm fecundity, having lower egg counts and reduced fecundity with the challenge infections. In contrast, no significant differences were observed in NOD SCID mice between primary and challenge infections. The immunology of the infection in humans has not been studied.

7.3 Family Diplostomidae

7.3.1 Background

The family Diplostomidae Poirier, 1886 comprises digenean parasites of numerous orders of birds and mammals. Recently, Niewiadomska (2002) accepted a total of 41 genera within this

family grouped into subfamilies according to host specificity. A total of the 11 genera reported in mammals were included in the subfamily Alariinae Hall and Wigdor, 1960.

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 (Hong et al. 1982). Definitive hosts become infected by the ingestion of the second intermediate host harboring metacercariae. Eggs typically hatch and penetrate the first intermediate host (Cribb et al. 2003). In some Diplostomidae, the life cycle is expanded to incorporate four hosts by inclusion of an unencysted larval stage known as mesocercariae (a form between the cercariae and the metacercariae). In this case, the definitive host becomes infected after ingestion of the second intermediate host or the paratenic host harbouring mesocercariae.

7.3.2 Species Reported in Humans and Geographical Distribution

At least members of three genera of Diplostomidae (*Alaria, Neodiplostomum,* and *Fibricola*) are known to parasitize man. However, in the case of *Alaria* spp., humans serve as a paratenic host harboring metacercariae in different tissues. Humans become infected after eating tainted frog meat (Fried et al. 2004). At the intestinal level, only *Fibricola cratera* and *Neodiplostomum seoulense* serve as parasites of humans.

Human infections with *F. cratera* can be considered as anecdotal since the only one report is an experimental infection of a human volunteer (Shoop 1989). This species is a parasite of wild mammals in North America. Frogs are the second intermediate host, and snakes act as paratenic hosts (Shoop 1989). A total of 100 metacercariae were inoculated in a human volunteer producing a patent infection that lasted 40 months. Symptoms of epigastric discomfort, loose stools, and flatulence occurred over the first year of infection but ameliorated thereafter (Shoop 1989). No further studies have been conducted in relation to the human studies with this species.

N. seoulense, formerly named *F. seoulensis* (Chai and Lee 1991), is a relatively common parasite of humans and animals in Korea (Chai and Lee 2002). Morphologically, it is characterized by a bisegmented body, a tribocytic organ, butterfly-shaped testes and a wide distribution of the vitellaria in the anterior body to the level of the ventral sucker (Fig. 7.2a) (Seo et al. 1964, 1988; Chai et al. 2009a). The freshwater snails *Hippeutis (Helicorbis) cantori, Segmentina (Polipylis) hemiaespherula,* and *Austropeplea ollula* serve as the first intermediate host (Seo

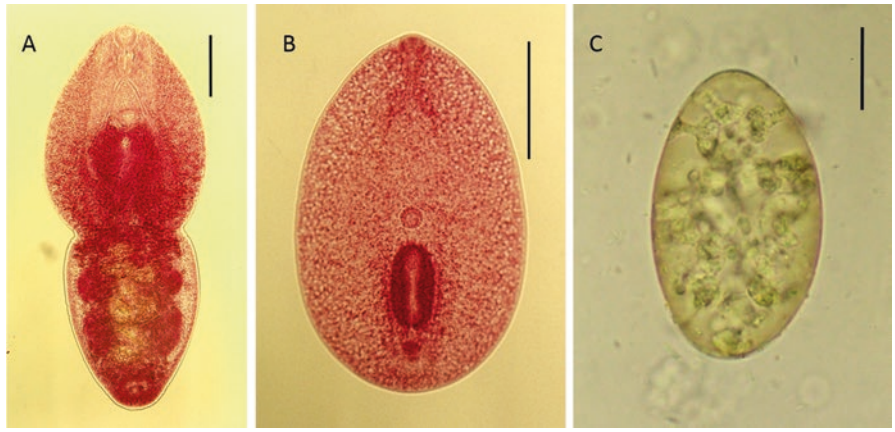

Fig. 7.2 *Neodisplostomum seoulense*: (**a**) adult specimen (Scale bar: 250 μm); (**b**) mesocercaria collected from a snake (Scale bar: 250 μm); and (**c**) egg (Scale bar: 25 μm). Photomicrographs courtesy of Woon-Mok Sohn

et al. 1988; Chung et al. 1996, 2002). Second intermediate hosts are tadpoles and frogs harboring metacercariae, and snakes may act as a paratenic host harboring the mesocercariae (Fig. 7.2b) (Hong et al. 1982; Seo et al. 1988). The site of infection in the definitive host is the duodenum but parasites may extend to the jejunum and ileum in heavy infections (Hong et al. 1983) (Fig. 7.2).

Human infections with *N. seoulense* only have been recorded in Korea (Chai et al. 2009a). This species was first implicated when a 25-year-old male suddenly suffered severe gastrointestinal symptoms (Seo et al. 1982). Another 26 cases were also reported among Korean soldiers, probably infected during their survival training. In all the cases, the infections were related with a history of consuming improperly cooked snakes and/or frogs (Seo et al. 1982; Hong et al. 1984a, b). Chai and Lee (2002) estimated the total number of human cases as 1000 in the Republic of Korea.

7.3.3 Clinical Manifestations and Pathology

The symptomatology of *N. seoulense* infections in humans has been scarcely studied and most of the current knowledge proceeds from studies on experimentally infected rodents (Toledo et al. 2006a).

The clinical manifestation and the pathology induced by *N. seoulense* is markedly related with the worm burden. It has been shown that the severity of the clinical symptoms and the mortality in experimentally infected mice is proportional to the cyst inoculum (Huh et al. 1988; Kook et al. 1998). In human infections, the symptomatology also has been shown to be dependent on the worm burden. Severe clinical manifestations only were reported in the first patient (Seo et al. 1982). This patient rapidly developed epigastric discomfort, fullness, and pain and anorexia. Thereafter, diarrhea, fever, and tenderness also appeared. After treatment with bithionol and magnesium purgation, a total of 79 adult worms were collected (Seo et al. 1982). In contrast, the remaining human cases had no clinical symptoms. The absence of clinical signs was attributed to the chronic and repeated infection with small amounts of metacercariae and a relatively low numbers of adults in the intestine (Hong et al. 1984a).

The parasite may cause mechanical and chemical damage. Each worm embraces a villous with the forebody which cause injury in the intestinal mucosa (Lee et al. 1985). Moreover, the tribocytic organ may pierce the host villi and secretes alkaline phosphatase which can lyse the villi (Huh et al. 1990; Huh and Hong 1993). The changes induced by *N. seoulense* were studied by Lee et al. (1985) in mice and rats. Shortening, widening, and fusion of the villi were observed. There was also a reduction in the number of goblet cells in the areas surrounding the worms, capillary congestion, lymphatic dilation, and inflammatory cell infiltration including lymphocytes, plasma cells, eosinophils, and occasional giant cells. In heavy infections, the changes were extended to the jejunum and gross bleeding was also observed (Lee et al. 1985). Pyo et al. (2012) showed that infection with *N. seoulense* downregulates the expression of the neuronal growth associated protein (GAP)-43 in mice suggesting that neuronal damage is induced by the parasite. Recently, Shin et al. (2016) demonstrated that *N. seoulense* reduced the fecundity in mice due to destruction of gonad tissues in a process that was infection intensity-dependent and progressive.

7.3.4 Host–Parasite Relationships and Immunology

There are some evidences supporting the existence of mechanisms of host protection against *N. seoulense*. For example, a significant reduction in worm recovery has been demonstrated in secondary infections in rats (Yu et al. 1995). In mice, it has been shown that worm survival is markedly dependent upon the genetic background of the host. The worm survival was markedly higher in BALB/c mice than in the C3H strain (Chai et al. 1998). However, little is known about the effector mechanisms of the host immune response in *N. seoulense* infections.

Several studies have shown that histamine and macrophages may have an important role on worm rejection. Histamine released by mast cells could facilitate worm expulsion by the increasing of intestinal motility and macrophages have been shown to kill worms in vitro (Shin et al. 2003, 2007). *N. seoulense* induces a mixed Th1/Th2 phenotype with overexpression of IFN-γ and IL-4 in mice (Shin et al. 2007). Antibody responses in mice are characterized by elevated levels of IgG, IgG2a, and IgA (Shin et al. 2007; Han et al. 2008). Major worm antigens appear to be located in the trybocytic organ, seminal vesicle, caeca, and vitelline follicles (Lee et al. 1997a). Han et al. (2008) detected by immunoblotting that the major antigens in the crude extract of adult worms had a molecular weight ranging from 26 to 94 kDa. Kim et al. (2008) identified two cystatin-binding cysteine proteinases, weighing 50 and 60 kDa, were recognized by the sera of humans infected with *N. seoulense*.

7.3.5 Diagnosis

Diagnosis of *N. seoulense* is usually done by detection of eggs in feces using traditional methods. The eggs are ellipsoid, thin shelled, with an inconspicuous operculum and measuring 86–99 × 55–63 μm (Seo et al. 1982) (Fig. 7.2c). They can be differentiated from other similar eggs, such as those of *Echinostoma* spp. by their clean shell surface and the absence of abopercular wrinkles at the posterior end (Chai et al. 2009a). Although immunological or DNA-based methods for diagnosis have not been developed, Kim et al. (2008) postulated that cystatin-binding cysteine proteinases could be putative antigens for serodiagnosis of *N. seoulense* infections in humans.

7.4 Family Echinostomatidae

7.4.1 Background

The family Echinostomatidae includes digeneans characterized by the presence of a prominent cephalic collar of spines (Fig. 7.3a). Adult echinostomatids are also characterized by the presence of spine-like structures, two post-ovarian testes in tandem located in the posterior part of the body and oral and ventral suckers that are close to each other.

Echinostomatids constitute a heterogeneous group of hermaphrodite trematodes that parasitize, as adult worms, numerous vertebrate hosts of all classes. The typical location is the intestine though species that parasitize other sites also exist (Toledo et al. 2009). Echinostomatids follow a three-host life cycle. The first intermediate hosts are aquatic snails in which a sporocyst, two generations of rediae and cercaria develop. Emerged cercariae freely swim and infect the second intermediate hosts, which may be several species of aquatic organisms such as snails, frogs, clams, and fishes. The definitive host, including humans, becomes infected after ingestion of the

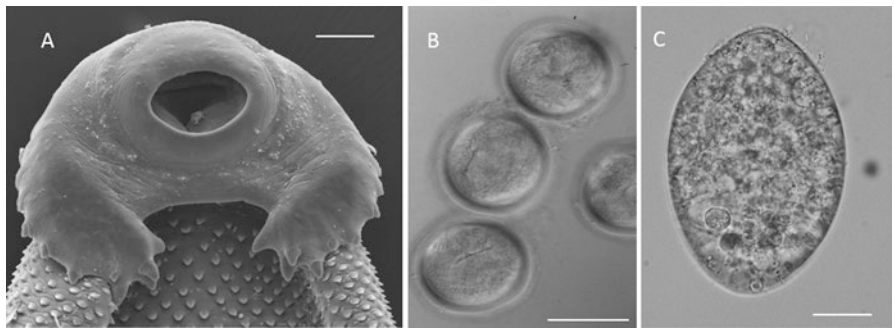

Fig. 7.3 (**a**) SEM microphotograph of the cephalic collar of spines of *Echinostoma* sp. (Scale bar: 100 μm); (**b**) metacercarial cysts of *Echinostoma* sp. (Scale bar: 100 μm); and (**c**) egg of *Echinostoma hortense* (Photomicrograph courtesy of In Sik Kim) (Scale bar: 25 μm)

second intermediate host harboring the encysted metacercariae (Fig. 7.3b). Finally, adults produce eggs that are released with the host's feces (Fig. 7.3c) (Toledo et al. 2009).

There is considerable confusion in relation to the taxonomy within the Echinostomatidae. This family has been viewed as a monophyletic taxon though the morphological similarity between its members and the diversity of the criteria adopted by the different authors for the classification have led to its division into an impressive number of taxa (Kostadinova and Gibson 2000; Fried and Toledo 2004; Kostadinova 2005; Esteban and Muñoz-Antoli 2009).

7.4.2 Species Reported in Humans and Geographical Distribution

Echinostomes commonly parasitize waterfowls and mammals associated with freshwater habitats. However, the specificity toward the definitive hosts is low determining that humans may become infected. Human echinostomiasis may occur worldwide though the distribution is usually focal though occasional cases are cited. Most of the cases are reported from East and Southeast Asia in relation to the eating habits in these areas (Graczyk and Fried 1998). Metacercariae, the infective stage, are ingested by humans in raw or undercooked freshwater fresh or brackish water molluscs, fishes, crustaceans, and amphibians (tadpoles or frogs). As a consequence, the infections are more prevalent in areas where traditional eating practices encourage the consumption of these types of foods. For example, in Philippines, human echinostome infections are related to eating raw fish or snails dipped in a salt and vinegar mixture, known as *kinilaw* and other methods of local fish preparations. In Cambodia, similar types of local food are commonly eaten which determine the prevalence observed in these areas (Belizario et al. 2007; Sohn et al. 2011a, b). Moreover, it has been suggested that drinking untreated water containing echinostome cercariae can be a source of human infection (Xiao et al. 1995).

The number and identity of the species causing human echinostomiasis is uncertain due to the absence of systematic surveys which determine that most of the available information is based on occasional case reports. Furthermore, the problematical taxonomy of the group complicates the situation since misidentifications are common. For example, *Artyfechinostomum malayanum* (Fig. 7.4a) was originally described in Malaysia as *Echinostoma malayanum*. Thereafter, *Artyfechinostomum sufrartyfex* has been considered by several authors as a synonym of *A. malayanum*. However, a recent study has suggested that the synonymy of these species is not valid (Tantrawatpan et al. 2013). Herein both species will be considered as separate taxa. Haseeb and Eveland (2000) listed a total of 21 species of echinostomes infecting humans, while Chai (2007, 2009) compiled 20 species and their identity differed with those reported by Haseeb and Eveland (2000) and Toledo and Esteban (2016) listed a total of 23 species. Table 7.2 compiled the main features of the echinostome species involved in human infections. Further details on the morphology and biology of these species can be found in the works by Chai and co-workers (Chai 2009; Chai et al. 2009a).

The highest incidence of echinostomiasis occurs in Asia, mainly in Southeast Asia (Toledo and Esteban 2016). In fact, all the species reported by Chai (2009) as causative of human echinostomiasis, with the exception of *Himasthla muehlensi*, have been reported in this area (Chai et al. 2009a).

A total of nine species have been reported infecting humans in China. Most of the cases occurred in the provinces of Fujian, Guangdong, Yunnan, Anhui, and Hubei. The mode of transmission could be the use of human excrements collected from latrines for fertilization of fish pond in these areas. Among the five members of the genus *Echinochasmus* reported in China (*E. fujianensis, E. perfoliatus, E. jiufoensis, E. liliputanus*, and *E. japonicus*), *E. fujianensis* is the most common. In several areas, the prevalence among residents reached the 3.2%, with the highest rates in children from 3 to 15 years

Fig. 7.4 Adult worms of (**a**) *Artyfechinostomum malayanum* (syn. *Echinostoma malayanum*) (Photomicrograph courtesy of Weerachai Saijuntha); (**b**) *Echinostoma hortense* (Photomicrograph courtesy of Woon-Mok Sohn); (**c**) *Echinoparyphium recurvatum*; and (**d**) *Hypoderaeum conoideum*. (Scale bars: 1 mm)

Table 7.2 Species of Echinostomatidae involved in human infections

Acanthoparyphium tyonense	Korea
Artyfechinostomum malayanum[a]	India, Indonesia, Lao PDR, Malaysia, Philippines, Singapore, Thailand
Artyfechinostomum oraoni	India
Echinoschasmus fujianensis	China
Echinoschasmus japonicus	China, Korea, Japanb, Lao PDR
Echinoschasmus jiufoensis	China
Echinoschasmus liliputanus	China
Echinochasmus perfoliatus	China, Japan
Echinoparyphium recurvatum	Egypt, Indonesia, Taiwan
Echinostoma angustitestis	China
Echinostoma cinetorchis	Japan, Korea, Taiwan
Echinostoma echinatum	Indonesia
Echinostoma hortense	China, Japan, Korea
Echinostoma ilocanum	Cambodia, India, Indonesia, Malaysia, Philippines, Thailand
Echinostoma macrorchis	Japan
Echinostoma revolutum	Cambodia, China, Egypt, Indonesia, Lao PDR, Russia, Thailand, Europe
Episthmium caninum	Thailand
Himasthla muehlensi	USA[c]
Hypoderaeum conoideum	Thailand
Isthmiophora melis	China, Rumania, Taiwan

[a]Syn. *Echinostoma malayanum*
[b]Experimental infection
[c]Imported infection

(Cheng et al. 1992; Yu and Mott 1994). The prevalence of *E. japonicus* in some counties of Guangdong and Fujian was 4.9%, whereas the prevalence of *E. perfoliatus* in these areas was lower (1.8%) (Yu and Mott 1994). Interestingly, prevalence of 13.4% of *E. liliputanus* was reported in Anhui (Xiao et al. 1992). A single case of human infection of *E. jiufoensis* has been reported during an autopsy of a 6-month-old girl in Guangzhou (Liang and Ke 1988). A total of three members of *Echinostoma* (*E. revolutum, E. hortense,* and *E. angustitestis*) have also been detected in China. *E. revolutum* has been reported in Yunnan and Guangdong provinces and two cases of *E. japonicus* infection were recorded in Fujian (Cheng et al. 1992; Chai 2009). In Liaoning Province, six patients with hepatitis were found to be infected with *E. hortense* (Fig. 7.4b) (Chen et al. 1993). More anecdotal is the infection with *Isthmiophora melis* (Chai 2009).

A total of five species of echinostomes have been reported in Indonesia infecting humans (*A. malayanum, Echinoparyphium recurvatum, Echinostoma echinatum, E. ilocanum,* and *E. revolutum*), mainly focused in the major Islands (Sumatra, Java, and Sulawesi) (Graczyk and Fried 1998). A high prevalence of *E. echinatum* was detected from 1937 to 1956 in the Lake Lindu Valley (Sulawesi). The average prevalence was 43%, but it reached 96% in some areas (Carney et al. 1980). Human infections appeared to be due to the habit of eating raw or insufficiently cooked lake molluscs, particularly bivalves (*Corbicula* spp.). Elimination of *Corbicula* clams from the diet significantly reduced the prevalences and a survey in the 1970s only revealed occasional presence of eggs (Clarke et al. 1974). In other areas, an increasing number of cases were reported in relation to the growing popularity of exotic food available in Korean and Japanese restaurants in Indonesia (Kusharyono and Sukartinah 1991; Graczyk and Fried 1998). Moreover, in the Indonesian area of Borneo (West Kalimantan), echinostome eggs are frequently observed in faces of the residents (Cross et al. 1976).

A total of four species have been recorded in Japan (*Echinochasmus perfoliatus*, *Echinostoma cinetorcis, E. hortense,* and *E. macrorchis*), Korea (*A. tyosenense, E. hortense, E. cinetorchis,* and *E. japonicus*), Thailand (*E. revolutum, E. ilocanum, Episthmium caninum,* and *Hypoderaeum conoideum*), Lao PDR (*A. malayanum, E. japonicus, E. revolutum,* and *Euparyphium* sp.), and India (*A. malayanum, A. oraoni, A sufrartyfex,* and *E. ilocanum*). However, some of these species only have been found occasionally. For example, in Japan only one case has been detected for *E. perfoliatus* and *Echinostoma macrorchis* in Japan (Majima 1927; Hirazawa 1928), while three cases were reported for *Episthmium caninum* in Thailand (Radomyos et al. 1985, 1991). In the case of *A. tyosenense* and *A. oraoni*, a total of 10 and 20 cases were reported in Korea and India, respectively (Bandyopadhyay and Nandy 1986; Bandyopadhyay et al. 1989; Chai et al. 2009a). Human infections with *E. cinetorchis* were first reported in Japan and in the Republic of Korea (Takahashi et al. 1930; Kawahara and Yamamoto 1933; Seo et al. 1980; Ryang et al. 1986; Lee et al. 1988; Jung et al. 2014). Human infections with *E. hortense* have been reported both in Japan and Korea (Chai 2009). An endemicity of this echinostome species has been reported among residents of Cheongsong-gun (Republic of Korea) with 22% of prevalence (Lee et al. 1988). *Hypoderaeum conoideum* (Fig. 7.4c) in humans only has been recorded in the northeast area of Thailand with a prevalence of 55% among the residents (Yokogawa et al. 1965).

A recent study in Laos PDR among 2074 residents in riparian villages along the Mekong River in the Khammouane Province demonstrated the presence of echinostome eggs in the feces of 1.1% of residents. A total of 55 specimens belonging to *E. revolutum, A. malayanum, E. japonicus,* and *Echinoparyphium* sp. were recovered after treatment and purgation (Chai et al. 2012). Sayasone et al. (2009a) detected three human cases of *E. japonicus*. Recently, human infections with *E. ilocanum* were recorded by the first time in Laos PDR in two riparian people from Savannakhet Province, confirming that echinostomiasis is prevalent in the Meking River (Chai et al. 2018). A prevalence of 6% was found in southern Lao PDR (Sayasone et al. 2011).

In Taiwan, three species (*E. revolutum, E. recurvatum,* and *I. melis*) were reported in humans (Lu 1982) and the prevalence of infection varied from 11 to 65% in some areas (Carney 1991). In Cambodia, *E. revolutum,* and *E. ilocanum* have been recently reported. Sohn et al. (2011a) demonstrated a prevalence of 1.0% of *E. ilocanum in* the Oddar Meanchey Province. Sohn et al. (2011b) determined a prevalence of 7.5–22.4% of *E. revolutum* in children from the Pursat Province. In the Philippines, *E. ilocanum* and *A. malayanum* infect humans with an overall prevalence of 3% though it reached 44% in some areas (Eduardo 1991). In Malaysia and Singapore, only human infections with *A. malayanum* have been recorded (Chai 2009). Recently, human echinostomiasis in Nepal was detected by the first time by Sah et al. (2018) who detected a single adult worm by endoscopy in a Hindu patient with gastrointestinal disorders. Coprological examination showed the presence of eggs of an echinostomatid.

The number of known reports outside of Asia is very limited. Only four species have been occasionally recorded. A human infection with *I. melis* was detected in 1916 in a diarrheic patient in Rumania (Beaver et al. 1984) and *E. recurvatum* (Fig. 7.4d) and *E. revolutum* have been sporadically reported in Egypt and Russia, respectively (Chai 2009). Imported cases in the USA also have been reported. *H. muehlensi* was originally described on the basis of five adult specimens from a German patient who lived in Colombia and traveled to New York, where he had eaten raw clams (Chai 2007). DeGirolami and Kimber (1983) recorded *Echinostoma* sp. from Asian refugees in the USA. Poland et al. (1985) reported 18 cases of imported echinostomiasis among a total of 20 American tourists to Kenya. A total of ten of the patients showed moderately severe abdominal cramps and loose or watery stools. A traveler group of seven Caucasians became infected with *Echinostoma* sp. In Tanzania, after ingesting raw fish from Lake Tanganika. The infection was diagnosed by finding eggs in stool samples at their return to Kenya. This constituted the first report of human echinostomiasis in East Africa (Chunge and Chunge 2017).

7.4.3 Clinical Manifestations and Pathology

The clinical symptoms of human echinostomiasis may be more severe than those produced by other intestinal trematodes though the clinical features greatly depend on the parasite load (Graczyk and Fried 1998; Chai 2007). Human morbidity and mortality due to echinostomiasis depend on a number of factors such as a prolonged latent phase, limited acute phase, and similarity with the symptomatology of other intestinal pathologies and, even, the existence of asymptomatic presentations (Graczyk and Fried 1998).

Epigastric and abdominal pain, easy fatigue, diarrhea, and weight loss are the most common symptoms in human echinostomiasis (Graczyk and Fried 1998; Chai and Lee 2002; Toledo et al. 2006a). Although this fact, other symptoms also can be detected. Several studies have shown that patients infected with *E. hortense* additionally suffered acid belching, anorexia, headache, nausea and vomiting, and urinary incontinence (Lee et al. 1986; Chai et al. 1994; Chang et al. 2005; Park and Kim 2006). Peripheral blood eosinophilia has been commonly reported (Poland et al. 1985; Lee et al. 1988). However, the levels of eosinophilia appear to be markedly dependent upon the worm load and ranged from 2 to 24% as demonstrated in *E. hortense* infections (Lee et al. 1988).

The intestinal pathology induced by echinostomes in humans has been poorly studied and most of the known data were obtained by gastroduodenal endoscopies in *E. hortense* infections. In general, the patients showed mucosal erosion and ulceration, bleeding in the stomach and the duodenum, signs of chronic gastritis, and infiltration of inflammatory cells including neutrophils (Chai et al. 1994; Chang et al. 2005; Cho et al. 2003). Interestingly, stage IIc or stage III early gastric cancer was determined by gastroduodenal fiberscopy (Chai et al. 1994). Cortés et al. (2015a) showed that echinostome infection in mice induces significant changes in the intestine affecting to the restoration of the intestinal epithelium and the control of homeostatic dysregulation, concomitantly with mitochondrial and cytoskeletal

proteins among others, which may conduct to malignant transformation.

Further details on the pathology of echinostome infections were obtained using laboratory rodents. The pathological effects of echinostomiasis are dependent on a wide variety of factors including the echinostome species, the host species, and the intensity of the infection (Toledo et al. 2006a). Echinostomes provoke inflammatory responses in the attachment sites. The surrounding areas showed marked dilation, erosion of the villi and lymphocytic infiltration (Huffman et al. 1988; Mabus et al. 1988; Toledo et al. 2006b, 2009; Muñoz-Antoli et al. 2007). Moreover, goblet cell hyperplasia, neutrophilia and infiltration of inflammatory cells, changes in the mucin expression and glycosylation, alterations of the intestine epithelial cell turnover and crypt hyperplasia with increased mitotic rates also occur (Fujino and Fried 1996; Toledo et al. 2006b; Cortés et al. 2015b, c). Cellular infiltration of lymphocytes, eosinophils, and plasma cells also were observed in the lamina propria and submucosa (Weinstein and Fried 1991; Toledo et al. 2006b).

7.4.4 Host–Parasite Relationships and Immunology

Immunology of echinostomiasis has been extensively studied in laboratory rodents. It has been shown that the rodent hosts are able to express various types of resistance to echinostome infections which suggest that the parasites can be spontaneously expulsed or, in contrast, develop a chronic infection depending on the host–parasite combination (Toledo and Fried 2005; Toledo et al. 2006a, 2009).

Although it has been shown that echinostomes alter several immunological parameters the role of these alterations in the course of the infections remains unclear (Chai 2009; Toledo 2009; Toledo et al. 2009). *Echinostoma* spp. induces changes at the cellular level and in the expression of certain glycoconjugates in the intestinal mucosa (Toledo et al. 2006a). Mastocytosis, eosinophilic infiltration, and increase in the goblet cells and mast cell populations have been commonly observed, though there are conflicting data in relation to their effect on the echinostome infections (Fujino and Fried 1993, 1996; Fujino et al. 1993, 1996a, b; Kim et al. 2000; Park et al. 2005; Toledo et al. 2006b; Muñoz-Antoli et al. 2007; Ryang et al. 2007). Furthermore, several studies have suggested that the alterations of the terminal sugar of the mucins produced by goblet cells may regulate the worm expulsion (Fujino and Fried 1993, 1996; Park et al. 2005).

Apart from the changes in cell populations, echinostomes also may induce energic antibody responses (Toledo 2009). However, they do not appear to alter the course of the infections. For example, *Echinostoma caproni* induces elevated responses of IgM, IgG, IgG1, and IgG3 in the serum of mice in which the parasite survives for more than 25 weeks (Graczyk and Fried 1994; Toledo et al. 2004; Sotillo et al. 2007). In contrast, low levels of antibodies were detected in the serum of *E. caproni*-infected rats concomitantly with an early expulsion of the worms (Sotillo et al. 2007). At the intestinal level, increases in the IgM, IgA, IgG1, and IgG2a levels were detected in mice (Sotillo et al. 2007). The response against *E. hortense* is characterized by an elevation of the serum levels of IgG1, IgE, and IgA (Cho et al. 2007). This can be explained, at least in part, by the fact that echinostomes are able to trap and degrade the surface-bound antibodies as a mechanism of immune evasion (Cortés et al. 2017a). The target antigens of these responses in *E. caproni* infections were studied by Sotillo et al. (2008). A total of four proteins (enolase, actin, HSP-70, and aldolase) appeared to be the major antigens in the *E. caproni* adult worms.

The cytokine profile in echinostome infections has been poorly studied. However, new information about this topic has been obtained in recent years. The production of cytokines in the splenocytes of mice infected with *E. hortense* has been studied (Cho et al. 2007; Ryang et al. 2007; Lee et al. 2009). These studies detected a predominance of the Th2 responses with elevated expression of IL-4 and IL-5. In *E. caproni* infections, Brunet et al. (2000) observed an elevated production of IFN-γ in the spleen cells of

experimentally infected mice. Comparative studies using hosts of different compatibility with *E. caproni* have provided further insight in the responses determining the course of the infection. The development of chronic infections appears to be related with the development of local Th1 responses with elevated levels of IFN-γ, whereas the early worm rejection is mediated by the development of a biased Th2/Th17 local phenotype (Sotillo et al. 2011; Trelis et al. 2011). Although, IFN-γ production has been associated with chronicity and elevated mucosal damage, this cytokine seems to play a dichotomous role in the infection facilitating the parasite establishment, but it may also benefit mice since it protects the mice from morbidity and mortality induced by the parasite (Cortés et al. 2014).

Recently, it has been shown that a primary infection with *E. caproni* in ICR mice induces partial resistance against subsequent homologous infections. This resistance was expressed as a reduced rate of infection, worm recovery, and worm size, indicating that primary infection induces changes in the host, making a hostile environment for the development of the parasite (Muñoz-Antoli et al. 2016a). Further studies on this topic indicated that susceptibility is determined by the lack of IL-25 expression in response to primary infection. In contrast, infection in an environment with elevated levels of IL-25, as occurs in challenge infection, results in a Th2 phenotype impairing parasite survival (Muñoz-Antoli et al. 2016b).

Interestingly, it has been found that the protein production of echinostome adult worms is markedly affected by the host milieu. Upregulation of proteins with stress or detoxification process has been observed in highly susceptible hosts which may serve to withstand the hostile Th1 environment generated in primary infections in these hosts (Cortés et al. 2016, 2018).

7.4.5 Diagnosis

Clinical diagnosis of human echinostomiasis is difficult since the infection may remain unapparent for a while or the symptoms, if present, are often unspecific. Laboratory diagnosis is based on the demonstration of eggs in feces. The eggs are oval, yellowish, thin shelled, and with an operculum at the anterior end which may be difficult to see. The size of the most human infecting echinostomes is in the range 66–145 × 43–90 mm though the eggs of several species may fall outside this range (Chai 2009). However, the difficulty entailed in the specific characterization of the eggs strongly recommends recovering the adult worms.

Although immunological methods for the diagnosis of human echinostomiasis have not been developed, several studies using laboratory rodents have shown that conventional ELISA and capture ELISA may be promising methods for the detection of human infections (Agger et al. 1993; Graczyk and Fried 1995; Toledo et al. 2003, 2004, 2005; Cho et al. 2007; Sotillo et al. 2007).

7.5 Family Fasciolidae

7.5.1 Background

The family Fasciolidae comprises large trematodes that inhabit the liver and bile ducts but members of two genera (*Fasciolopsis* and *Parafasciolopsis*) are intestinal parasites. The life cycle of the members of this family includes a metacercarial stage that encysts on pasture and other vegetation.

7.5.2 Species Reported in Humans and Geographical Distribution

Fasciolopsis buski is the only fasciolid species reported infecting the intestine of humans. This is the largest trematode parasitizing humans (8–10 × 1–3 cm) (Fig. 7.5a) and a common intestinal parasite of human and pigs in Asia (Fried et al. 2004).

In humans, *F. buski* inhabits the duodenum and the jejunum though it can extend to almost the complete intestine and, even, the stomach in heavy infections. Adult worms produce over

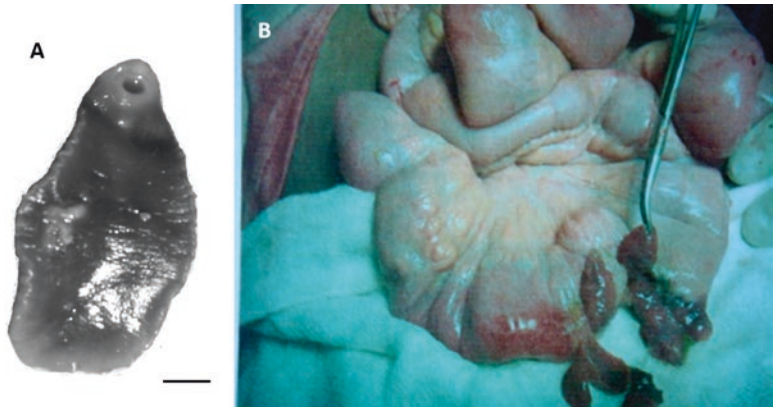

Fig. 7.5 (**a**) Unstained specimen of *Fasciolopsis buski* (Scale bar: 0.5 mm) (Photograph courtesy of Deepak Bagga); (**b**) *Fasciolopsis buski* emerging from perforation site of the ileum (Scale bar: 5 μm) (Photograph courtesy of Umesh Chandra Singh)

25,000 eggs every day. Unembryonated eggs are discharged into the intestine and stool. Eggs become embryonated in water and release miracidia, which invade a suitable snail intermediate host. Several species of genera *Segmentina* and *Hippeutis* serve as intermediate hosts. In the snail, the parasite undergoes several developmental stages (sporocysts, rediae, and cercariae). The cercariae are released from the snail and encyst as metacercariae on aquatic plants such as water chestnut, water caltrop, lotus, bamboo, and other edible plants. The mammalian final host becomes infected by ingesting metacercariae on the aquatic plants. After ingestion, the metacercariae excyst in the duodenum in about 3 months and attach to the intestinal wall. There they develop into adult flukes in approximately 3 months, attached to the intestinal wall of the mammalian hosts (humans and pigs). The adults have a life span of about 1 year.

Fasciolopsiasis is confined and endemic in Far East and Southeast Asia (Fried et al. 2004). The disease occurs focally and is linked to freshwater habitats and is associated with common social and agricultural practices and promiscuous defecation (Mas-Coma et al. 2005). Humans commonly become infected by eating raw or undercooked aquatic plants, but infection can also be contracted by the drinking or use of contaminated water or processing of the water-derived plants, e.g., using teeth to peel plants (Gilman et al. 1982; Weng et al. 1989; Fried et al. 2004). Fasciolopsiasis can be aggravated by social and economic factors such as poverty, malnutrition, and uninspected and poorly sanitized food markets (Mas-Coma et al. 2005). In fact, differences in the incidence within the same area have been found in relation to the economic status, educational background, or the standard of health and/or way of life (Jaroonvesama et al. 1986). The infection predominantly occurs in children and the worm burden may exceed 800 flukes/child (Gilman et al. 1982; Weng et al. 1989). In foci of transmission, the prevalence of infection in children ranged from 10 to 60% in countries such as China, Taiwan, India, Bangladesh, or Thailand (Muttalib and Islam 1975; Rahman et al. 1981; Bunnag et al. 1983; Shyu et al. 1984; Weng et al. 1989). Pigs may play an important role in the transmission of the parasite. The pig is the main reservoir and different infection rates have been reported, ranging from 10% in China to 52% in Taiwan (Mas-Coma et al. 2005). Fresh aquatic green fodder and untreated water used to raise pigs appear to be the source of infection in farm animals (Weng et al. 1989; D'Souza et al. 2001).

In China, infections have been reported in ten provinces reaching 85% of prevalence in some of them (Weng et al. 1989). In Bangladesh, the prevalence in schoolchildren in an endemic focus reached 50% (Gilman et al. 1982). In India, 60% of people were found to be infected and harboring 1–57 worms in Assam (Buckley 1939). In Thailand, the central area is the main endemic area with an estimation of 20% of infected people (Manning et al. 1971). Infection has also been reported from Lao PDR, Vietnam, Cambodia,

Indonesia, the Philippines, Kampuchea, Burma, and Malaysia (Mas-Coma et al. 2005). Moreover, imported cases from the Far East have been recorded in Korea, Japan, the USA, Venezuela, Australia, Guatemala, Israel, and Cuba (Mas-Coma et al. 2005).

Although several studies showed that human fasciolopsiasis decreased in the 1980s, this tendency was not maintained since people continue eating raw vegetables. Recently, Quang et al. (2008) suggested the possibility of an emergence of human fasciolopsiasis in Lao PDR.

7.5.3 Clinical Manifestations and Pathology

Clinical symptoms in *F. buski* infections in humans are related to parasite load and can be fatal in heavy infections (Gilman et al. 1982; Weng et al. 1989). In light infections, symptomatology may include anemia, eosinophilia, dizziness, and gastrointestinal symptoms. In moderate and heavy infections, there may appear severe epigastric and abdominal pain, diarrhea or bowel obstruction, nausea, acute ileus, anasarca, and eosinophilia and leucocytosis (Gilman et al. 1982; Fried et al. 2004). Eventually, it may cause intestinal perforation or obstruction and may produce apendicitis (Fig. 7.5b) (Bhattacharjee et al. 2009; Cao et al. 2015).

Moreover, adult flukes damage the intestinal mucosa and cause extensive duodenal erosions, ulceration, hemorrhage, abscesses, and catarrhal inflammation. Absorption of toxic and allergic worm metabolites causes ascitis, general edema, and facial edema (Jaroonvesama et al. 1986; Fried et al. 2004).

7.5.4 Diagnosis

Laboratory diagnosis is based on the demonstration of eggs in feces. The eggs are ellipsoidal, operculated, non-embryonated, and measuring 130–140 × 80–85 mm (Gilman et al. 1982). Immunological or molecular methods have not been developed.

7.6 Family Gastrodiscidae

7.6.1 Background

The family Gastrodiscidae contains trematodes relatively large, i.e., approximately 8–14 mm in length. They are intestinal parasites of terrestrial mammals, including man, and have been distinguished by a dorsoventrally flattened body, which has the appearance of being divided into two parts.

7.6.2 Species Reported in Humans and Geographical Distribution

Only one species of Gastrodiscidae, *Gastrodiscoides hominis*, has been found infecting humans. Adult of *G. hominis* are large flukes (8–14 × 5.5–7.5 mm). Moreover, this species is characterized by a short and cylindrical anterior part, large and discoidal posterior part, subterminal pharynx, testes lobed and in tandem, a post-testicular ovary, an ascending uterus, and a ventral genital pore.

The life cycle is not completely understood. Adult worms inhabit the caecum and colon of humans, pigs, small, monkeys, and other mammals. Unembryonated eggs are laid and, in a freshwater environment, the miracidium hatches and infects the first intermediate host. Only the snail *Helicorbis coenosus* is known to act as the first intermediate host. After the development of mother and daughter rediae, the cercaria emerges and encysts in aquatic plants, snails, tadpoles, frogs, or crayfish. Definitive host becomes infected after swallowing metacercariae with tainted vegetables or raw or undercooked crustaceans, molluscs, or amphibians (Mas-Coma et al. 2006).

G. hominis has been detected infecting humans in India, Burma, Pakistan, Myanmar, Vietnam, the Philippines, Thailand, China, Kazakhstan, Indian immigrants in Guyana, Zambia, Nigeria, and the Volga Delta in Russia (Mas-Coma et al. 2005). Although *G. homins* is mainly a parasite of pigs, high prevalence in humans has been detected in some areas. For

example, Buckley (1939) detected a prevalence of 41% in children from Assam (India).

Pathology and symptomatology of humans *G. hominis* infections are not well known. Heavy infections may induce headache, epigastric pain, and diarrhea that may be a reaction to metabolites released by the parasite (Marty and Andersen 2000). Acetabulum of the adult worm is found to drag the mucosa like a plug causing inflammation (Mas-Coma et al. 2005, 2006). In human infection, a picture similar to that detected in pigs might be expected. Surface desquamation, infiltration with eosinophils, lymphocytes, and plasma cells appear in sections of the lesions caused by the fixation of the parasites to the mucosa. Hypersecretion of mucus and necrosis of the mucous glands are also observed. The lamina propria shows infiltration of eosinophils, lymphocytes, macrophages, and plasma cells (Mas-Coma et al. 2005, 2006).

Diagnosis of human gastrodiscoidiasis is feasible by detection of eggs in feces. Egg size is about 4–6 × 5–10 mm and it is deep yellow, operculated, non-embryonated, and measuring about 150 × 70 mm (Mas-Coma et al. 2006).

7.7 Family Gymnophallidae

7.7.1 Background

Gymnophalloidiasis is the intestinal infection caused by *Gymnophalloides seoi*, belonging to the family members of the family Gymnophallidae. This family consists of a small group of marine digeneans. Most members use molluscs as the first intermediate host; the metacercariae never encysts and are usually parasitic in bivalves. With rare exceptions, charadriiform and anseriform birds are the definitive host which becomes infected after ingestion of the second intermediate host harboring the metacercariae (Cremonte et al. 2013). Although a total of five genera are accepted in the family, only *G. seoi* has been found infecting humans.

7.7.2 Species Infecting Humans and Geographical Distribution

Human infections with *G. seoi* only have been recorded in the Republic of Korea (Fried et al. 2004; Chai et al. 2009a; Toledo et al. 2012). This fluke was first discovered in 1988 in a Korean woman suffering pancreatitis and gastric discomfort (Lee et al. 1993a; Chai et al. 2003). This woman lived in the southwestern coastal island of Aphae in the Shinan County, which was subsequently found to be an endemic area. Then, more than 25 villages in western and southwestern coastal islands and 3 mainland coastal villages have been reported as endemic foci with prevalences ranging from 9.3 to 57.9% (Lee et al. 1996; Chai et al. 1997, 2009a, b; Fried et al. 2004; Guk et al. 2006; Chai 2007; Cho et al. 2010).

The adult parasite is small (0.4–0.5 × 0.2–0.3 mm) and characterized by a large oral sucker and small ventral sucker, short caeca, and a unique ventral pit (Fig. 7.6a) (Chai et al. 2003). The first intermediate host remains unknown but the second intermediate host was found to be the oyster *Crassostrea gigas* (Lee et al. 1995; Sohn et al. 1998). Humans become infected by eating raw or undercooked oysters harboring the metacercariae (Fig. 7.6b). The parasite inhabits the small intestine though a case of mucosal tissue invasion has been reported. An adult worm was incidentally found in a surgical pathology specimen of the lymph node around the colon of a 65-year-old Korean patient (Seo et al. 2006).

The symptomatology induced by *G. seoi* may vary greatly between individual patients. Gastrointestinal discomfort and indigestion are the most common symptoms. Moreover, fever, anorexia, weight loss, easy fatigue, and weakness can also appear (Chai et al. 2003). It has also been suggested that gymnophalloidiasis increases the levels of amylase in the serum and urine. In two patients, the gymnophalloidiasis was accompanied by diabetes mellitus suggesting a relationship between both diseases (Lee et al. 1995).

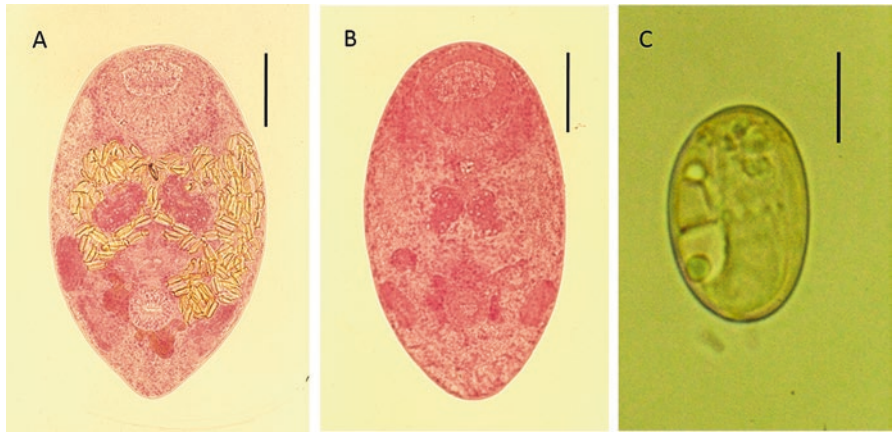

Fig. 7.6 *Gymnophalloides seoi*: (**a**) adult worm (Scale bar: 100 μm); (**b**) metacercaria collected from a naturally infected oyster (Scale bar: 100 μm); and (**c**) egg (Scale bar: 10 μm). Photomicrographs courtesy of Woon-Mok Sohn

7.7.3 Host–Parasite Relationships and Immunology

Apparently, *G. seoi* induces less pathological damage than other intestinal trematodes. Adult worms inhabit the duodenum and jejunum where they are attached by grasping or pinching the epithelial layer with the oral sucker. This induces villous atrophy and crypt hyperplasia with inflammation of the villous stroma and the crypt (Chai et al. 2001; Toledo et al. 2006a). A marked goblet cell hyperplasia dependent on CD4+ T-helper cells along the villous epithelia was observed, mainly in the jejunum (Chai et al. 2001; Guk et al. 2009). These changes were resolved at 2–3 weeks post-infection. However, it is suspected that *G. seoi* invades the pancreatic duct of humans which may cause further complications which may require medical attention. Acute pancreatitis was diagnosed in one patient of gymnophallodiasis and diabetes mellitus was accompanied in other two patients (Lee et al. 1993a, 1995).

In mice, *G. seoi* infections are spontaneously expelled within 3 weeks post-infection but the course of the infection differs depending on the strain of mice (Lee et al. 1997b; Chai et al. 1999) indicating a genetic background in the resistance to infection. Moreover, comparative studies on the development of *G. seoi* in immunocompetent and immunosuppressed mice indicated that the differential susceptibility is dependent on the host immune response (Lee et al. 1997b; Chai et al. 1999; Seo et al. 2003). Little is known about the immune effector mechanisms responsible for the short-term parasitism of *G. seoi* observed in mice. However, the observation of a strong proliferation of goblet cells previous to the worm expulsion in *G. seoi*-infected mice suggested that this can be one of the effector mechanisms involved in worm rejection (Chai et al. 2001). Lee et al. (2013) showed that *G. seoi i*nfection in mice induces overexpression of STAT6 and IL-13 enhancing goblet cell hyperplasia responsible for the worm expulsion. Increased intestinal epithelial cell turnover, and increased intestinal motility should be important mucosal defense mechanisms in *G. seoi*-infected mice (Lee et al. 2014). However, recent studies have shown that T-cell-independent mechanisms also mediate in the expulsion of *G. seoi*. In this context, alteration of the mucin quality with changes in the terminal sugar chain and elevated levels of IL-4 and IL-5 expression appears to be of importance (Guk et al. 2009). Furthermore, it has been demonstrated that *G. seoi* antigens upregulate Toll-like receptors and mucin-related genes (MUC2) in human intestinal epithelial cells via IFN-γ (Lee et al. 2010).

7.7.4 Diagnosis

Diagnosis can be done by detection of eggs in the feces. However, the detection and identification may be difficult because of: (1) low-laying capacity of the parasite and (2) identification of *G. seoi* eggs. The daily egg output/worm is estimated in 2–84 in the human host (Chai et al. 2003) which makes difficult the isolation of the eggs unless heavy infections occur. Another difficulty entailed with the diagnosis of the gymnophalloidiasis is the identification of the eggs. They are very small (approximately 23 × 13 μm) and thin and transparent shelled (Fig. 7.6c) (Lee et al. 2012). Due to their small size, the eggs can be overlooked by an inexpert analyst or misdiagnosed as a bubble or other artifacts. Moreover, differential diagnosis with other digeneans may be difficult (Lee et al. 2012).

7.8 Family Heterophyidae

7.8.1 Background

Heterophyiasis is caused by the members of the family Heterophyidae. This family contains small egg-shaped trematodes with infective metacercariae that are usually encysted in fish second intermediate hosts. The heterophyids are characterized by the possession of a gonotyle or genital sucker (Yamaguti 1971). This author accepted 13 subfamilies within the family which may be differentiated on the basis of the body morphology, the presence of a circumoral crown of spines, extension of the vitellaria and uterus, morphology of the testes, and location of the genital pore.

The definitive host becomes infected by eating raw or poorly cooked fish harboring metacercariae. 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 (species of *Melanoides, Semisulcospira,* and others), and hatch within the snail's intestine. Intramolluscan development comprises sporocyst and redial stages and cercariae are released into the water where they typically penetrate shrimps or shore-fish, such as cunners, gudgeon, and charr, and encyst on the surface or muscles of the fish. Metacercariae may remain viable for years (Fried et al. 2004; Toledo et al. 2006a; Sohn 2009).

7.8.2 Species Infecting Humans and Geographical Distribution

A total of 26 species of heterophyids have been reported infecting humans (Table 7.3). Humans become infected by eating fish or shrimps harboring viable metacercariae, which mature into adults in 5–10 days. Adult heterophyids attach and live embedded the intestinal mucosa producing embryonated eggs that reach water reservoir due to the lack or non-use of sanitary latrines which allow for the maintenance of the life cycle.

Probably, *Heterophyes heterophyes* and *Metagonimus yokogawai* (Fig. 7.7a, b) are the most relevant species from the standpoint of human disease. *H. heterophyes* was first discovered in an Egyptian and is a common parasite in the Nile Delta around the lakes Manzala, Borollos, and Edco (Yu and Mott 1994). In the period 1984–1991, the prevalence in the five governorates of the Delta ranged from 0.001 to 1% and the population at risk was estimated as 933,000. People became infected by consuming recently salted or insufficiently baked fish (Sheir and Aboul-Enein 1970). In the villages of Khuzestan (Iran), the prevalence in humans ranged from 2 to 24% (Yu and Mott 1994). In Asia, several foci have been identified but this parasite could be confused with *Heterophyes nocens* (Chai et al. 2009a). Imported cases in Japan were reported from people who returned from Egypt, Saudi Arabia, and Sudan (Chai et al. 2009a). In Western Europe, human infections with *H. heterophyes* have been recorded sporadically. For example, Martínez-Alonso et al. (1999) described the infection of a woman after eating raw fish in a Chinese restaurant in Spain. *M. yokogawai* is probably the most common intestinal fluke infecting humans in the Far East. It has been recorded in China, Japan, Korea, Taiwan,

Table 7.3 Species of Heterophyidae involved in human infections

Genus	Species	Geographical distribution of human cases
Apophallus	*A. donicus*	USA
Ascocotyle	*A. longa*[a]	Brazil[a]
Centrocestus	*C. armatus*	Japan, Korea
	C. caninus	Thailand
	C. cuspidatus	Egypt
	C. formosanus	Japan, Taiwan, Vietnam, Lao PDR
	C. kurokawai	Japan
Cryptocotyle	*C. lingua*	Greenland
Haplorchis	*H. pleurolophocerca*	Egypt
	H. pumilio	China, Egypt, Korea, Lao PDR, Philippines, Taiwan, Thailand, Vietnam
	H. taichui	China, Lao PDR, Philippines, Thailand, Vietnam
	H. vanissimus	Philippines
	H. yokogawai	China, Egypt, India, Indonesia, Lao PDR, Malaysia, Philippines, Taiwan, Thailand
Heterophyes	*H. dispar*	Korea[b]
	H. heterophyes	Egypt, Iran, Korea[b], Sudan
	H. nocens	China, Japan, Korea
Heterophyopsis	*H. continua*	Japan, Korea
Metagonimus	*M. miyatai*	Japan, Korea
	M. takahashii	Korea
	M. yokogawai	All Far East, India, Balkan states, Israel, Siberia, Spain
Procerovum	*P. calderoni*	Africa, China, Philippines
Pygidiopsis	*P. summa*	Japan, Korea
Stellanchasmus	*S. falcatus*	Hawaii, Japan, Korea, Palestine, Philippines, Thailand, Vietnam
	S. pseudocirratus	Hawaii, Philippines
Stictodora	*S. fuscata*	Korea
	S. lari	Korea

[a]Referred to as *Phagicola* sp. by Chieffi et al. (1992) in Brazil
[b]Imported cases

and Indonesia (Yu and Mott 1994). In Korea, the prevalence reached 4.8% (Seo et al. 1981), with special incidence in riverside communities where raw sweetfish is commonly eaten (Youn 2009). In particular, the eastern parts of Gyeongbuk Province, Gangjingun (Tamjin River), Boseong (Boseong River), Hadonggun (Seomjin River), and Samcheok (Osip Stream) were the most important endemic areas with a prevalence ranging from 20 to 70% (Chai and Lee 2002; Lee et al. 2008). In China, *M. yokogawai* is distributed in Guangdong, Anhui, Hubei and Zhejiang (Chai et al. 2009a). In India, only three cases have been reported, the first two cases occurred with a Muslim family in upper Assam (Mahanta et al. 1995) and more recently an isolated case was found in a 6-year-old female in New Delhi (Uppal and Wadhwa 2005).

Another ten species of Heterophyidae have been recorded in the Republic of Korea (Table 7.3). *H. nocens* commonly infects humans though the identity of this species is confused and may be a synonym of *H. heterophyes*. Prevalence ranging from 17 to 70% was detected among residents in the southwestern coastal areas (Chai et al. 2004). Human infections with *Metagonimus miyatai* (Fig. 7.7c) have been detected in people from Gum, Namhan, and Hantaan rivers (Chai et al. 2009a). In the upper reaches of Namhan River have also recorded human infections with *Metagonimus takahashi* (Chai et al. 1993). *Pygidiopsis summa* is widely distributed in the western and southwestern coastal areas of Korea (Eom et al. 1985). Recently, a prevalence of 4.9% of heterophyid infections was described in South Jeolla Province

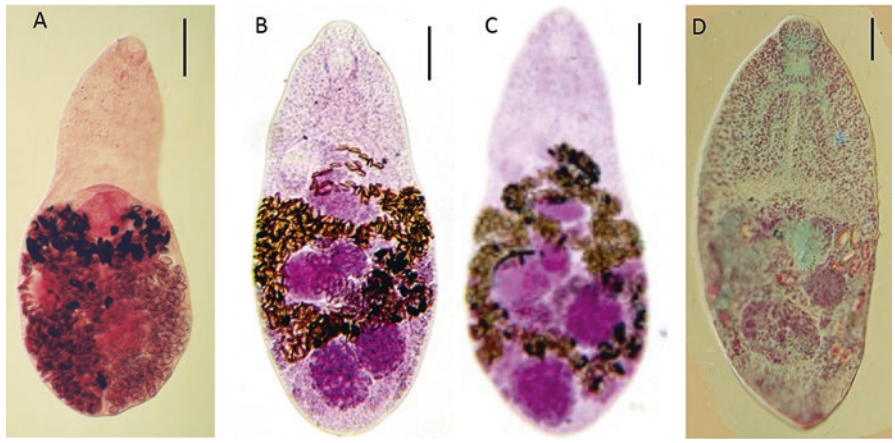

Fig. 7.7 Adult worms of (**a**) *Heterophyes heterophyes* (Scale bar: 50 μm); (**b**) *Metagonimus yokogawai* (Scale bar: 150 μm) (Photomicrograph courtesy of Hideto Kino); (**c**) *Metagonimus miyatai* (Scale bar: 75 μm) (Photomicrograph courtesy of Hideto Kino); and (**d**) *Haplorchis taichui*. (Scale bar: 100 μm)

(Cho et al. 2010). Sporadic cases of *Centrocestus armatus* (1 case), *Haplorchis pumilio* (1) *Heterophiopsis continua* (10), *Stellantchasmus falcatus* (4), *Stictodora fuscata* (14), and *S. lari* (6) have also been recorded (Chai and Lee 2002; Chai et al. 2009a; Chung et al. 2011). Furthermore, two imported cases of *Heterophyes dispar* infections were reported in men returning from Saudi Arabia (Chai et al. 1986). A recent study demonstrated that intestinal trematodiasis constituted an unrecognized food safety risk in Vietnam and heterophyids were the most prevalent intestinal trematodes. Elevated prevalence was detected for *H. pumilio, Haplorchis taichui* (Fig. 7.3d), *Haplorchis yokogawai,* and *S. falcatus* in several areas (Trung Dung et al. 2007).

In Japan, seven additional species of heterophyids have been recorded infecting humans (Table 7.3) (Chai et al. 2009a; Yu and Mott 1994). Of particular interest are the endemic foci of *H. nocens* detected in Shizuoka Prefecture (Kino et al. 2006). In Lao PDR and Vietnam, several studies have shown the *Haplorchis* spp. (*H. pumilio, H. taichui,* and *H. yokogawai*) and *Centrocestus formosanus* have a significant impact among residents (Trung Dung et al. 2007; Chai et al. 2009b, 2013; Sayasone et al. 2009b; De and Le 2011).

7.8.3 Clinical Manifestations and Pathology

Commonly, low-grade infections with heterophyids are of no clinical consequences. Heavy infections are associated with diarrhea, mucus-rich feces, abdominal pain, dyspepsia anorexia, nausea, and vomiting (Toledo et al. 2006a). Symptoms frequently subside spontaneously after 1 month although the flukes may remain for more than 1 year, and only episodic diarrhea may appear (Sripa et al. 2010). Several complications are associated with heterophyiasis. Anaphylaxis was developed by a woman after infection with *H. heterophyes* after eating raw fish in Spain (Martínez-Alonso et al. 1999). Occasionally worm eggs may enter the circulatory system via the crypts of Lieberkhün which may be fatal (Marty and Andersen 2000; Sripa et al. 2010). Eggs can be transported to other organs and they have been found in cardiac muscles in autopsies, spinal cord or brain, where they become trapped and elicit granulomatous lesions and fibrosis. Signs of heterophyid myocarditis may include subepicardial edema, epicardium hemorrhage, fragmentation of muscle fibers, cardiac enlargement, embolism of the capillaries, cough, dyspnea, cyanosis, fatigue, edema and ascites,

palpitation, loss of reflexes, and abnormal heart sounds. Eggs or worms in the spinal cord or brain may cause neurological disease, transverse myelitis, and loss of sensory and motor function (Sripa et al. 2010). Recently, *H. taichui* has been identified as a possible etiologic causative agent of irritable Bowel syndrome-like symptoms (Watthanakulpanich et al. 2010).

The adult worms parasitize mainly the duodenum though they may extend more distally in heavy infections (Lee et al. 1981). The pathology induced by heterophyids has been mainly studied in laboratory animals. Adult worms live embedded in the intestinal mucosa causing villous atrophy, hypertrophy of crypts of Lieberkhün, enlargement of mesenteric lymph nodes, and inflammatory responses with cell infiltration (Toledo et al. 2006a). Ashour et al. (2014) showed that atrophic villi covered by poorly differentiated epithelial cells were observed in the second week post-infection in mice with *H. heterophyes*. Moreover, marked hyperplasia of the intestinal crypts with abundant inflammatory cellular infiltrate in the lamina propria, as well as apoptosis of cells lining the intestinal villi was noted. Both caspase-3 and NF-jB showed positive staining in the intestinal epithelial cells with varying grades of intensity over the length of infection. In humans, Chi et al. (1988) studied the pathology of a human case of metagonimiasis. The parasitism was incidentally detected in an intestinal segment that was removed surgically for treating intestinal perforation related to a malignant histiocytosis. The adult worms of *M. yokogawai* were found free in the jejunal lumen as well as impacted in the intervillous spaces. The pathological findings in this human case were similar to those in the recovering phase of infection in experimental animals. The main histological lesions were massive lymphoplasmacytic and eosinophilic infiltration in the stroma, erosion of the enterocytes in the areas surrounding the worms, goblet cell depletion, and occasional villous edema. Sukontason et al. (2005) studied the pathology induced by *H. taichui* on three human cases revealing mucosal ulceration, mucosal and submucosal hemorrhages, fusion and shortening villi, chronic inflammation, and fibrosis of the submucosa.

7.8.4 Host–Parasite Relationships and Immunology

The fact that pathology induced by heterophyids is spontaneously restored indicated the development of host protective immunity. Several effector mechanisms have been suggested to act against heterophyids (Toledo et al. 2006a). Intraepithelial lymphocytes and the lamina propria lymphocytes acting as CD8+ cytotoxic and IgA producing cells, respectively, may be of importance. Moreover, mucosal mast cells and goblet cells appear to be also involved in the worm expulsion in rodents (Chai et al. 2009a). Regarding antibody responses, it is known that both systemic and local antibody responses occur in these infections. The specific IgG levels in the serum of *M. yokogawai*-infected cats rise from 7 dpi and the maximum levels were observed at 2–4 wpi with both antigens (Cho et al. 1987). Elevated levels of IgG, IgM, and IgE have been detected in the serum of humans infected with *H. heterophyes* (El-Ganayni et al. 1989; Martínez-Alonso et al. 1999; Pica et al. 2003). In the intestine, the levels of IgG, IgM, and IgA were increased (El-Ganayni et al. 1989). Systemic IgG response has also been detected in humans infected with *H. taichui* and *M. yokogawai*, respectively (Ditrich et al. 1991; Lee et al. 1993b). There is some controversy on the potential role on the antibody response in the course of heterophyid infections. An inverse relationship between the antibody levels and the load of the infection has been often observed, suggesting that humoral response may be involved in worm expulsion (El-Ganayni et al. 1989; Ditrich et al. 1991). In contrast to these findings, Cho et al. (1987) detected that specific IgG levels were directly related to the number of *M. yokogawai* adult worms in experimentally infected cats.

7.8.5 Diagnosis

Diagnosis can be done by detection of eggs in the feces. However, the detection and identification may be difficult because of: (1) similarity of the eggs of the heterophyids; (2) low-laying capacity of the parasite and (3) existence of extraintestinal

cases. Due to the similarity of the eggs of heterophyids, when eggs are detected it is usually referred to as "heterophyid infection." The specific diagnosis may require the recovery of adult worms after treatment and purgation (Chai et al. 2009a). Because of this fact, close examination of the egg sizes may be of help (Lee et al. 2012). Moreover, light infections can be easily missed since the egg laying capacity of the heterophyids is low (Sripa et al. 2010). For example, the daily egg output of *H. taichui* is estimated as 82 eggs/worm (Sato et al. 2010). More problematic is the diagnosis of the extraintestinal cases which only can be detected by surgery or autopsy.

Few studies have been done concerning the immunodiagnosis of heterophyid infections. ELISA and western-blot methods showed a good level of sensitivity but cross-reactivity with other trematodes occurred (Ditrich et al. 1991; Lee et al. 1993b). Similarly, several molecular-based methods for the diagnosis have been developed recently (Van Van et al. 2009; Wongsawad and Wongsawad 2009; Wongsawad et al. 2009a, b; Sato et al. 2010; Thaenkham et al. 2011).

7.9 Other Families of Intestinal Trematodes Infecting Humans

7.9.1 Family Cathaemaciidae

Only one species belonging to this family, *Cathaemacia cabrerai*, has been recorded infecting humans. A single case was reported in the Philippines (Jueco and Monzon 1984). No further information about this parasite is available.

7.9.2 Family Lecithodendriidae

The lecithodendriids are characterized by possessing an oval body and a spinose tegument. The testes are opposite and a cirrus sac is present. The position of the genital pore is variable, the vitellaria are in lateral clusters, and the eggs are operculate and embryonated when laid. Adults of these digeneans are parasites of the digestive tract of amphibians, birds, or mammals (typically bats). Three species of this family have been found infecting humans: *Prosthodendrium molenkampi, Phaneropsolus bonnei,* and *P. spinicirrus* (Fried et al. 2004).

P. molenkampi was first discovered infecting humans in Indonesia but thereafter, high prevalence was detected in Thailand reaching 19% (Radomyos et al. 1998). Moreover, human infections have been reported in Lao PDR (Chai et al. 2009b; Sayasone et al. 2009b). *P. bonnei* was first discovered concurrently with *P. molenkampi* in humans in Indonesia. Later, it was recorded in Malaysia, India, Thailand and, more recently, in Lao PDR (Chai et al. 2009a; Sayasone et al. 2009b). Metacercariae of both species have been detected in dragonflies and it is thought that people become infected after eating this odonata which is common in these areas (Chai et al. 2009a). This may explain that concurrent infections have been commonly reported (Sayasone et al. 2009b). Only one human infection by *P. spinicirrus* has been recorded in Thailand (Kaewkes et al. 1991). In general, lecithodendriids do not appear to produce clinical disease in humans (Fried et al. 2004).

7.9.3 Family Microphallidae

This family includes trematode parasites of vertebrates and is characterized by possession of a pyriform body, spinose tegument, short intestinal caeca, testes opposite in the hindbody, vitellaria posterior to the testes and the uterus confined to the hindbody. Eggs are operculated and non-embryonated. The life cycle includes three hosts: a mollusc, an arthropod, and a vertebrate. In some species, the cycle is abbreviated by eliminating the second intermediate (arthropod) host. In these species, the cercaria encysts within the sporocyst and the infected mollusc is eaten by the definitive host (Fried et al. 2004).

Two species of this family have been recorded in humans: *Spelotrema brevicaeca* and *Gynaecotyla squatarolae*. In *S. brevicaeca*, cercariae encyst in the abdomen and cephalothorax of shrimps or crabs, which appears to be the source of human infections. This species was reported as the cause of fatal involvement in the

heart and spinal cord of humans in the Philippines. Fluke eggs were found in the heart, brain, and spinal cord of persons who died of acute cardiac dilatation (Fried et al. 2004; Chai et al. 2009a).

The first human infection of *G. squatarolae* was recently reported in Korea (Chung et al. 2011). A 50-year-old female was found to be infected with this trematode concomitantly with other trematodes such as *G. seoi* and *H. pumilio*. It is known that *G. squatarolae* metacercariae encyst in crabs (*Macrophthalmus* spp.) and the woman had usually eaten brackish crabs in soy sauce.

7.9.4 Family Nanophyetidae

Only one species belonging to this family, *Nanophyetus salmincola*, has been recorded in humans. This parasite is characterized by a pyriform body and the presence of two large testes in the posterior half of the body (Fig. 7.8a). The life cycle of *N. salmincola* involves the release of non-embryonated eggs in the feces of the mammalian host. The miracidium hatches and penetrates the freshwater snail, *Oxytrema silicula*. Cercariae emerge from snails, penetrate the skin and gills of salmonid fishes, and encyst in the muscles and connective tissue. The adults develop in the intestine of mammals following ingestion of the infected fish (Fried et al. 2004).

Human nanophyetiasis is endemic in the far-eastern part of Russia with an average prevalence of 5% in some areas (Yu and Mott 1994). In local ethnic minorities, the prevalence is higher and reaches 60% in some localities (Chai et al. 2004). In the northwest USA, Eastburn et al. (1987) reported ten human infections. Patients became infected after eating improperly cooked salmon or trout. The main symptoms were gastrointestinal complaint of abdominal discomfort, diarrhea, nausea, and vomiting. Peripheral blood eosinophilia was also observed.

It should be noted that *Nanophyetus schikhobalowi* was described from natives of far-eastern Siberia though this fluke is currently considered as a subspecies of *N. salmincola* (Chai 2007).

7.9.5 Family Paramphistomidae

The family Paramphistomidae is restricted to paramphistomoid digeneans, parasitic in mammals, which lack pharyngeal sacs, a cirrus sac and a ventral pouch. However, this family has often been used as a repository with a varying number of subfamilies and there is confusion on its taxonomy.

Members of Paramphistomidae rarely infect humans. Only two species, *Fischoederius elongatus* and *Watsonius watsoni*, have been recorded. Information about *F. elongatus* is scarce. This is a parasite of humans in China (Yu and Mott 1994).

W. watsoni was first discovered in a patient from West Africa who died of severe diarrhea (Beaver et al. 1984). In both species, the source of infection it is suspected to be the ingestion of aquatic plants.

7.9.6 Family Plagiorchiidae

The members of this family are characterized by having a spinose tegument, the ventral sucker in the anterior half of the body, long intestinal caeca, testes opposite, tandem or oblique and located in the hindbody. A cirrus sac is present and the genital pore is anterior to the acetabulum. The ovary is pretesticular and the vitelline follicles are adjacent to the intestinal ceca and are variable in length (Fig. 7.8b). The eggs are operculate and embryonated (Schell 1985). The life cycle typically involves embryonated eggs being eaten by pulmonate snails, in which sporocysts develop and produce xiphidiocercariae. Emerged cercariae encyst in insect larvae, but encystment in freshwater fishes also occurs. Definitive hosts become infected after eating the second intermediate host harboring the cysts.

Occasional human infections with *Plagiorchis harinasutai* (in Thailand), *P. javensis* (in Indonesia), *P. muris* (Fig. 7.8b) (in Japan and Korea), and *P. philippinensis* (in the Philippines) have been recorded (Asada et al. 1962; Radomyos et al. 1989; Yu and Mott 1994; Hong et al. 1996).

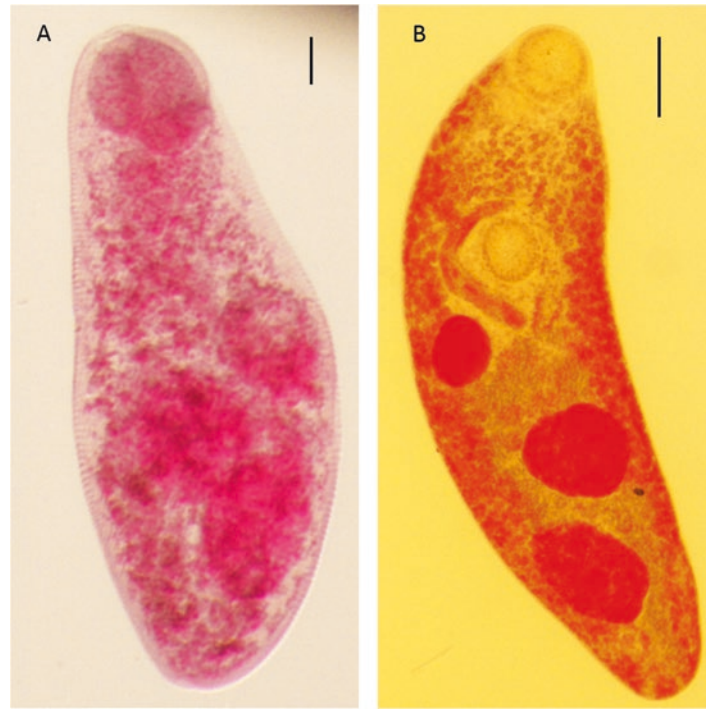

Fig. 7.8 Adult worms of (**a**) *Nanophyetus salmincola* (Scale bar: 100 μm); and (**b**) *Plagiorchis muris* (Scale bar: 200 μm)

7.9.7 Family Strigeidae

The family Strigeidae includes trematodes with a cup-shaped forebody and a tribocytic organ (an accessory sucker). The hindbody is cylindrical or ovoid and contains the reproductive organs. The short uterus contains operculate, non-embryonated eggs. The adult flukes are parasites of the intestine of birds and mammals (Fried et al. 2004).

Within this family only *Cotylurus japonicus* has been recorded parasitizing the intestine of humans. The first infection was reported from a 13-year-old girl in China (Chen and Cai 1985). Further details about the human infections are scarce and the source of the infection is unknown (Marty and Andersen 2000).

7.10 Treatment of Intestinal Trematode Infections

At present, the drug of choice for the treatment of intestinal trematode infections is praziquantel. Although the exact mechanism of action of praziquantel has not been elucidated, it has been postulated that a disruption of Ca2+ homestasis occurs as praziquantel induces a rapid contraction of the trematodes (Greenberg 2005). Praziquantel exhibits a broad spectrum against trematodes and has an excellent safety profile (Keiser and Utzinger 2004). All treatment schedules with praziquantel are well tolerated, with only few adverse events including abdominal pain, dizziness, headache, vomiting, nausea, and urticaria and less commonly may also appear rash, hypotension, and sudden expulsion of the worms which may produce obstruction (Keiser and Utzinger 2004; Fürst et al. 2012b). Furthermore, there are no reports of food-borne trematodes resistant to praziquantel. All the used treatments consist of a single dose of 25 mg/kg for echinostomiasis and heterophyiasis and 10 mg/kg for gymnophalloidiasis (Keiser and Utzinger 2004, 2010; Toledo et al. 2011, 2012). Other drugs such as mebendazole, thymol, carbon tetrachloride, and tetrachloroethylene also have been used.

The limited number of available drugs for food-borne trematodiases, together with the concerns in

relation to the development of resistance against these drugs, has encouraged 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 intestinal trematodiases using *Echinostoma caproni* as an experimental model (Keiser and Utzinger 2010). Artemisinin, the active constituent of the herb *Artemisia annua*, is a sesquiterpene lactone that contains an unusual peroxide bridge. 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. In trematodes, it has been shown that artemether disrupts the tegument (Keiser and Utzinger 2009). Complete healing of *E. caproni* infections was achieved in mice using a single oral dose of 200 mg/kg (Keiser et al. 2006a). Recently, artesunate has successfully studied against heterophyids in experimentally infected mice (Fathy 2011). Due to the problems that artemisinins have in relation to their bioavailability, preparation, and pharmacokinetics, many synthetic peroxide analogues have been prepared and investigated. One of them, ozonide OZ78, has shown to be an effective flukicide in the rodent model. Complete *E. caproni* worm burden reduction was achieved in acute and chronic infections in mice using a dose of 1000 mg/kg (Keiser et al. 2006b). The increasing interest in medicinal plants as new sources of antiparasitic drugs has led to study several extracts as flukicides. Recently, Ferreira et al. (2011) showed that ethanolic extracts of *Artemisia annua, A. absinthium, and Asimina triloba* kill *E. caproni,* probably in relation to their elevated content in artemisinin and acetogenins. Garlic (*Allium sativum*) has been shown to have certain degree of efficacy against echinostomatids (Cortés et al. 2017b).

7.11 Control of the Intestinal Trematode Infections

Eating raw or improperly cooked freshwater fish and fresh or brackish water snails, snakes, bivalves, or aquatic vegetables should be avoided to prevent intestinal trematode infections. There is evidence that various types of marinades and food preparations commonly used may not affect the viability of the metacercariae. Various physical and chemical factors have been studied to determine their effects on the viability of encysted metacercariae of *E. caproni*. Viability was equated with chemical excystation in an alkaline trypsin-bile salts medium. Of numerous marinades tested, the one that was most harmful to isolated and in situ cysts was vinegar. Concentrated solutions of NaCl and sucrose had no effect on the viability of isolated and in situ cysts, suggesting that their use in food preparations for molluscs would not be effective in killing echinostomatid cysts in tainted snail tissues (Saxton and Fried 2009). Similarly, Wongsawad et al. (2005) studied the effect of several factors on the viability of the metacercaria of *S. falcatus*. The authors concluded that the worms were killed in NaCl at 20, 30, and 40% within 12, 6, and 2 h, respectively. Acetic acid at 5 and 10% killed the metacercaria within 12 and 6 h while at 20 and 30%, within 2 h. The killing effect of 3% vinegar was found within 18 h and of 5% vinegar within 12 h. Lemon juice showed no killing effect.

The nature of intestinal trematode infections does not justify the establishment of a separate control program because it can be controlled along with other food-borne diseases for which there are sustained WHO control programs. The control of human intestinal trematodiasis via blocking or interruption of the life cycle can be achieved through proper diagnosis, followed by pharmacologic treatment and prevention of reinfection. The control should be focused predominantly on a reduction or elimination of the transmission of the disease. In theory, the means of control in endemic regions can include: a reduction of the sources of infection, particularly human beings, through effective treatment; the protection of fish ponds and aquaculture systems from contamination with feces from people and other definitive hosts; the treatment or sterilization of feces; the control of snail host populations; and the implementation of education campaigns. Therefore, in principle, the prevention and control is relatively simple. Since infec-

tion of the definitive host is only contracted through the ingestion of metacercariae, the most practical measure for preventing and controlling human infection is to eliminate the consumption of raw, undercooked aliments susceptible to harbor the metacercariae. However, this strategy may be difficult to implement in some endemic regions because of the ancient eating traditions. Thus, only adequate cooking will render safe for human consumption. Nonetheless, together with education awareness programs focused particularly on teaching young children about the parasites, its life cycle, and the disease it causes, prevention and control could be successful. Targeting children may have the advantage that they are less entrenched than adults in their customs and eating habits. Significant changes for the control of intestinal trematodiasis may include: (1) the development of effective broad-spectrum anthelmintic, (2) an understanding by WHO of the differences between intestinal helminths and arthropod-borne infectious agents, and (3) the implementation of control programs in school-age children, with strong community therapy programs delivering multiple treatments against concurrent helminthic infections. A decreasing pattern in some food-borne trematode diseases along with industrialization, health education, and alteration of environment has been observed in certain areas of Southeast Asia. This is particularly true for Taiwan and mainland China, where industrial developments and wastewater discharges pollute streams and rivers, practically destroying those aquatic animals involved in trematode life cycles. The WHO control programs operating through the essential components of diagnosis, treatment, and prevention for the control of human zoonotic trematodiases have not been successful against several intestinal trematodiases although they have been for other trematode diseases.

7.12 Concluding Remarks

Despite the significant public health impact of the intestinal trematodiases, these diseases are among the most neglected tropical diseases. There was a general lack of knowledge about these infections and their causative species. Traditionally, intestinal trematode infections were considered as minor diseases confined to low-income countries, mainly in Asia, and the presence in the press media and funding research were practically inexistent. In recent years, this picture is changing by a number of factors. The number of infected is counted in the millions and an elevated percentage of the world's population is considered to live at risk for infection. Currently, 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, new eating habits in developed countries and demographic changes. Moreover, the number of species causing human intestinal trematode infections is high which makes difficult their correct knowledge, several severe endemic foci have been described and some species can cause serious health problems. This is aggravated since as occurs with other neglected tropical diseases, there are several gaps in our knowledge on the intestinal trematode infections.

In this context, new approaches to these diseases are needed. Current control strategies are reasonable and logical though approaches based on new technologies for training of health workers, treatment, diagnosis, and control of these diseases should be implemented. At present, a limited number of drugs are available, which is aggravated by the little incentive to invest in the discovery and development of new trematodicidal drugs. Moreover, the identification of parasite-specific proteins could clearly facilitate the design of new tools for rapid and cheap diagnosis, which may help to control the transmission of the parasite. In this context, the identification of potential targets for vaccination seems to be one of the best ways to control these parasite infections. The emergence of the intestinal trematodiases associated with the new risks of transmission introduced by the "globalized world" makes necessary the development of updated maps of distributions and risk of transmission and all these measures should also be accompanied by programs of training of health personnel and the population in general to change

risk eating habits. These approaches can provide the tools necessary for a proper understanding and control of the intestinal trematode infections.

Acknowledgements This work was supported supported by Ministerio de Economía y Competitividad (Madrid, Spain) (grant number: BFU2016-75639-P) and by Ministerio de Sanidad y Consumo (Madrid, Spain) (No. RD12/0018/0013, Red de Investigación Cooperativa en Enfermedades Tropicales—RICET, IV National Program of I+D+I 2008-2011, ISCIII—Subdirección General de Redes y Centros de Investigación Cooperativa and FEDER).

References

Agger MK, Simonsen PE, Vennervald BJ (1993) The antibody response in serum, intestinal wall and intestinal lumen of NMRI mice infected with *Echinostoma caproni*. J Helminthol 67:169–178

Asada JI, Otagaki H, Morita M, Takeuchi T, Sakai Y, Kojishi T, Okahashi K (1962) A case report on the human infection with *Plagiorchis muris* Tanabe, 1922 in Japan. Jpn J Parasitol 11:512–516

Ashour DS, Othman AA, Radi DA (2014) Insights into regulatory molecules of intestinal epithelial cell turnover during experimental infection by *Heterophyes heterophyes*. Exp Parasitol 143:48–54

Bandyopadhyay AK, Nandy A (1986) A preliminary observation on the prevalence of echinostomes in a tribal community near Calcutta. Ann Trop Med Parasitol 80:373–375

Bandyopadhyay AK, Manna B, Nandy A (1989) Human infection of *Artyfechinostomum oraoni* n. sp. (Paryphostominae: Echinostomatidae) in a tribal community, "Oraons" in West Bengal, India. Indian J Parasitol 13:191–196

Beaver PC, Jung RC, Cupp EW (1984) Clinical parasitology, 9th edn. Lea and Febiger, Philadelphia

Belizario VY, Geronilla GG, Anastacio MBM, de Leon WU, Suba-an AU, Sebastian AC, Bangs MJ (2007) *Echinostoma malayanum* infection, in the Philippines. Emerg Infect Dis 13:1130–1131

Bhattacharjee HK, Yadav D, Bagga D (2009) Fasciolopsis presenting as intestinal perforation: a case report. Trop Gastroenterol 30:40–41

Brunet LR, Joseph S, Dunne DW et al (2000) Immune responses during the acute stages of infection with the intestinal trematode *Echinostoma caproni*. Parasitology 120:565–571

Buckley JJC (1939) Observations on *Gastrodiscoides hominis* and *Fasciolopsis buski* in Assam. J Helminthol 17:1–12

Bunnag D, Radomyos P, Harinasuta T (1983) Field trial on the treatment of fasciolopsiasis with praziquantel. Southeast Asian J Trop Med Public Health 14:216–219

Butcher AR, Grove DI (2001) Description of the life-cycle stages of *Brachylaima cribbi* n. sp. (Digenea: Brachylaimidae) derived from eggs recovered from human faeces in Australia. Syst Parasitol 49:211–221

Butcher AR, Talbot GA, Norton RE, Kirk MD, Cribb TH, Forsyth JR, Knight B, Cameron AS (1996) Locally acquired *Brachylaima* sp. (Digenea: Brachylaimidae) intestinal fluke infection in two South Australian infants. Med J Aust 164:475–478

Butcher AR, Parasuramar PS, Thompson CS, Grove DI (1998) First report of the isolation of an adult worm of the genus *Brachylaima* (Digenea: Brachylaimidae), from the gastrointestinal tract of a human. Int J Parasitol 28:607–610

Butcher AR, Palethorpe HM, Grove DI (2002) Effects of sex and age on the susceptibility of C57BL/6J mice to infection with *Brachylaima cribbi* and the course of the infection in NOD SCID mice. Parasitol Res 88:668–674

Butcher AR, Palethorpe HM, Grove DI (2003) Response to re-infection with *Brachylaima cribbi* in immunocompetent and immunodeficient mice. Parasitol Int 52:219–228

Cao YH, Ma YM, Qiu F, Zhang XQ (2015) Rare cause of appendicitis: mechanical obstruction due to *Fasciolopsis buski i*nfestation. World J Gastroenterol 21:3146–3149

Carney WP (1991) Echinostomiasis—a snail-borne intestinal disease. Southeast Asian J Trop Med Public Health 22:206–211

Carney WP, Sudomo M, Purnomo A (1980) Echinostomiasis: a disease that disappeared. Trop Geogr Med 32:101–105

Chai JY (2007) Intestinal flukes. In: Murrell KD, Fried B (eds) Food-borne parasitic zoonoses: fish and plant-borne parasites, world class parasites, vol 1. Springer, New York, pp 53–115

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

Chai JY, Lee SH (1991) Intestinal trematodes infecting humans in Korea. Southeast Asian J Trop Med Public Health 22:163–170

Chai JY, Lee SH (2002) Food-borne intestinal trematode infections in the Republic of Korea. Parasitol Int 51:129–154

Chai JY, Seo BS, Lee SH, Hong SJ, Sohn WM (1986) Human infections by *Heterophyes heterophyes* and *H. dispar* imported from Saudi Arabia. Korean J Parasitol 24:82–88

Chai JY, Huh S, Yu JR (1993) An epidemiological study of metagonimiasis along the upper reaches of the Namhau River. Korean J Parasitol 31:99–108

Chai JY, Hong ST, Lee SH, Lee GC, Min YI (1994) A case of echinostomiasis with ulcerative lesions in the duodenum. Korean J Parasitol 32:201–204

Chai JY, Kim IM, Seo M, Guk SM, Kim JL, Sohn WM, Lee SH (1997) A new focus of *Heterophyes nocens*, *Pygidiopsis summa*, and other intestinal flukes in a

coastal area of Muan-gun, Chollanam-do. Korean J Parasitol 35:233–238

Chai JY, Kim TK, Cho WH, Seo M, Kook J, Guk SM, Lee SH (1998) Intestinal mastocytosis and goblet cell hyperplasia in BALB/c and C3H mice infected with *Neodiplostomum seoulense*. Korean J Parasitol 36:109–119

Chai JY, Chung WJ, Kook J, Seo M, Park YK, Guk SM, Choi MH, Lee SH (1999) Growth and development of *Gymnophalloides seoi* in immunocompetent and immunosupressed C3H/HeN mice. Korean J Parasitol 37:21–26

Chai JY, Lee HS, Hong SJ, Yoo JH, Guk SM, Seo M, Choi MH, Lee SH (2001) Intestinal histopathology and in situ postures of *Gymnophalloides seoi* in experimentally infected mice. Korean J Parasitol 39:31–41

Chai JY, Choi MH, Yu JR, Lee SH (2003) *Gymnophalloides seoi*, a new human intestinal trematode. Trends Parasitol 19:109–112

Chai JY, Park JH, Han ET, Shin EH, Kim JL, Guk SM, Hong KS, Lee SH, Rim HJ (2004) Prevalence of *Heterophyes nocens* and *Pygydiopsis summa* infections among residents in Western and Southern coastal islands of the Republic of Korea. Am J Trop Med Hyg 71:617–622

Chai JY, Shin EH, Lee SH, Rim HJ (2009a) Foodborne intestinal flukes in Southeast Asia. Korean J Parasitol 47:S69–S102

Chai JY, Han ET, Shin EH, Sohn WM, Yong TS, Eom KS, Min DY, Um JY, Park MS, Hoang EH, Phommasack B, Insisiengmay B, Lee SH, Rim HJ (2009b) High prevalence of *Haplorchis taichui, Phaneropsolus molenkampi,* and other helminth infections among people in Khammouane province, Lao PDR. Korean J Parasitol 47:243–247

Chai JY, Sohn WM, Yong TS, Eom KS, Min DY, Hoang EH, Phammasack B, Insisiengmay B, Rim HJ (2012) Echinostome flukes recovered from humans in Khammouane Province, Lao PDR. Korean J Parasitol 50:269–272

Chai JY, Sohn WM, Yong TS, Eom KS, Min DY, Lee MY, Lim H, Insisiengmay B, Phommasack B, Rim HJ (2013) Centrocestus formosanus (Heterophyidae): human infections and the infection source in Lao PDR. J Parasitol 99:531–536

Chai JY, Sohn WM, Cho J, Eom KS, Yong TS, Min DY, Hoang EH, Phommasack B, Insisiengmay B, Rim HJ (2018) *Echinostoma ilocanum* infection in two residents of Savannakhet Province, Lao PDR. Korean J Parasitol 56:75–79

Chang YD, Sohn WM, Ryu JH, Kang SY, Hong SJ (2005) A human infection of *Echinostoma hortense* in duodenal bulb diagnosed by endoscopy. Korean J Parasitol 43:57–60

Chen CE, Cai GD (1985) Morphological testimony: demonstration of a case of human infestation with *Cotylurus japonicus* Ishii, 1932 (Trematoda, Strigeidae). Bull Hum Med 10:31–34

Chen XQ, Feng GX, Qian ZF (1993) Survey on infection due to *Echinostoma hortense* in Liaoning Province. Chin J Parasitol Parasit Dis 11:226

Cheng YZ, Lin JX, Fang YY, Lin AQ, Chen BJ, Zhunag HJ (1992) Discovery of human infection with *Echinostoma angustitestis*. Chin J Zoonoses 8:7–8

Chi JG, Kim CW, Kim JR, Hong ST, Lee SH (1988) Intestinal pathology in human metagonimiasis with ultrastructural observations of parasites. J Korean Med Sci 3:171–177

Chieffi PP, Gorla MC, Torres DM, Dias RM, Mangini AC, Monteiro AV, Woiciechovski E (1992) Human infection by *Phagicola* sp. (Trematoda, Heterophyidae) in the *municipality* of Registro, São Paulo State, Brazil. J Trop Med Hyg 95:346–348

Cho SY, Kim SI, Kang SY (1987) Specific IgG responses in experimental cat metagonimiasis. Korean J Parasitol 25:149–153

Cho CM, Tak WY, Kweon YO, Kim SK, Choi YH, Kong HH, Chung DI (2003) A human case of *Echinostoma hortense* (Trematoda: Echinostomatidae) infection diagnosed by gastroduodenal endoscopy in Korea. Korean J Parasitol 41:117–120

Cho YK, Ryang YS, Kim IS, Park SK, Im JA, Lee KJ (2007) Differential immune profiles following experimental *Echinostoma hortense infection* in BALB/c and C3H/HeN mice. Parasitol Res 100:1053–1061

Cho SH, Cho PY, Lee DM, Kim TS, Kim IS, Hwang EJ, Na BK, Sohn WM (2010) Epidemiological survey on the infection of intestinal flukes in residents of Muan-gun, Jeollanam-do, the Republic of Korea. Korean J Parasitol 48:133–138

Chung PR, Jung Y, Kim DS (1996) *Segmentina (Polypylis) hemisphaerula* (Gastropoda: Planorbidae): a new molluscan intermediate host of a human intestinal fluke *Neodiplostomum seoulensis* (Trematoda: Diplostomatidae) in Korea. J Parasitol 82:336–338

Chung PR, Jung Y, Park YK, Hwang MG (2002) Host specificity of *Austropeplea ollula* (Gastropoda: Lymnaeidae) to miracidial infection with a human intestinal fluke *Neodiplostomum seoulense* (Trematoda: Diplostomatidae) in Korea. J Parasitol 88:630–631

Chung OS, Lee HJ, Kim YM, Sohn WM, Kwak SJ, Seo M (2011) First report of human infection with *Gynaecotyla squatarolae* and first Korean record of *Haplorchis pumilio* in a patient. Parasitol Int 60:227–229

Chunge RN, Chunge CN (2017) Infection with *Echinostoma* sp. in a group of travellers to Lake Tanganyika, Tanzania, in January 2017. J Travel Med 24(5): 1–3

Clarke MD, Carney WP, Cross JH, Hadidjaja P, Oemijati S, Joesoef A (1974) Schistosomiasis and other human parasitoses of Lake Lindu in Central Suwalesi (Celebes), Indonesia. Am J Trop Med Hyg 23:385–392

Cortés A, Sotillo J, Muñoz-Antoli C, Fried B, Esteban JG, Toledo R (2014) Intestinal IFN-γ production is associated with protection from clinical signs, but not with elimination of worms, in *Echinostoma caproni* infected-mice. Parasitol Res 113:2037–2045

Cortés A, Sotillo J, Muñoz-Antoli C, Fried B, Esteban JG, Toledo R (2015a) Altered protein expression in the ileum of mice associated with the development of chronic infections with *Echinostoma caproni* (Trematoda). PLoS Negl Trop Dis 9:e0004082

Cortés A, Muñoz-Antoli C, Martín-Grau C, Esteban JG, Grencis RK, Toledo R (2015b) Differential alterations in the small intestine epithelial cell turnover during acute and chronic infection with *Echinostoma caproni* (Trematoda). Parasit Vectors 8:334

Cortés A, Muñoz-Antoli C, Sotillo J, Fried B, Esteban JG, Toledo R (2015c) *Echinostoma caproni* (Trematoda): differential in vivo mucin expression and glycosylation in high- and low-compatible hosts. Parasite Immunol 37:32–42

Cortés A, Sotillo J, Muñoz-Antolí C, Trelis M, Esteban JG, Toledo R (2016) Definitive host influences the proteomic profile of excretory/secretory products of the trematode *Echinostoma caproni*. Parasit Vectors 9:185

Cortés A, Sotillo J, Muñoz-Antolí C, Molina-Durán J, Esteban JG, Toledo R (2017a) Antibody trapping: a novel mechanism of parasite immune evasion by the trematode *Echinostoma caproni*. PLoS Negl Trop Dis 11:e0005773

Cortés A, García-Ferrús M, Sotillo J, Esteban JG, Toledo R, Muñoz-Antolí C (2017b) Effects of dietary intake of garlic on intestinal trematodes. Parasitol Res 116:2119–2129

Cortés A, Muñoz-Antolí C, Álvarez-Izquierdo M, Sotillo J, Esteban JG, Toledo R (2018) Adaptation of the secretome of *Echinostoma caproni* may contribute to parasite survival in a Th1 milieu. Parasitol Res 117:947–957

Cremonte F, Pina S, Gilardoni C, Rodrigues P, Chai JY, Ituarte C (2013) A new species of gymnophallid (Digenea) and an amended diagnosis of the genus *Gymnophalloides* Fujita, 1925. J Parasitol 99:85–92

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

Cross JH, Clarke MD, Cole WC (1976) Parasitic infection in humans in West Kalimantan (Borneo) Indonesia. Trop Geogr Med 28:121–130

De NV, Le TH (2011) Human infections of fish-borne trematodes in Vietnam: prevalence and molecular specific identification at an endemic commune in Nam Dinh province. Exp Parasitol 129:355–361

DeGirolami PC, Kimber J (1983) Intestinal parasites among Southeast Asian refugees in Massachusetts. Am J Clin Pathol 79:502–504

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

D'Souza PE, Jagannath MS, Prasanna KM (2001) A note on the occurrence of *Fasciolopsis buski* in pigs. Cheiron 30:178–179

Eastburn RL, Fritsche TR, Terhune CA (1987) Human intestinal infection with *Nanophyetus salminocola* from salmonoid fishes. Am J Trop Med Hyg 36:586–591

Eduardo SL (1991) Food-borne zoonoses in the Philippines. Southeast Asian J Trop Med Public Health 22:16–22

El-Ganayni GA, Youssef ME, Handousa AE, Bou-Zakham AA, Hegazi MM (1989) Serum and intestinal immunoglobulins in heterophyiasis. J Egypt Soc Parasitol 19:219–223

Eom KS, Son SY, Lee JS, Rim HJ (1985) Heterophyid trematodes (*Heterophyopsis continua, Pygidiopsis summa* and *Heterophyes heterophyes nocens*) from domestic cats in Korea. Korean J Parasitol 23:197–202

Esteban JG, Muñoz-Antoli 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

Fathy FM (2011) Anthelmintic effect of artesunate in experimental heterophyiid infection. J Egypt Soc Parasitol 41:469–483

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*. Parasitol Res 109:1585–1582

Fried B, Toledo R (2004) Criteria for species determination in the "*revolutum*" group of *Echinostoma*. J Parasitol 90:917

Fried B, Graczyk TK, Tamang L (2004) Food-borne intestinal trematodiases in humans. Parasitol Res 93:159–170

Fujino T, Fried B (1993) Expulsion of *Echinostoma trivolvis* (Cort, 1914) Kanev, 1985 and retention of *E. caproni* Richard, 1964 (Trematoda, Echinostomatidae) in C3H mice: pathological, ultrastructural, and cytochemical effects on the host intestine. Parasitol Res 79:286–292

Fujino T, Fried B (1996) The expulsion of *Echinostoma trivolvis* from C3H mice: differences in glycoconjugates in mouse versus hamster small intestinal mucosa during infection. J Helminthol 70:115–121

Fujino T, Fried B, Tada I (1993) The expulsion of *Echinostoma trivolvis*: worm kinetics and intestinal cytopathology in conventional and congenitally athymic BALB/c mice. Parasitology 106:297–304

Fujino T, Fried B, Ichikawa H, Tada I (1996a) Rapid expulsion of the intestinal trematodes *Echinostoma trivolvis* and *E. caproni* from C3H mice by trapping with increased goblet cell mucins. Int J Parasitol 26:319–324

Fujino T, Ichikawa H, Fried B, Fukuda K (1996b) The expulsion of *Echinostoma trivolvis*: suppressive effects of dexamethasone on goblet cell hyperplasia and worm rejection in C3H/HeN mice. Parasite 3:283–289

Fürst T, Keiser J, Utzinger J (2012a) Global burden of human food-borne trematodiasis: a systematic review and meta-analysis. Lancet Infect Dis 12:210–221

Fürst T, Sayasone S, Odermatt P, Keiser J, Utzinger J (2012b) Manifestations, diagnosis, and management of foodborne trematodiasis. Br Med J 26:e4093

Gibson DI, Bray RA (1994) The evolutionary expansion and host-parasite relationship of the Digenea. Int J Parasitol 24:1213–1226

Gilman RH, Mondal G, Maskud M, Alam K, Rutherford E, Gilman JB, Khan MU (1982) Endemic focus of *Fasciolopsis buski* in Bangladesh. Am J Trop Med Hyg 31:796–802

Graczyk TK, Fried B (1994) ELISA method for detecting anti-*Echinostoma caproni* (Trematoda: Echinostomatidae) immunoglobulins in experimentally infected ICR mice. J Parasitol 80:544–549

Graczyk TK, Fried B (1995) An enzyme-linked immunosorbent assay for detecting anti-*Echinostoma trivolvis* (Trematoda) IgG in experimentally infected ICR mice. Cross-reactivity with *E. caproni*. Parasitol Res 81:710–712

Graczyk TK, Fried B (1998) Echinostomiasis: a common but forgotten food-borne disease. Am J Trop Med Hyg 58:501–504

Greenberg RM (2005) Are Ca2+ channels targets of praziquantel action? Int J Parasitol 35:1–9

Guk SM, Park JH, Shin EH, Kim JL, Lin A, Chai JY (2006) Prevalence of *Gymnophalloides seoi* infection in coastal villages of Haenamgun and Yeongam-gun, Republic of Korea. Korean J Parasitol 44:1–5

Guk SM, Lee JH, Kim HJ, Kim WH, Shin EH, Chai JY (2009) CD4+ T-cell-dependent goblet cell proliferation and expulsion of *Gymnophalloides seoi* from the intestine of C57BL/6 mice. J Parasitol 95:581–590

Han ET, Chen JH, Chai JY (2008) Antibody responses in sera of different mouse strains experimentally infected with *Neodiplostomum seoulense*. Korean J Parasitol 46:279–283

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, pp 83–98

Hirazawa I (1928) On *Echinochasmus perfoliatus* (Ratz) found in man. Tokyo Iji Shinshi 2577:1328–1334

Hong ST, Hong SJ, Lee SH, Seo BS, Chi JG (1982) Studies on intestinal trematodes in Korea: VI. On the metacercaria and the second intermediate host of *Fibricola seoulensis*. Korean J Parasitol 20:101–111

Hong SJ, Lee SH, Seo BS, Hong ST, Chai JY (1983) Studies on intestinal trematodes in Korea: IX. Recovery rate and development of *Fibricola seoulensis* in experimental animals. Korean J Parasitol 21:224–233

Hong ST, Chai JY, Lee SH (1984a) Ten human cases of *Fibricola seoulensis* infection and mixed one with *Stellantchasmus* and *Metagonimus*. Korean J Parasitol 24:95–97

Hong ST, Cho TK, Hong SJ, Chai JY, Lee SH, Seo BS (1984b) Fifteen human cases of *Fibricola seoulensis* infection in Korea. Korean J Parasitol 22:61–65

Hong SJ, Woo HC, Chai JY (1996) A human case of *Plagiorchis muris* (Tanabe, 1922: Digenea) infection in the Republic of Korea: freshwater fish as a possible source of infection. J Parasitol 82:647–649

Huffman JE, Iglesias D, Fried B (1988) *Echinostoma revolutum*: pathology of extraintestinal infection in the golden hamster. Int J Parasitol 18:873–874

Huh S, Hong ST (1993) Transmission electron microscopic findings of the tribocytic organ of *Fibricola seoulensis*. Korean J Parasitol 31:315–320

Huh S, Chai JY, Hong ST, Lee SH (1988) Clinical and histopathological findings in mice heavily infected with *Fibricola seoulensis*. Korean J Parasitol 26:45–53

Huh S, Lee SH, Seo BS (1990) Histochemical findings of the tribocytic organ and tegument of *Fibricola seoulensis*. Korean J Parasitol 28:155–160

Jaroonvesama N, Charoenlarp K, Areekul S (1986) Intestinal absorption studies in *Fasciolopsis buski* infection. Southeast Asian J Trop Med Public Health 17:582–586

Jueco NL, Monzon RB (1984) *Cathaemacia cabrerai* sp. n. (Trematoda: Cathaemaciidae) a new oarqasite of man in the Philippines. Southeast Asian J Trop Med Public Health 15:427–429

Jung WT, Lee KJ, Kim HJ, Kim TH, Na BK, Sohn WM (2014) A case of *Echinostoma cinetorchis* (Trematoda: Echinostomatidae) infection diagnosed by colonoscopy. Korean J Parasitol 52:287–290

Kaewkes S, Elkins DB, Haswell-Elkins MR, Sithithaworn P (1991) *Phaneropsolus spinicirrus* n. sp. (Digenea: Lecithodendriidae), a human parasite in Thailand. J Parasitol 77:514–516

Kawahara S, Yamamoto E (1933) Human cases of *Echinostoma cinetorchis*. Tokyo Iji Shinshi 2840:1794–1796

Keiser J, Utzinger J (2004) Chemotherapy for major food-borne trematodes: a review. Expert Opin Pharmacother 5:1711–1726

Keiser J, Utzinger J (2009) Food-borne trematodiases. Clin Microbiol Rev 22:466–483

Keiser J, Utzinger J (2010) The drugs we have and the drugs we need against major helminth infections. Adv Parasitol 73:197–230

Keiser J, Brun R, Fried B, Utzinger J (2006a) Trematocidal activity of praziquantel and artemisinin derivatives: in vitro and in vivo investigations on adult *Echinostoma caproni*. Antimicrob Agents Chemother 50:803–805

Keiser J, Utzinger J, Tanner M, Dong Y, Vennerstrom JL (2006b) The synthetic peroxide OZ78 is effective against *Echinostoma caproni* and *Fasciola hepatica*. J Antimicrob Chemother 58:1193–1197

Kim I, Im JA, Lee KJ, Ryang YS (2000) Mucosal mast cell responses in the small intestine of rats infected with *Echinostoma hortense*. Korean J Parasitol 38:139–143

Kim TY, Han KY, Shin EH, Chai JY (2008) Antigenic properties of cystatin-binding cysteine proteinases from *Neodiplostomum seoulense*. J Parasitol 94:654–658

Kino H, Suzuki Y, Oishi H, Suzuki S, Yamagiwa S, Ishiguro M (2006) Geographical distribution of *Metagonimus yokogawai,* and *M. miyatai* in Shizuoka Prefecture, Japan, and their site preferences in the sweetfish, *Plecoglossus altivelis*, and hamsters. Parasitol Int 55:201–206

Kook J, Nawa Y, Le SH, Chai JY (1998) Pathogenicity and lethality of a minute intestinal fluke, *Neodiplostomum seoulense*, to various strains of mice. Korean J Parasitol 84:1178–1183

Kostadinova A (2005) Family Echinostomatidae Looss, 1899. In: Gibson DI, Jones A, Bray RA (eds) Keys to the Trematoda, vol 2. CABI Publishing, Wallingford, pp 9–64

Kostadinova A, Gibson DI (2000) The systematics of the Echinostomes. In: Fried B, Graczyk TK (eds) Echinostomes as experimental models for biological research. Kluwer Academic Publishers, Dordrecht, pp 31–57

Kusharyono C, Sukartinah S (1991) The current status of foodborne parasitic zoonoses in Indonesia. Southeast Asian J Trop Med Public Health 22:8–10

Lee JB, Chi JG, Lee SK, Cho SY (1981) Study on the pathology of metagonimiasis in experimentally infected cat intestine. Korean J Parasitol 19:109–129

Lee SH, Yoo BH, Hong ST, Chai JY, Seo BS, Chi JG (1985) A histopathological study on the intestine of mice and rats experimentally infected by *Fibricola seoulensis*. Korean J Parasitol 23:58–72

Lee SK, Chung NS, Ko IH, Ko HI, Chai JY (1986) Two cases of natural human infection by *Echinostoma hortense*. Kisaengchunghak Chapchi 24:77–81

Lee SK, Chung NS, Ko IH (1988) An epidemiological survey of *Echinostoma hortense* infection in Chongsong-gun, Kyongbuk Province. Korean J Parasitol 26:199–206

Lee SH, Chai JY, Hong ST (1993a) *Gymnophalloides seoi* n. sp. (Digenea: Gymnophallidae), the first report of human infection by a gymnophallid. Korean J Parasitol 79:677–680

Lee SC, Chung YB, Kong Y, Kang SY, Cho SY (1993b) Antigenic protein fractions of *Metagonimus yokogawai* reacting with patient sera. Korean J Parasitol 31:43–48

Lee SH, Chai JY, Seo M, Choi MH, Kim DC, Lee SK (1995) Two cases of *Gymnophalloides seoi* infection accompanied by diabetes mellitus. Korean J Parasitol 33:61–64

Lee SH, Sohn WM, Hong SJ, Huh S, Seo M, Choi MH, Chai JY (1996) A nationwide survey of naturally produced oysters for infection with *Gymnophalloides seoi* metacercariae. Korean J Parasitol 34:107–112

Lee JC, Kong Y, Lee SU, Huh S (1997a) Localization of worm antigen in *Neodiplostomum seoulense* by immuno-electronmicroscopy. Korean J Parasitol 35:95–104

Lee SH, Park SK, Seo M, Guk SM, Choi MH, Chai JY (1997b) Susceptibility of various species of animals and strains of mice to *Gymnophalloides seoi* infection and the effects of immunosuppression in C3H/HeN mice. J Parasitol 83:883–886

Lee JJ, Kim HJ, Kim MJ, Yi Lee JW, Jung BK, Lee JY, Shin EH, Kim JL, Chai JY (2008) Decrease of *Metagonimus yokogawai* endemicity along the Tamjin River basin. Korean J Parasitol 46:289–291

Lee KJ, Jin D, Chang BS, Teng YC, Kim DH (2009) The immunological effects of electrolyzed reduced water on the *Echinostoma hortense* infection in C57BL/6 mice. Biol Pharm Bull 32:456–462

Lee KD, Guk SM, Chai JY (2010) Toll-like receptor 2 and Muc2 expression on human intestinal epithelial cells by *Gymnophalloides seoi* adult antigen. J Parasitol 96:58–66

Lee JJ, Jung BK, Lim H, Lee MY, Choi SY, Shin EH, Chai JY (2012) Comparative morphology of minute intestinal fluke eggs that can occur in human stools in the Republic of Korea. Korean J Parasitol 50:207–213

Lee JJ, Kim D, Pyo KH, Kim MK, Kim HJ, Chai JY, Shin EH (2013) STAT6 expression and IL-13 production in association with goblet cell hyperplasia and worm expulsion of *Gymnophalloides seoi* from C57BL/6 mice. Korean J Parasitol 51:589–594

Lee SH, Jung BK, Park JH, Shin EH, Chai JY (2014) Increased intestinal epithelial cell turnover and intestinal motility in *Gymnophalloides seoi*-infected C57BL/6 mice. Korean J Parasitol 52:273–280

Liang C, Ke XL (1988) *Echinochasmus jiufoensis* sp. nov., a human parasite from Guangzhou (Trematoda: Echinostomatidae). Acta Zootaxa Sin 13:4–8

Lu SC (1982) Echinostomiasis in Taiwan. Int J Zoonoses 9:33–38

Mabus J, Huffman JE, Fried B (1988) Humoral and cellular response to infection with *Echinostoma revolutum* in the golden hamster, *Mesocricetus auratus*. J Helminthol 62:127–132

Mahanta J, Narain K, Srivastava VK (1995) Heterophyid eggs in human stool samples in Assam: first report for India. J Commun Dis 27:142–145

Majima M (1927) On *Echinostoma macrorchis* found in man. Kumamoto Igakkai Zasshi 2252:2260

Manning GS, Brockelman WY, Yiyant V (1971) An analysis of the prevalence of *Fasciolopsis buski* in central Thailand using catalyitic models. Am J Epidemiol 93:354–360

Martínez-Alonso JC, Armentia A, Vega JM, Callejo A, Gómez A (1999) Anaphylactic reaction concurrent with *Heterophyes heterophyes* infestation. Rev Esp Alergol Immunol Clín 14:37–39

Marty AM, Andersen EM (2000) Fasciolopsiasis and other intestinal trematodiasis. In: Meyers WM, Neafie RC, Marty A, Wear DJ (eds) Pathology of infectious diseases, vol I. Helminthiases. Armed Forces Institute of Pathology, American Registry of Pathology, Washington, DC

Mas-Coma S, Bargues MD, Valero MA (2005) Fascioliasis and other plant-borne trematode zoonoses. Int J Parasitol 35:1255–1278

Mas-Coma S, Bargues MD, Valero MA (2006) Gastrodiscoidiasis, a plant-borne zoonotic dis-

ease caused by the intestinal amphistome fluke *Gastrodiscoides hominis* (Trematoda: Gastrodiscidae). Rev Iber Parasitol 66:75–81

Muñoz-Antoli C, Sotillo J, Monteagudo C, Fried B, Marcilla A, Toledo R (2007) Development and pathology of *Echinostoma caproni* in experimentally infected mice. J Parasitol 93:854–859

Muñoz-Antoli C, Cortés A, Martín-Grau C, Fried B, Esteban JG, Toledo R (2016a) Partial resistance to homologous challenge infections of the digenean *Echinostoma caproni* in ICR mice. J Helminthol 90:428–433

Muñoz-Antoli C, Cortés A, Santano R, Sotillo J, Esteban JG, Toledo R (2016b) Interleukin-25 induces resistance against intestinal Trematodes. Sci Rep 6:34142

Muttalib MA, Islam N (1975) *Fasciolopsis buski* in Bangladesh: a pilot study. J Trop Med Hyg 78:135–137

Niewiadomska K (2002) Family Diplostomidae Poirier, 1886. In: Gibson DI, Jones A, Bray RA (eds) Keys to the Trematoda, vol 1. CABI Publishing, Wallingford, pp 167–198

Park CJ, Kim J (2006) A human case of *Echinostoma hortense* infection diagnosed by endoscopy in area of southwestern Korea. Korean J Med 71:229–234

Park KY, Lee KJ, Kim IS (2005) Reaction of mast cells and goblet cells in the small intestine of C57BL/6 and C3H/HeN mice infected with *Echinostoma hortense*. Korean J Biomed Lab Sci 11:259–266

Pica R, Castellano C, Cilia C, Errico FO (2003) Intestinal fluke infections: the heterophyids. Clin Ter 154:61–63

Poland GA, Navin TR, Sarosi GA (1985) Outbreak of parasitic gastroenteritis among travelers returning from Africa. Arch Intern Med 145:2220–2221

Pyo KH, Kang EY, Jung BK, Moon JH, Chai JY, Shin EH (2012) Depressed neuronal growth associated protein (GAP)-43 expression in the small intestines of mice experimentally infected with *Neodiplostomum seoulense*. Korean J Parasitol 50:89–93

Quang TD, Duong TH, Richard-Lenoble D, Odermatt P, Khammanivong K (2008) [Emergence in humans of fascioliasis (from *Fasciola gigantica*) and intestinal distomatosis (from *Fasciolopsis buski*) in Laos]. Sante 18:119–124 (in French)

Radomyos P, Bunnag D, Harinasuta T (1985) Report of *Episthmium caninum* (Verma, 1935) Yamaguti, 1958 (Digenea: Echinostomatidae) in man. Southeast Asian J Trop Med Public Health 16:508–511

Radomyos P, Bunnag D, Harinasuta C (1989) A new intestinal fluke, *Plagiorchis harinasutai*, n. sp. Southeast Asian J Trop Med Public Health 20:101–107

Radomyos P, Charoenlarp P, Harinasuta T (1991) Human *Episthmium caninum* (Digenea: Echinostomatidae) infection: report of two more cases. J Trop Med Parasitol 14:48–50

Radomyos B, Wongsaroj T, Wilrairatana P, Radomyos P, Praevanich R, Meesomboon V, Jongsuksuntikul P (1998) Opisthorchiasis and intestinal fluke infections in northern Thailand. Southeast Asian J Trop Med Public Health 29:123–127

Rahman KM, Idris M, Khan AKA (1981) A study of fasciolopsiasis in Bangladesh. J Trop Med Hyg 84:81–86

Ryang YS, Ahn YK, Kim WT, Shin KC, Lee KW, Kim TS (1986) Two cases of human infection by *Echinostoma cinetorchis*. Kisaengchunghak Chapchi 24:71–76

Ryang YS, Yang EJ, Kim JL, Lee KJ, Sung HJ, Kim JB, Kim IS (2007) Immune response and inhibitory effect of ketotifen on the BALB/c and C3H/HeN mice infected with *Echinostoma hortense*. Parasitol Res 101:1103–1110

Sah R, Khadka S, Hamal R, Poudyal S (2018) Human echinostomiasis: a case report. BMC Res Notes 11:17

Sato M, Pongvongsa T, Sanguankiat S, Yoonuan T, Dekumyoy P, Kalambaheti T, Keomoungkhoun M, Phimmayoi I, Boupha B, Moji K, Waikagul J (2010) Copro-DNA diagnosis of *Opisthorchis viverrini* and *Haplorchis taichui* infection in an endemic area of Lao PDR. Southeast Asian J Trop Med Public Health 41:28–35

Saxton T, Fried B (2009) An update on metacercarial excystment of trematodes. Parasitol Res 105:1185–1191

Sayasone S, Tesana S, Utzinger J, Hatz C, Akkhavong K, Odermatt P (2009a) Rare human infection with the trematode *Echinochasmus japonicus* in Lao PDR. Parasitol Int 58:106–109

Sayasone S, Vonghajack Y, Vanmany M, Rasphone O, Tesana S, Utzinger J, Akkhavong K, Odermatt P (2009b) Diversity of human intestinal helminthiasis in Lao PDR. Trans R Soc Trop Med Hyg 103:247–254

Sayasone S, Mak TK, Vanmany M, Rasphone O, Vounatsou P, Utzinger J, Akkhavong K, Odermatt P (2011) Helminth and intestinal protozoa infections, multiparasitism and risk factors in Champasack province, Lao People's Democratic Republic. PLoS Negl Trop Dis 5:e1037

Schell SC (1985) Trematodes of North America, north of Mexico. University Press of Idaho, Idaho

Seo BS, Rim HJ, Lee CW (1964) Studies on the parasitic helmiths of Korea: I. Trematodes of rodents. Korean J Parasitol 2:20–26

Seo ES, Cho SY, Chai JY (1980) Studies on intestinal trematodes in Korea. I. A human case of *Echinostoma cinetorchis* infection with an epidemiological investigation. Seoul J Med 21:21–29

Seo BS, Lee SH, Cho SY, Chai JY, Hong ST, Han IS, Sohn JS, Cho BH, Ahn SR, Lee SK, Chung SC, Kang KS, Shim HS, Hwang IS (1981) An epidemiologic study on clonorchiasis and metagonimiasis in riverside areas in Korea. Korean J Parasitol 19:137–150

Seo BS, Lee SH, Hong ST, Hong SJ, Kim CY, Lee HY (1982) Studies on intestinal trematodes in Korea: V. A human case infected by *Fibricola seoulensis* (Trematoda: Diplostomatidae). Korean J Parasitol 20:93–99

Seo BS, Lee SH, Chai JY, Hong SJ, Hong ST (1988) The life cycle and larval development of *Fibricola seoulensis* (Trematoda: Diplostomatidae). Korean J Parasitol 26:179–188

Seo M, Guk SM, Han ET, Chai JY (2003) Role of intestinal goblet cells in the expulsion of *Gymnophalloides seoi* from mice. J Parasitol 89:1080–1082

Seo M, Chun H, Ahn G, Jang KT, Guk SM, Chai JY (2006) A case of colonic lymphoid tissue invasion by *Gymnophalloides seoi* in a Korean man. Korean J Parasitol 44:87–89

Sheir ZM, Aboul-Enein ME (1970) Demographic, clinical and therapeutic appraisal of heterophyiasis. J Trop Med Hyg 73:148–152

Shin EH, Kim TH, Hong SJ, Park JH, Guk SM, Chai JY (2003) Effects of anti-allergic drugs on intestinal mastocytosis and worm expulsion of rats infected with *Neodiplostomum seoulense*. Korean J Parasitol 41:81–87

Shin EH, Lee SH, Kim JL, Chai JY (2007) T-helper-1 and T-helper-2 immune responses in mice infected with the intestinal fluke *Neodiplostomum seoulense*: their possible roles in worm expulsion and host fatality. J Parasitol 93:1036–1045

Shin EH, Im TK, Park YK, Cho J, Kim JL, Chai JY (2016) Fecundity reduction of BALB/c mice after survival from lethal *Neodiplostomum seoulense* infection. Parasitol Res 115:2051–2059

Shoop WL (1989) Experimental human infection with *Fibricola cratera* (Trematoda: Neodiplostomidae). Korean J Parasitol 27:249–252

Shyu LY, Lee HH, Chen ER (1984) A preliminary study on epidemiology of fasciolopsiasis in Tainan Hsien, south Taiwan. Chin J Microbiol Immunol 17:118–120

Sohn WM (2009) Fish-borne zoonotic trematode metacercariae in the Republic of Korea. Korean J Parasitol 47:S103–S113

Sohn WM, Ryang YS, Chai JY, Lee SH (1998) Discovery of *Gymnophalloides seoi* metacercariae in oysters from islands of the West Sea known as the habitats of paleartic oystercatchers. Korean J Parasitol 36:163–169

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 (2011a) *Echinostoma ilocanum* infection in Oddar Meanchey Province, Cambodia. Korean J Parasitol 49:187–190

Sohn WM, Chai JY, Yong TS, Eom KS, Yoon CH, Sinuon M, Socheat D, Lee SH (2011b) *Echinostoma revolutum* infection in children, Pursat Province, Cambodia. Emerg Infect Dis 17:117–119

Sotillo J, Muñoz-Antoli C, Marcilla A, Fried B, Guillermo Esteban J, Toledo R (2007) *Echinostoma caproni*: kinetics of IgM, IgA and IgG subclasses in the serum and intestine of experimentally infected rats and mice. Exp Parasitol 116:390–398

Sotillo J, Valero L, Sánchez del Pino MM, Fried B, Esteban JG, Marcilla A, Toledo R (2008) Identification of antigenic proteins from *Echinostoma caproni* (Trematoda: Echinostomatidae) recognized by mouse immunoglobulins M, A and G using an immunoproteomic approach. Parasite Immunol 30:271–279

Sotillo J, Trelis M, Cortés A, Fried B, Marcilla A, Esteban JG, Toledo R (2011) Th17 responses in *Echinostoma caproni* infections in hosts of high and low compatibility. Exp Parasitol 129:307–311

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

Sukontason K, Unpunyo P, Sukontason KL, Piangiai S (2005) Evidence of *Haplorchis taichui* infection as pathogenic parasite: three case reports. Scand J Infect Dis 37:388–390

Takahashi S, Ishii T, Ueno N (1930) A human case of *Echinostoma cinetorchis*. Tokyo Iji Shinshi 2757:141–144

Tantrawatpan C, Saijuntha W, Sithithaworn P, Andrews RH, Petney TN (2013) Genetic differentiation of Artyfechinostomum *malayanum* and *A. sufrartyfex* (Trematoda: Echinostomatidae) based on internal transcribed spacer sequences. Parasitol Res 112:437–441

Thaenkham U, Phuphisut O, Pakdee W, Homsuwan N, Sa-nguankiat S, Waikagul J, Nawa Y, Dung do T (2011) Rapid and simple identification of human pathogenic heterophyid intestinal fluke metacercariae by PCR-RFLP. Parasitol Int 60:503–506

Toledo R (2009) Immunology and pathology of echinostomes 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

Toledo R, Esteban JG (2016) An update on human echinostomiasis. Trans R Soc Trop Med Hyg 110:37–45

Toledo R, Fried B (2005) Echinostomes as experimental models for interactions between adult parasites and vertebrate hosts. Trends Parasitol 21:251–254

Toledo R, Espert A, Muñoz-Antoli C, Marcilla A, Fried B, Esteban JG (2003) Development of an antibody-based capture enzyme-linked immunosorbent assay for detecting *Echinostoma caproni* (Trematoda) in experimentally infected rats: kinetics of coproantigen excretion. J Parasitol 89:1227–1231

Toledo R, Espert A, Muñoz-Antoli C, Marcilla A, Fried B, Esteban JG (2004) Kinetics of *Echinostoma caproni* (Trematoda: Echinostomatidae) antigens in feces and serum of experimentally infected hamsters and rats. J Parasitol 90:752–758

Toledo R, Espert A, Muñoz-Antoli C, Marcilla A, Fried B, Esteban JG (2005) Kinetics of antibodies and antigens in serum of mice experimentally infected with *Echinostoma caproni* (Trematoda: Echinostomatidae). J Parasitol 91:978–980

Toledo R, Esteban JG, Fried B (2006a) Immunology and pathology of intestinal trematodes in their definitive hosts. Adv Parasitol 63:285–365

Toledo R, Monteagudo C, Espert A, Fried B, Esteban JG, Marcilla A (2006b) *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

Toledo R, Esteban JG, Fried B (2009) Recent advances in the biology of echinostomes. Adv Parasitol 69:147–204

Toledo R, Bernal MD, Marcilla A (2011) Proteomics of foodborne trematodes. J Proteome 74:1485–1503

Toledo R, Esteban JG, Fried B (2012) Current status of food-borne trematode infections. Eur J Clin Microbiol Infect Dis 31:1705–1718

Trelis M, Sotillo J, Monteagudo C, Fried B, Marcilla A, Esteban JG, Toledo R (2011) *Echinostoma caproni* (Trematoda): differential in vivo cytokine responses in high and low compatible hosts. Exp Parasitol 127:387–397

Trung Dung D, Van De N, Waikagul J, Dalsgaard A, Chai JY, Sohn WM, Murrell KD (2007) Fishborne zoonotic intestinal trematodes, Vietnam. Emerg Infect Dis 13:1828–1833

Uppal B, Wadhwa V (2005) Rare case of *Metagonimus yokogawai*. Indian J Med Microbiol 23:61–62

Van Van K, Dalsgaard A, Blair D, Le TH (2009) *Haplorchis pumilio* and *H. taichui* in Vietnam discriminated using ITS-2 DNA sequence data from adults and larvae. Exp Parasitol 123:146–151

Watthanakulpanich D, Waikagul J, Maipanich W, Nuamtanong S, Sanguankiat S, Pubampen S, Praevanit R, Mongkhonmu S, Nawa Y (2010) *Haplorchis taichui* as a possible etiologic agent of irritable bowel syndrome-like symptoms. Korean J Parasitol 48:225–229

Weinstein MS, Fried B (1991) The expulsion of *Echinostoma trivolvis* and retention of *Echinostoma caproni* in the ICR mouse: pathological effects. Int J Parasitol 21:255–257

Weng YL, Zhuang ZL, Jiang HP, Lin GR, Lin JJ (1989) Studies on ecology of *Fasciolopsis* buski and control strategy of fasciolopsiasis. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi 7:108–111

Wongsawad P, Wongsawad C (2009) Development of PCR-based diagnosis of minute intestinal fluke, *Haplorchis taichui*. Southeast Asian J Trop Med Public Health 40:919–923

Wongsawad C, Kawin S, Wongsawad P, Paratasilpin T (2005) Some factors affecting *Stellantchasmus falcatus* metacercaria in laboratory. Southeast Asian J Trop Med Public Health 36:11711–11719

Wongsawad C, Wongsawad P, Chai JY, Anuntalabhochai S (2009a) *Haplorchis taichui*, Witenberg, 1930: development of a HAT-RAPD marker for the detection of minute intestinal fluke infection. Exp Parasitol 123:158–161

Wongsawad C, Wongsawad P, Chuboon S, Anuntalabhochai S (2009b) Copro-diagnosis of *Haplorchis taichui* infection using sedimentation and PCR-based methods. Southeast Asian J Trop Med Public Health 40:924–928

Xiao X, Wang TP, Lu DB, Gao JF, Xu LF, Wu WD, Mei JD, Xu FL (1992) The first record of human infection with *Echinochasmus liliputanus*. Chin J Parasitol Parasit Dis 10:132–135

Xiao X, Dabing L, Tianping W, Gao J, Zhu C, Zhang B, An J, Pong H, Xu M, Wu W (1995) Studies on mode of human infection with *Echinochasmus liliputanus*. Chin J Parasitol Parasit Dis 13:197–199

Yamaguti S (1971) Synopsis of digenetic trematodes of vertebrates. Keigaku Publishing Company, Japan

Yamaguti S (1975) A synoptical review of the life histories of digenetic trematodes of vertebrates: with special reference to the morphology of their larval forms. Keigaku Publishing Company, Japan

Yokogawa M, Harinasutam C, Charoenlarp P (1965) *Hypoderaeum conoideum* (Block, 1782) Diez, 1909, a common intestinal fluke of man in the north-east Thailand. Jpn J Parasitol 14:148–153

Youn H (2009) Review of zoonotic parasites in medical and veterinary fields in the Republic of Korea. Korean J Parasitol 47:S133–S141

Yu S, Mott K (1994) Epidemiology and morbidity of food-borne intestinal trematode infections. Trop Dis Bull 91:R125–R152

Yu JR, Hong ST, Chai JY, Lee SH (1995) The effect of reinfection with *Neodiplostomum seoulensis* on the histopathology and activities of brush border membrane bound enzymes in the rat small intestine. Korean J Parasitol 33:37–43