

Echinostomes and Other Intestinal Trematode Infections

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Abstract

Intestinal trematodes are among the most common types of parasitic worms. About 76 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. These parasites are diverse in regard to their morphology, geographical distribution and life cycle, which make it difficult to study the parasitic diseases that they cause. Many of these intestinal trematodes have been considered as endemic parasites in the past. 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, new eating habits in developed countries and demographic changes. These factors make it necessary to better understand intestinal trematode infections. This chapter describes the main features of human intestinal trematodes in relation to their biology, epi-

demiology, host–parasite relationships, pathogenicity, clinical aspects, diagnosis, treatment and control.

Keywords

Digenea · Trematode infection · Intestinal trematodes · Biology · Epidemiology · Host–parasite relationships · Pathogenicity · Diagnosis · Treatment · Control

8.1 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 7 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 aliments are involved in the transmission of intestinal flukes (Table 8.1) and the eating habits are essential to determine the distribution of these parasitic diseases. High incidence of intestinal trematodiasis is strongly associated with popula-

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Table 8.1 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 ^b
Brachylaimidae	1	Terrestrial snails	Oceania
Cathaemaciidae	1	Not known	Asia
Diplostomidae	2 ^a	Snakes, frogs, tadpoles	Asia
Echinostomatidae	23	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

^aOne of them (*Fibricola cratera*) only in experimental infections

^bImported cases are not considered

tions 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 trematodiasis, 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.

8.2 Family Echinostomatidae

8.2.1 Background

The family Echinostomatidae includes digeneans characterized by the presence of a prominent cephalic collar of spines (Fig. 8.1a). 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). Recently, Miao et al. (2021) reported an ectopic case in Zhejiang

(China). A single adult of *Echinostoma* was found in the urinary bladder of a 67-year-old male by cystoscopy. The patient did not complain from intestinal symptoms but did suffer from hematuria and dysuria.

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, become infected after ingestion of the second intermediate host harbouring the encysted metacercariae (Fig. 8.1b). Finally, adults produce eggs that are released with the host's faeces (Fig. 8.1c) (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).

8.2.2 Species Reported in Humans and Geographical Distribution

Echinostomes commonly parasitize waterfowls and mammals associated with freshwater habitats. However, the specificity towards 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; Toledo et al. 2019, 2022). 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 preva-

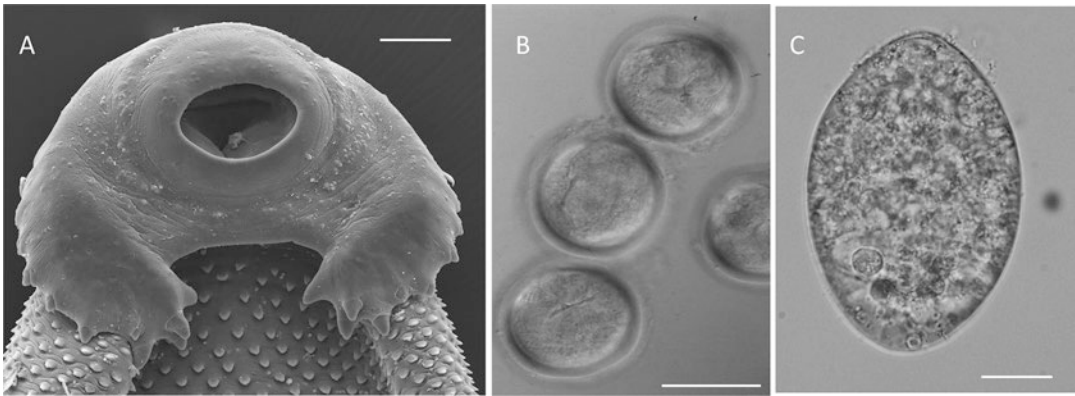


Fig. 8.1 (a) SEM microphotograph of the cephalic collar of spines of *Echinostoma* sp. (Scale bar: 100 µm); (b) metacercarial cysts of *Echinostoma* sp. (Scale bar:

100 µm); and (c) egg of *Isthmiophora hortensis*. (Photomicrograph courtesy of In Sik Kim) (Scale bar: 25 µm)

lent in areas where traditional eating practices encourage the consumption of these types of foods. For example, in the 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 with uncertain, incomplete or unavailable specific identification, even in recent reports. For example, Sah et al. (2018) reported the first case of human echinostome infection in Nepal in a 62-year-old male from Gorkha district caused by an unidentified member of the genus *Echinostoma*. Similarly, Duan et al. (2019) reported a human case caused by a member of the family Echinostomatidae in a 40-year-old patient with bipolar disorder patient and complaining gastrointestinal symptoms in China. Also in China, Li et al. (2019) detected eggs of an echinostome that was simply diagnosed as a member of Echinostomatidae in the

faeces of a 42-year-old male. In India, Khanna et al. (2019) recorded a single case attributed to *Echinostoma* sp. in a 3-year-old male from Karnataka complaining severe anaemia. This latest case appeared to be related to the consumption of undercooked fish and snails. In Thailand, Martviset et al. (2023) revised the prevalence of helminth infections in the parasitic endemic areas of rural Northeastern Thailand, though no specific diagnosis was reported for the human echinostome infections.

Furthermore, the problematical taxonomy of the group complicates the situation since misidentifications are common. The situation becomes more complicated since due to all these problematics, the synonymies between different species are continuous. For example, *Artyfechinostomum malayanum* (Fig. 8.2a) 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). Kostadinova and Gibson (2002) revised several species of the genus *Echinostoma* and *Echinostoma hortense* was redescribed and nominated as *Isthmiophora hortensis*. Similarly, *Episthmium caninum* was considered as a synonym of the new combination



Fig. 8.2 Adult worms of (a) *Artyfechinostomum malayanum* (syn. *Echinostoma malayanum*) (Photomicrograph courtesy of Weerachai Saijuntha); (b) *Isthmiophora hor-*

tensis. (Photomicrograph courtesy of Woon-Mok Sohn); (c) *Echinoparyphium recurvatum*; and (d) *Hypoderaeum conoideum*. (Scale bars: 1 mm)

Echinochasmus caninus using adult worm specimens collected from human infections in Lao PDR (Chai et al. 2019).

All these facts make it difficult to determine the exact number of echinostome species that infect humans. 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. Toledo et al. (2019) listed a total of 20 species, whereas Chai and Jung (2020) considered a total of 23 species, 15 of them having more than 6 human reporters each. Herein, we follow the paper by Toledo et al. (2022) in which a total of 23 species are considered, including the latest synonyms and new combinations (Table 8.2). Further details on the morphology and biology of several echinostome 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 muelhensi*, have been reported in this area (Chai et al. 2009a).

A total of 12 species have been reported infecting humans in China (Table 8.2). 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. jiufuensis*, *E. liliputanus* and *E. japonicus*), *E. fujianensis* is the most common. In several areas, the prevalence among residents reached 3.2%, with the highest rates in children from 3 to 15 years (Cheng et al. 1992; Yu and Mott 1994). The prevalence of *E. japonicus* in some counties of Guangdong and

Table 8.2 Species of Echinostomatidae involved in human infections

<i>Acanthoparyphium tyosenense</i>	Korea
<i>Artyfechinostomum malayanum</i> ^a	Cambodia, India, Indonesia, Lao PDR, Malaysia, Philippines, Singapore, Thailand
<i>Artyfechinostomum oraoni</i>	India
<i>Artyfechinostomum sufrartyfex</i>	India
<i>Echinoschasmus caninus</i> ^b	Lao PDR, Thailand
<i>Echinoschasmus fujianensis</i>	China
<i>Echinoschasmus japonicus</i>	China, Korea, Lao PDR
<i>Echinoschasmus jiufoensis</i>	China
<i>Echinoschasmus liliputanus</i>	China
<i>Echinochasmus perfoliatus</i>	China, Japan, Korea
<i>Echinoparyphium recurvatum</i>	Egypt, Indonesia, Korea, Taiwan
<i>Echinostoma aegyptica</i>	China
<i>Echinostoma angustitestis</i>	China
<i>Echinostoma cinetorchis</i>	China, Japan, Korea, Taiwan
<i>Echinostoma ilocanum</i>	Cambodia, China, India, Indonesia, Malaysia, Philippines, Thailand
<i>Echinoastoma lindoense</i> ^c	Indonesia, Lao PDR
<i>Echinostoma macrorchis</i>	Japan, Korea, Lao PDR
<i>Echinostoma mekongi</i>	Cambodia
<i>Echinostoma revolutum</i>	Cambodia, China, Egypt, Indonesia, Lao PDR, Russia, Europe
<i>Himasthla muelhensi</i>	USA ^d
<i>Hypoderaeum conoideum</i>	Thailand
<i>Isthmiophora hortensis</i> ^e	China, Japan, Korea
<i>Isthmiophora melis</i>	China, Rumania, Taiwan

^aSyn. *Echinostoma malayanum*

^bSyn. *Episthmium caninum*

^cSyn. *Echinostoma echinatum*

^dImported infection

^eSyn. *Echinostoma hortense*

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 four members of *Echinostoma* (*E. angustitestis*, *E. ilocanum*, *E. japonicus* and *E. revolutum*) have been also detected in China. *E. revolutum* has been reported in Yunnan and Guandong 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 *Isthmiophora hortense* (Fig. 8.2b) (Chen et al. 1993). More anecdotal is the infection with *I. 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*

cinetorchis, *E. macrorchis* and *I. hortense*), Thailand (*E. revolutum*, *E. ilocanum*, *Episthmium caninum* and *Hypoderaeum conoideum*), and India (*A. malayanum*, *A. oraoni*, *A. sufrartyfex* and *E. ilocanum*) and seven in Korea (*A. tyosenense*, *E. cinetorchis*, *E. japonicus*, *E. perfoliatus*, *E. recurvatum*, *E. macrorchis* and *I. hortensis*). 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 *Echinochasmus caninus* 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 then 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 *I. hortense* have been reported in both 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. 8.2c) 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 total of seven echinostome species have been reports in Lao PDR infecting humans (*A. malayanum*, *Echinochasmus caninus*, *E. japonicus*, *E. ilocanum*, *E. lindoense*, *E. macrorchis* and *E. revolutum*). Sayasone et al. (2011) detected a prevalence of 6% of echinostome human infections which was found in southern Lao PDR. A more recent study in Laos PDR found, out of a total of 6178 residents in 102 villages of 9 provinces that were between 2007 and 2011, the presence of echinostome eggs in the faeces of 1.1% of residents (Eom et al. 2014). 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*. 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 Mekong River (Chai et al. 2018). Recently, adult specimens of *Echinochasmus caninus* (syn. *Episthmium caninum*) were recovered from 11 riparian people who resided along the Mekong River in Khammouane Province (Chai et al. 2019).

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. Moreover, *Echinostoma mekongi* was recently described on the basis of a total 256 flukes collected from the diarrhoeic stools of six villagers from Kratie and Takeo Provinces after praziquantel treatment and purging (Cho et al. 2020).

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 echinostome.

In India, two deaths have been historically attributed to *A. sufrartyfex* in the states of Assam and Tamil Nadu (Lane 1915; Reddy and Varmah 1950). During 2004–2017, several cases of echinostome infection were reported in children at Shri Shubh Lal Hospital and Research Centre in Bihar (Prasad et al. 2019). A total of 170 cases of *A. sufrartyfex* infection occurred in northern Bihar, India, mostly in children under 12 years of age. Symptoms were diarrhoea with watery or

mucus-bound stool, vomiting, loss of appetite, weakness, passage of red worms in stool or vomit, swelling of the feet and the entire body, fever, cough, breathlessness, night blindness and urticarial rashes (Prasad et al. 2019).

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 diarrhoeic patient in Rumania (Beaver et al. 1984) and *E. recurvatum* (Fig. 8.2c) 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 travelled 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 10 patients showed moderately severe abdominal cramps and loose or watery stools. A traveller 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).

8.2.3 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; Toledo et al. 2022). 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, diarrhoea 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 the 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 fibroscopy (Chai et al. 1994). Cortés et al. (2015a) showed that echinostome infection in mice induces significant changes in the intestine affecting 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. Recently, severe anaemia was been reported in a 3-year-old male child from India in concomitants infections with *Echinostoma* sp., *Trichuris trichiura* and *Ascaris lumbricoides* (Khanna et al. 2019).

In ectopic locations such as the urinary bladder, the patient suffered haematuria accompanied by urgency and dysuria. The values for haematuria were 305 red blood cells per μl (normal 1–13), 297 white blood cells per μl (normal 0–9) and a positive result of 2+ for occult blood in the urine (Miao et al. 2021).

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).

8.2.4 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 expelled 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 energetic 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. Interestingly, echinostomes can trap and degrade antibodies bound to their surface by secreted cathepsin L-like peptidases (Cortés et al. 2017a, 2019).

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* infec-

tions, 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).

Factors determining resistance to echinostome infections have been studied in mice. 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. Resistance was associated with local hyperplasia of tuft cells and production of IL-25 (Muñoz-Antoli et al. 2016b). However, IL-25 appears to act independently of the Th2 mechanisms. Development of Th2 responses, in absence of IL-25, is not sufficient to generate resistance against infections. Although effector mechanism remains unknown, IL-25 appears to operate by facilitating alternative activation of macrophages and the maintenance of the epithelial homeostasis that could be ultimately responsible for parasite rejection (Cortés et al. 2017b; Álvarez-Izquierdo et al. 2020a, b). Furthermore,

resident microbiota play a major role in the production of IL-25, and the appearance of members of the phylum Verrucomicrobia is essential (Álvarez-Izquierdo et al. 2022).

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).

8.2.5 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 faeces. 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 are in the range 66–145 $\times$ 43–90 μ m, 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).

8.3 Family Brachylaimidae

8.3.1 Background

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

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.

8.3.2 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. 8.3). The first human infections with this trematode were described in two young children (21-month-olds) 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 diarrhoea 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 helcid 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 diarrhoea 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.

8.3.3 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 than 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 is 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 faecal eggs per gram of faeces 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.

8.4 Family Diplostomidae

8.4.1 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

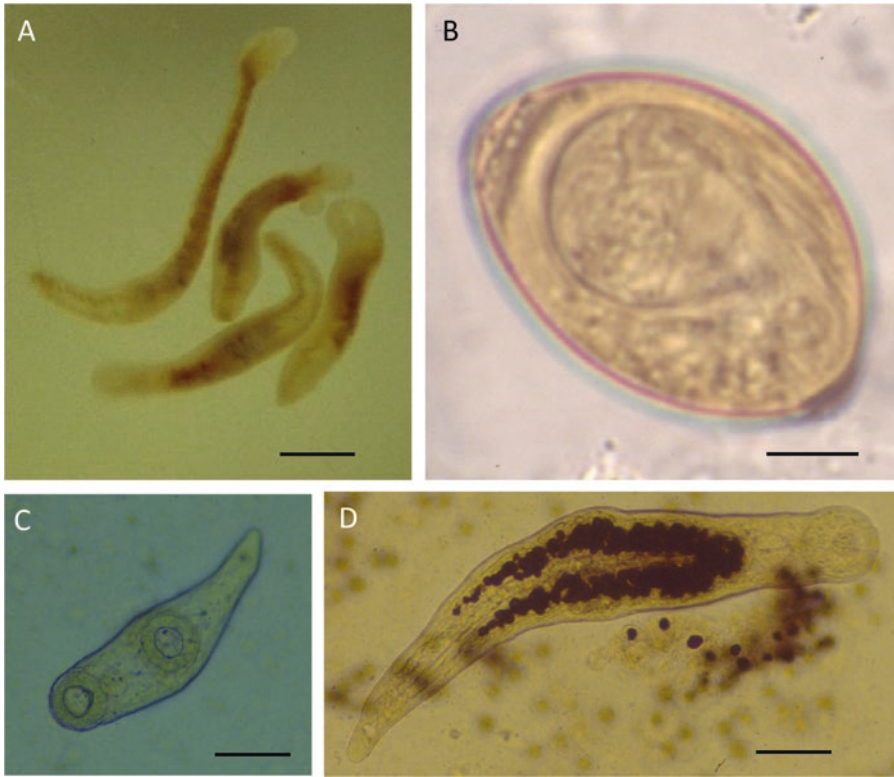


Fig. 8.3 Unstained specimens of *Brachylaima cribbi*: (a) adults (Scale bar: 1 mm); (b) fertile egg (Scale bar: 5 μ m); (c) cercaria (Scale bar: 25 μ m); and (d) metacercaria

(Scale bar: 500 μ m). (Photomicrographs courtesy of Andrew R. Butcher)

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 harbouring 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.

8.4.2 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 harbouring 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. 8.4a) (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 et al. 1988; Chung et al. 1996, 2002). Second intermediate hosts are tadpoles and frogs harbouring metacercariae, and snakes may act as a paratenic host harbouring the mesocercariae (Fig. 8.4b) (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).

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.

8.4.3 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).



Fig. 8.4 *Neodiplostomum seoulense*: (a) adult specimen (Scale bar: 250 μ m); (b) mesocercaria collected from a snake (Scale bar: 250 μ m); and (c) egg (Scale bar: 25 μ m). (Photomicrographs courtesy of Woon-Mok Sohn)

The clinical manifestation and the pathology induced by *N. seoulense* are 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, pain and anorexia. Thereafter, diarrhoea, 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 remainder 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* down-regulates 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.

8.4.4 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 increasing 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 tribocytic 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, which were recognized by the sera of humans infected with *N. seoulense*.

8.4.5 Diagnosis

Diagnosis of *N. seoulense* is usually done by detection of eggs in faeces using traditional methods. The eggs are ellipsoid, thin-shelled, with an inconspicuous operculum and measuring 86–99 $\times$ 55–63 μ m (Seo et al. 1982) (Fig. 8.4c). They can be differentiated from other similar eggs, such as those of *Echinostoma* spp. by their clean shell surface and the absence of abopercu-

lar 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.

8.5 Family Fasciolidae

8.5.1 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.

8.5.2 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. 8.5a) 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 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 be also contracted by the drinking or use of contaminated water or processing of the water-derived plants, for example, 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).

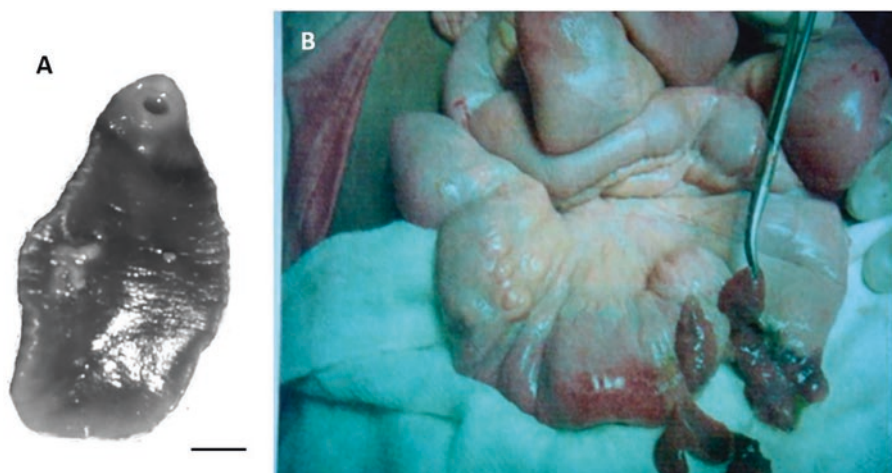


Fig. 8.5 (a) Unstained specimen of *Fasciolopsis buski* (Scale bar: 0.5 mm). (Photograph courtesy of Deepak Bagga); (b) *Fasciolopsis buski* emerging from perforation

site of the ileum (Scale bar: 5 μ m). (Photograph courtesy of Umesh Chandra Singh)

In China, infections have been reported in 10 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). Wu et al. (2020) reported a human infection of a 15-year-old girl from Guizhou Province, China, presenting with recurrent upper middle abdominal pain that was misdiagnosed as a choledochal cyst. In India, 60% of people were found to be infected and harbouring 1–57 worms in Assam (Buckley 1939). Jha and Jha (2019) reported a case of a 40-year-old Indian female presented with a 2-week history of epigastric pain, vomiting and passage of flesh-like material in the stool. She provided a history of consumption of raw singhara (water caltrop). She used to wash the kitchen utensils and raw vegetables in pond water. Blood examination showed haemoglobin of 10.5 g/dL and normal total eosinophil count. Stool examination was normal. Esophagogastroduodenoscopy showed multiple live worms diagnosed as *F. buski* attached to the pyloric and duodenal mucosa. Recent studies suggest that human infections with *F. buski* in India are more common than previously thought (Saurabh and Ranjan 2017). Saikia et al. (2022) detected a total of 12

3–12-year-old children infected with *F. buski* from a total of 14 children studied during the period 2012–2021 in a hospital from Bihar. The first case of autochthonous transmission of fasciolopsiasis in Nepal was described by Sah et al. (2019a) in a 22-year-old man who was admitted in the hospital with abdominal pain, distension and loss of appetite for a month. He had previously diagnosed with acute viral hepatitis, but his abdominal pain was not resolving despite improvement in his liver function and general condition.

In Thailand, the central area is the main endemic area with an estimation of 20% infected people (Manning et al. 1971). Infection has been also reported from Lao PDR, Vietnam, Cambodia, Indonesia, the Philippines, Kampuchea, Burma and Malaysia (Mas-Coma et al. 2005; Ridha et al. 2021). 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.

8.5.3 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, diarrhoea 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 appendicitis (Fig. 8.5b) (Bhattacharjee et al. 2009; Cao et al. 2015).

Moreover, adult flukes damage the intestinal mucosa and cause extensive duodenal erosions, ulceration, haemorrhage, abscesses and catarrhal inflammation. Absorption of toxic and allergic worm metabolites causes ascites, general oedema and facial oedema (Jaroonvesama et al. 1986; Fried et al. 2004). Recently, a case of mechanical biliary obstruction caused by *F. buski* has been described. Biliary obstruction caused by *F. buski* infection leading to surgery is not uncommon, but it is very rare in modern society (Wu et al. 2020).

8.5.4 Diagnosis

Laboratory diagnosis is based on the demonstration of eggs in faeces. 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.

8.6 Family Gastrodiscidae

8.6.1 Background

The family Gastrodiscidae contains trematodes relatively large, that is, approximately 8–14 mm in length. They are intestinal parasites of terres-

trial mammals, including man, and have been distinguished by a dorsoventrally flattened body, which has the appearance of being divided into two parts.

8.6.2 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 emerge and encyst 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; Toledo et al. 2022).

Although *G. hominis* 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). *G. hominis* has been detected infecting humans in India, Burma, Pakistan, Myanmar, Vietnam, the Philippines, Thailand, China, Nepal, Kazakhstan, Indian immigrants in Guyana, Zambia, Nigeria and the Volga Delta in Russia (Mas-Coma et al. 2005). A recent case was reported in Nepal in a 66-year-old Nepali man without history of travels outside of Nepal who was admitted in the hospital with pain in upper abdomen for 4 months with history of pas-

sage of black-tarry stools, anaemia and eosinophilia (Sah et al. 2019b).

Pathology and symptomatology of humans *G. hominis* infections are not well known. Heavy infections may induce headache, epigastric pain and diarrhoea 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 faeces. Egg size is about $4\text{--}6 \times 5\text{--}10$ mm and it is deep yellow, operculated, non-embryonated and measuring about 150×70 mm (Mas-Coma et al. 2006).

8.7 Family Gymnophallidae

8.7.1 Background

Gymnophalloidiasis in 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 that becomes infected after ingestion of the second intermediate host harbouring 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.

8.7.2 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; Fried et al. 2004; Guk et al. 2006; Chai 2007; Cho et al. 2010).

The adult parasite is small ($0.4\text{--}0.5 \times 0.2\text{--}0.3$ mm) and characterized by a large oral sucker and small ventral sucker, short caeca and a unique ventral pit (Fig. 8.6a) (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 harbouring the metacercariae (Fig. 8.6b). 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 been also 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).

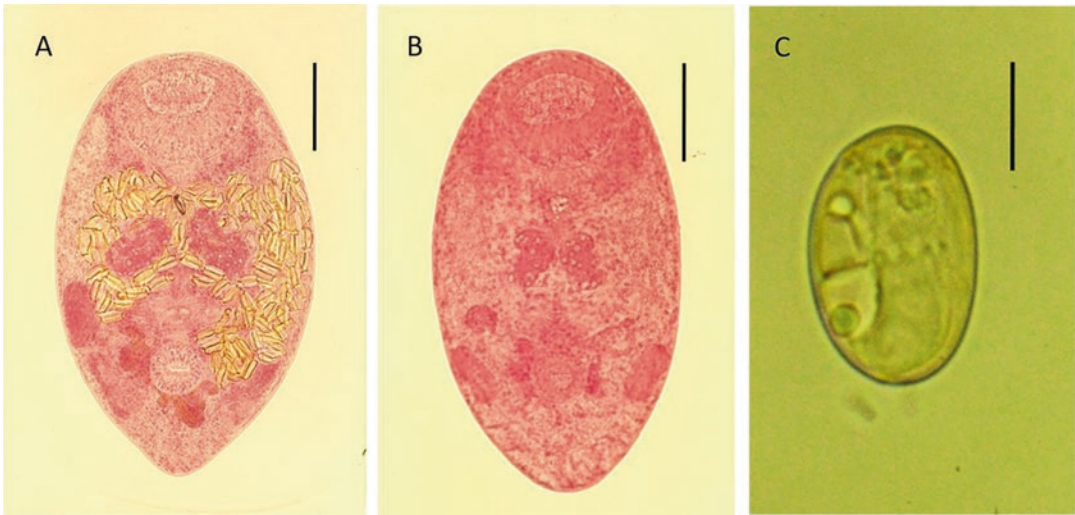


Fig. 8.6 *Gymnophalloides seoi*: (a) adult worm (Scale bar: 100 μ m); (b) metacercaria collected from a naturally infected oyster (Scale bar: 100 μ m); and (c) egg (Scale bar: 10 μ m). (Photomicrographs courtesy of Woon-Mok Sohn)

8.7.3 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 that 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* infection 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 defence 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). Song et al. (2018) reported that expulsion of adult worms from C57BL/6 mice is probably related to operation of mucosal innate immunity due to expression of TLR2 and MUC2 in epithelial cells in response to *G. seoi*-secreted exosomes.

8.7.4 Diagnosis

Diagnosis can be done by detection of eggs in the faeces. However, the detection and identification may be difficult because of (i) low-laying capacity of the parasite and (ii) 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 gymnohalloidiidiasis is the identification of the eggs. They are very small (approximately $23 \times 13 \mu\text{m}$) and thin and transparent shelled (Fig. 8.6c) (Lee et al. 2012). Due to their small size, the eggs can be overlooked by an inexperienced analyst or misdiagnosed as a bubble or other artefacts. Moreover, differential diagnosis with other digeneans may be difficult (Lee et al. 2012).

8.8 Family Heterophyidae

8.8.1 Background

Heterophyasis 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 gonostyle or genital sucker (Yamaguti 1971). This author accepted 13 subfamilies within the family which may be differentiated on the basis of the body morphology, 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 harbouring 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).

8.8.2 Species Infecting Humans and Geographical Distribution

About 33 species of heterophyids have been reported infecting humans (Table 8.3). Humans become infected by eating fish or shrimps harbouring viable metacercariae, which mature into adults in 5–10 days. Adult heterophyids live attached and embedded to 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. 8.7a, 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). A total prevalence of 1.4% of *H. heterophyes* was estimated in preschool and school children from Egypt by Elmonir et al. (2021).

Table 8.3 Species of Heterophyidae involved in human infections

Genus	Species	Geographical distribution of human cases
<i>Acanthotrema</i>	<i>A. felis</i>	Korea
<i>Apophallus</i>	<i>A. donicus</i>	USA
<i>Ascoctyle</i>	<i>A. longa</i>	Brazil ^a
<i>Centrocestus</i>	<i>C. armatus</i>	Japan, Korea
	<i>C. caninus</i>	Thailand
	<i>C. cuspidatus</i>	Egypt
	<i>C. formosanus</i>	Japan, Taiwan, Vietnam, Lao PDR
	<i>C. kurokawai</i>	Japan
<i>Cryptocotyle</i>	<i>C. lingua</i>	Greenland
<i>Haplorchis</i>	<i>H. pleurolophocerca</i>	Egypt
	<i>H. pumilio</i>	China, Egypt, Iran, Korea, Lao PDR, Philippines, Taiwan, Thailand, Vietnam
	<i>H. taichui</i>	China, Iran, Lao PDR, Philippines, Thailand, Vietnam
	<i>H. vanissimus</i>	Philippines
	<i>H. yokogawai</i>	China, Egypt, India, Indonesia, Lao PDR, Malaysia, Philippines, Taiwan, Thailand
<i>Heterophyes</i>	<i>H. dispar</i>	Korea ^b
	<i>H. heterophyes</i>	Egypt, India, Iran, Korea ^b , Sudan
	<i>H. nocens</i>	China, Japan, Korea
<i>Heterophyopsis</i>	<i>H. continua</i>	Japan, Korea
<i>Metagonimus</i>	<i>M. katsudurai</i>	Japan, Russia
	<i>M. minutus</i>	Taiwan
	<i>M. miyatai</i>	Japan, Korea
	<i>M. pusills</i>	Russia
	<i>M. sufinensis</i>	Russia
	<i>M. takahashii</i>	Korea
	<i>M. yokogawai</i>	All Far East, India, Balkan states, Israel, Iran, Korea, Siberia, Spain
<i>Procerovum</i>	<i>P. calderoni</i>	Africa, China, Philippines
	<i>P. varium</i>	India, Lao PDR
<i>Pygidioopsis</i>	<i>P. genata</i>	Egypt
	<i>P. summa</i>	Japan, Korea
<i>Stellanchasmus</i>	<i>S. falcatus</i>	Hawaii, Japan, Korea, Palestine, Philippines, Thailand, Vietnam
	<i>S. pseudocirratius</i>	Hawaii, Philippines
<i>Stictodora</i>	<i>S. fuscata</i>	Korea
	<i>S. lari</i>	Korea

^aReferred to as *Phagicola* sp. by Chieffi et al. (1990, 1992) in Brazil

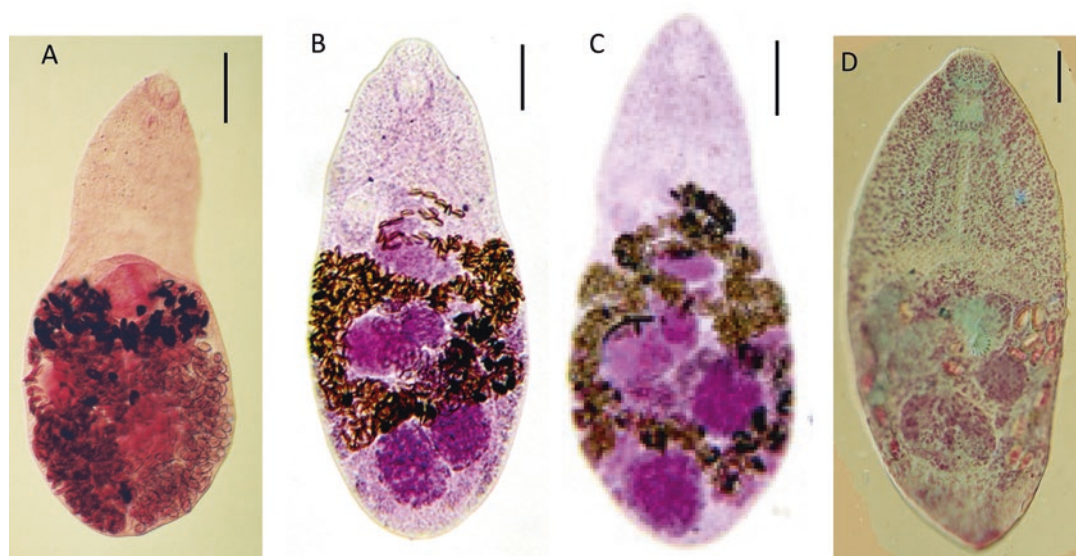


Fig. 8.7 Adult worms of (a) *Heterophyes heterophyes* (Scale bar: 50 μ m); (b) *Metagonimus yokogawai* (Scale bar: 150 μ m). (Photomicrograph courtesy of Hideto

Kino); (c) *Metagonimus miyatai* (Scale bar: 75 μ m). (Photomicrograph courtesy of Hideto Kino); and (d) *Haplorchis taichui*. (Scale bar: 100 μ m)

Recently, an infection of a 9-year-old boy, non-vegetarian, from rural Northern India was diagnosed by endoscopy. The patient complained severe emaciation, dehydration and severe acute malnutrition (Prasad et al. 2021). An overall prevalence of 2.4% of heterophyiasis has been reported in Southern Philippines (Moendeg et al. 2021). The risk of infection was associated with raw freshwater fish consumption, undercooked grilled fish consumption and proximity to rivers or creeks.

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, Iran, Japan, Korea, Taiwan and Indonesia (Yu and Mott 1994; Toledo et al. 2019; Nazarbeigy et al. 2021). 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 Guandong, Anhui, Hubei and Zhejiang (Chai et al. 2009a). Bahk et al. (2018) detected a prevalence of infection with *M. yokogawai* of 0.61% in the Hadong-gun county of Korea. 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 6-year-old female in New Delhi (Uppal and Wadhwa 2005).

Another 10 species of Heterophyidae have been recorded in the Republic of Korea (Table 8.3). *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. 8.7c) 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). *Pygidioopsis 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 (Cho et al. 2010). Sporadic cases of *Centrocestus armatus* (1 case), *Haplorchis pumilio* (2), *Heterophiopsis continua* (10), *Stellantchasmus falcatus* (4), *Stictodora fuscata* (14) and *S. lari* (6) have been also recorded (Chai and Lee 2002; Chai et al. 2009a; Chung et al. 2011; Nazarbeigy et al. 2021). 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. 8.7d), *Haplorchis yokogawai* and *S. falcatus* in several areas (Trung Dung et al. 2007). Similarly, high prevalences of *H. taichui* and *H. yokogawai* have been found in Lao PDR and Thailand (Chai et al. 2010; Wijit et al. 2013).

In Japan, seven additional species of heterophyids have been recorded infecting humans (Table 8.3) (Chai et al. 2009a; Yu and Mott 1994). Of particular interest are the endemic foci of *H. nocos* 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). Human infections with *Ascocotyle longa* (referred to as *Phagicola* sp.) have been reported in Brazil (Chieffi et al. 1990, 1992).

8.8.3 Clinical Manifestations and Pathology

Commonly, low-grade infections with heterophyids are of no clinical consequences. Heavy infections are associated with diarrhoea, mucus-rich faeces, 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 diarrhoea 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 Lieberkühn 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 oedema, epicardium haemorrhage, fragmentation of muscle fibres, cardiac enlargement, embolism of the capillaries, cough, dyspnoea, cyanosis, fatigue, oedema 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 (Wathanakulpanich et al. 2010). Interestingly, Arya et al. (2016) described a total of 42 cases of paediatric granulomatous eye disease caused by *Pocerozum varium* cercariae in 42 children with a history of recreationally exposure to village ponds and river waters in South India.

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 Lieberkühn, 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 were noted. Both caspase-3 and NF- κ B 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 oedema. Sukontason et al. (2005) studied the pathology induced by *H. taichui* on three human cases revealing mucosal ulceration, mucosal and submucosal haemorrhages, fusion and shortening villi, chronic inflammation, and fibrosis of the submucosa.

8.8.4 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 pro-

pria 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 been also 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.

8.8.5 Diagnosis

Diagnosis can be done by detection of eggs in the faeces. However, the detection and identification may be difficult because of (i) similarity of the eggs of the heterophyids, (ii) low-laying capacity of the parasite and (iii) 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).

8.9 Other Families of Intestinal Trematodes Infecting Humans

8.9.1 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.

8.9.2 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, 2010; Sayasone et al. 2009b). Metacercariae of both species have been detected in dragonflies and it is thought that people become infected after eating this odonate 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).

8.9.3 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.

8.9.4 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. 8.8a). The life cycle of *N. salmincola* involves the release of non-embryonated eggs in the faeces 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 10 human infections. Patients became infected after eating improperly cooked salmon or trout. The main symptoms were gastrointestinal complaint of abdominal discomfort, diarrhoea, nausea and vomiting. Peripheral blood eosinophilia was also observed.

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

8.9.5 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 diarrhoea (Beaver et al. 1984). In both species, the source of infection is suspected to be the ingestion of aquatic plants.

8.9.6 Family Plagiorchiidae

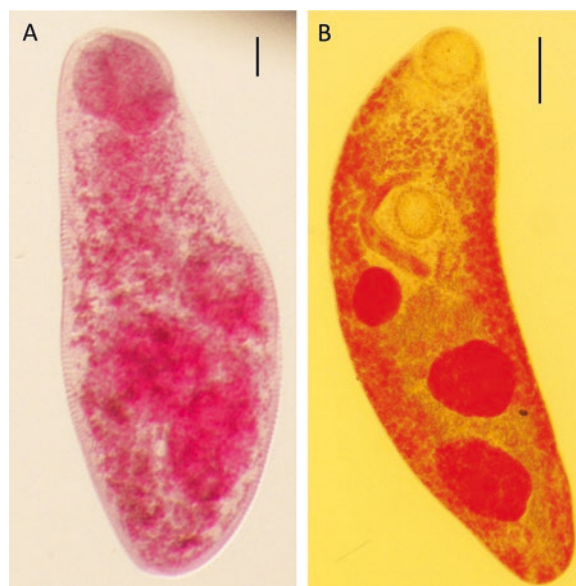
The members of this family are characterized by having a spinose tegument, the ventral sucker in the anterior half of the body, long intestinal caeca, testes opposite, tandem or oblique and located in the hindbody. A cirrus sac is present and the genital pore is anterior to the acetabulum. The ovary is pretesticular and the vitelline follicles are adjacent to the intestinal ceca and are variable in length (Fig. 8.8b). 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 harbouring the cysts.

Occasional human infections with *Plagiorchis harinasutai* (in Thailand), *P. javensis* (in Indonesia), *P. muris* (Fig. 8.8b) (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).

8.9.7 Family Strigeidae

The family Strigeidae includes trematodes with a cup-shaped forebody and a tribocytic organ (an accessory sucker). The hindbody is cylindrical or

Fig. 8.8 Adult worms of (a) *Nanophyetus salmincola* (Scale bar: 100 μ m); and (b) *Plagiorchis muris* (Scale bar: 200 μ m)



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).

8.10 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 Ca^{2+} homeostasis 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 gymnohalloidiasis (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 trematodiasis, 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 trematodiasis 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 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. 2017c).

8.11 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 programme because it can be controlled along with other food-borne diseases for which there are sustained WHO control programmes. 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 faeces from people and other definitive hosts; the treatment or sterilisation of faeces; the control of snail host populations; and the implementation of education campaigns. Therefore, in principle, the prevention and control are relatively simple. Since infection 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 harbour 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 programmes focused par-

ticularly 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 programmes in school-age children, with strong community therapy programmes 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 programmes operating through the essential components of diagnosis, treatment and prevention for the control of human zoonotic trematodiasis have not been successful against several intestinal trematodiasis, although they have been for other trematode diseases.

8.12 Concluding Remarks

Despite the significant public health impact of the intestinal trematodiasis, 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 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 trematocidal 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 trematodiasis 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 be also accompanied by programmes 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.

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