Trans R Soc Trop Med Hyg 2016; **110**: 37–45
doi:10.1093/trstmh/trv099

REVIEW

An update on human echinostomiasis

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Received 14 July 2015; revised 20 August 2015; accepted 2 September 2015

Echinostomiasis, caused by trematodes belonging to the family Echinostomatidae, is an important intestinal foodborne parasitic disease. Humans become infected after ingestion of raw or insufficiently cooked molluscs, fish, crustaceans and amphibians, thus, understanding eating habits is essential to determine the distribution of the disease. Despite the public health impact of echinostomiasis, it has been neglected for years. Traditionally, echinostomiasis has been considered as a minor disease confined to low-income areas, mainly in Asia. However, the geographical boundaries 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 improve our understanding of intestinal trematode infections. Herein, we review the main features of human echinostomiasis in relation to their biology, epidemiology, host-parasite relationships, pathogenicity, clinical aspects, diagnosis, treatment and control.

Keywords: Echinostomatidae, Echinostomiasis, Echinostomes, Foodborne trematodes, Intestinal trematodes, Parasitic diseases

Introduction

Humans suffer from numerous foodborne parasitic zoonoses, many of which are caused by trematodes (phylum Platyhelminthes: Digenea). Echinostomiasis, caused by trematodes belonging to the family Echinostomatidae or echinostomes, is an important intestinal foodborne parasitic disease. Echinostomes constitute a heterogeneous group of intestinal hermaphrodite trematodes that, as adult worms, parasitise numerous vertebrate hosts of all classes, including humans. Humans become infected after ingestion of raw or insufficiently cooked molluscs, fish, crustaceans and amphibians. High incidence of intestinal trematodiasis is strongly associated with populations living near freshwater bodies and the practice of eating raw or undercooked aquatic products. Although human infections are commonly considered as tropical diseases with several endemic foci in Asia, the population at risk and the distribution of the disease is currently expanding and changing in relation to factors such as international markets, improved transportation systems, changes in eating practices in Western countries and demographic changes.[1–5]

Currently, human echinostomiasis is attributed to at least 24 species (Table 1) endemic to Southeast Asia and the Far East. Most of these endemic foci are located in China, India, Indonesia, Korea, Malaysia, the Philippines or Thailand. Moreover, occasional cases have also been reported in other countries. In this paper, we will review the biology, medical and epidemiological features, and current treatment and diagnostic tools of this parasitic disease.

Morphology of echinostomes

Members of the family Echinostomatidae are characterised by the presence of a head collar with collar spines around the oral sucker (Figure 1A). The spines of the cephalic collar may be arranged in one or two circles. Considerable variation exists in the size of echinostomes and may vary from 5 mm to more than 10 mm in length.[6] The tegument contains scale-like spines on both dorsal and ventral surfaces, though the number and size of the spines is reduced in the posterior part of the body. The oral and ventral suckers are close to each other (Figure 1B). The two testes, usually in tandem, are posterior to the ovary. The uterus is intercaecal and normally pre-ovarian. The vitellarium is follicular, in two lateral fields, usually in the hindbody but may extend into the forebody (Figure 1C).[6]

Echinostomes are immersed in a long-ranging controversy regarding their systematic classification, particularly within the genus *Echinostoma*.[6] Recently, the characters traditionally used for identification have been critically revised.[7] Moreover, several attempts have been made to clarify this group based on molecular studies, though further studies are required.[7–15]

Life cycle and sources of human infection

Echinostome adults are hermaphroditic digeneans that live in the intestine and bile ducts of numerous vertebrate hosts, particularly aquatic or semi-aquatic birds and mammals, including humans. Figure 2 shows a generalised scheme of the life cycle

Table 1. Source of infection and geographical distribution of the major species involved in human echinostomiasis (modified from Toledo et al.[5])

Echinostome species	Geographical distribution	Possible source of infection
Acanthoparyphium tyosenense	Japan, Korea	Bivalves
Artyfechinostomum malayanum	China, India, Indonesia, Lao PDR, Malaysia, Philippines, Singapore, Thailand	Snails
Artyfechinostomum mehrai	India	Snails
Artyfechinostomum oraoni	India, Thailand	Snails
Artyfechinostomum sufrartyfex	India	Snails
Echinochasmus fujianensis	China	Not known
Echinochasmus japonicus	China, Japan, Korea, Lao PDR	Not known
Echinochasmus jiufoensis	China	Freshwater fishes
Echinochasmus liliputanus	China, Egypt, Palestina, Syria	Freshwater fishes, Untreated water
Echinochasmus perfoliatus	China, Denmark, Egypt, Hungary, Italy, Japan, Korea, Romania, Russia, Taiwan	Freshwater fishes
Echinoparyphium recurvatum	Egypt, Indonesia, Taiwan	Freshwater snails, tadpoles and frogs
Echinostoma angustitestis	China	Freshwater fishes
Echinostoma cinetorchis	China, Japan, Korea, Taiwan	Freshwater fishes
Echinostoma echinatum	Brazil, Europe, Indonesia, Japan, Lao PDR, Thailand	Mussels
Echinostoma hortense	China, Japan, Korea	Freshwater fishes
Echinostoma ilocanum	Cambodia, China, India, Indonesia, Java, Malaysia, Philippines, Thailand	Snails
Echinostoma macrorchis	Indonesia, Japan, Korea, Lao PDR, Taiwan	Snails and frogs
Echinostoma revolutum	Australia, Cambodia, Egypt, Lao PDR, Russia, Taiwan, Thailand	Snails, tadpoles and clams
Episthmium caninum	India, Thailand	Freshwater fishes
Euparyphium sp.	Lao PDR	Not known
Himasthla muehlensi	Colombia, USA	Clams
Hypoderaeum conoideum	Thailand	Snails and tadpoles
Isthmiophora melis	China, Taiwan, Romania, USA	Tadpoles

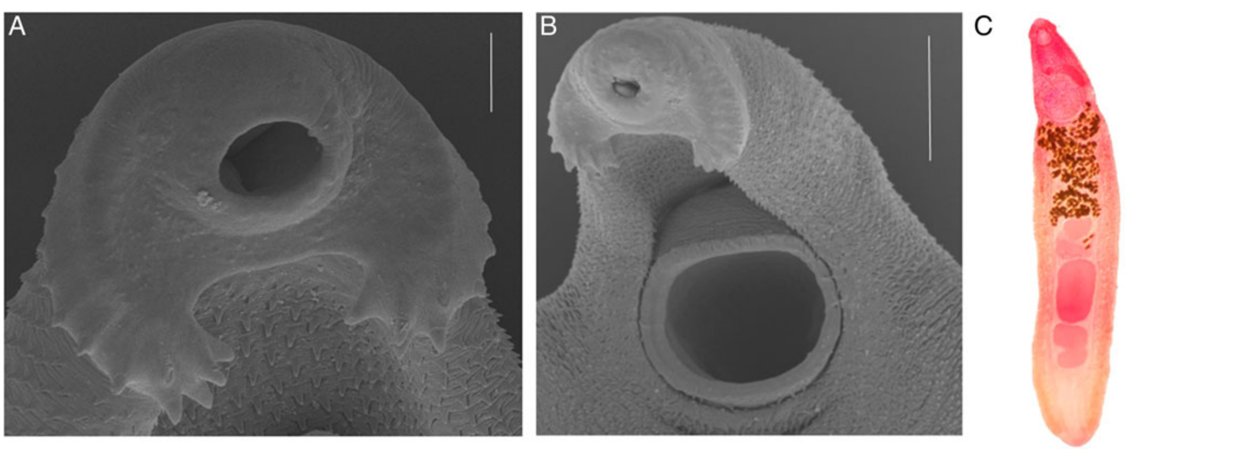

Figure 1. (A) Low-magnification scanning electron micrographs of the cephalic collar of spines of *Echinostoma* sp. adult worm (scale bar: 100 μm). (B) Low-magnification scanning electron micrographs of anterior part of the body *Echinostoma* sp. adult worm (scale bar: 200 μm). (C) General view of adult worm of *Echinoparyphium recurvatum* (scale bar: 1 mm). This figure is available in black and white in print and colour at Transactions online.

of echinostomes. The definitive host releases undeveloped eggs within faeces into ponds, streams and lakes, which take about 2–3 weeks at 22°C to reach the fully developed miracidial stage. Miracidia hatch from eggs and actively locate the first intermediate snail host. Several species of planorbids, lymnaeids and bulinids have been recorded as first intermediate hosts of echinostomes. Miracidia usually enter the head foot region of the snail and transform into sporocysts in the heart. The primary

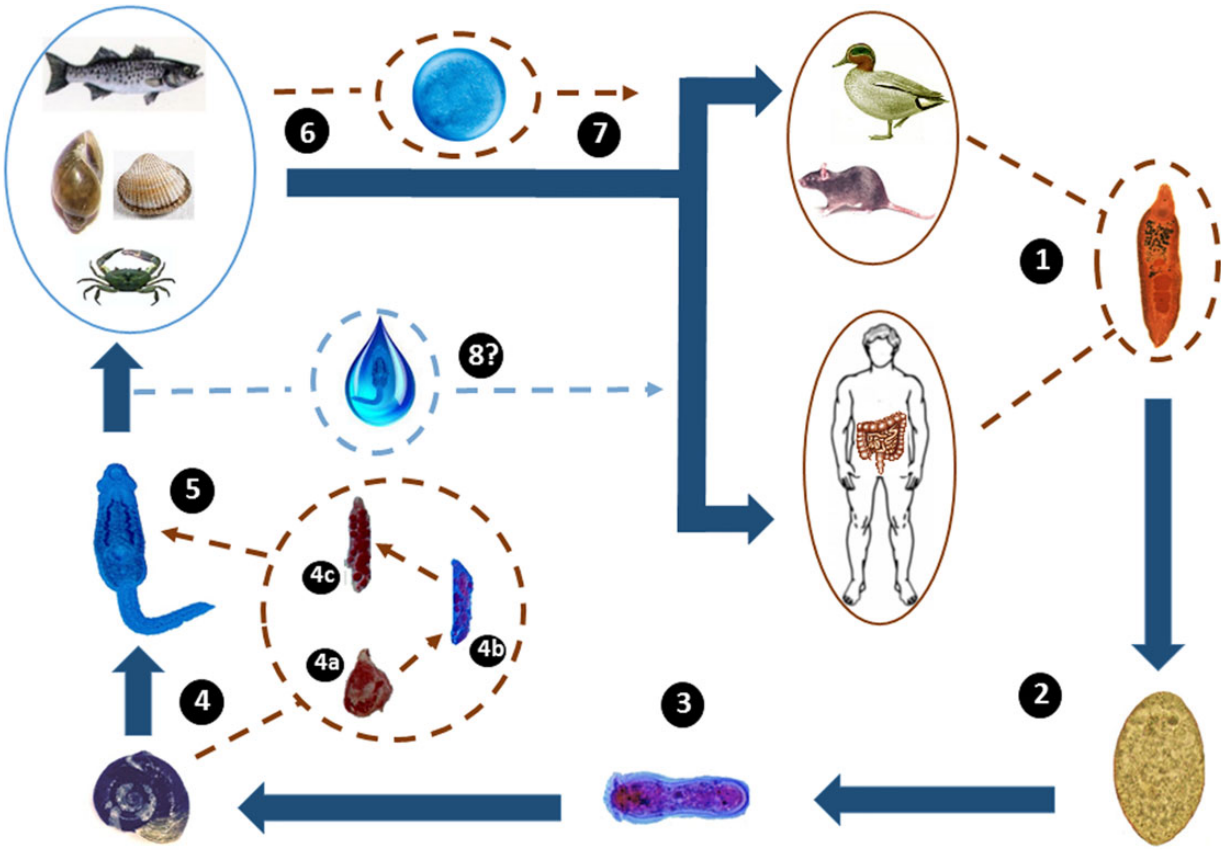

Figure 2. Generalised life cycle of echinostomes. (1) Adult worms inhabiting the small intestine of several vertebrate hosts, including humans; (2) eggs are voided with host faeces; (3) miracidia hatch in freshwater and actively infect the snail first intermediate host; (4) intramolluscan stages, i.e., sporocysts (4a) mother rediae (4b) and daughter rediae (4c) develop within the snail; (5) cerariae are released by the first intermediate host and swim to locate the second intermediate host (snails, amphibians, bivalves, fishes) which they penetrate; (6) cercariae become metacercariae after encystation within the second intermediate host; (7) metacercariae are ingested by the definitive host and excyst to become adults; (8) it has been suggested that drinking water containing cercariae is also a source of human infection.[22,23] This figure is available in black and white in print and in colour at Transactions online.

germinal cells of the sporocyst start to develop into mother rediae. Mother rediae reproduce asexually and produce daughter rediae, which develop in the digestive gland-ovotesis complex of the snail. Cercariae emerge and locate the second intermediate host. Several species of snails, frogs, tapdoles and fish serve as secondary intermediate hosts. Cercariae penetrate the second intermediate host in which they encyst to become metacercariae.

Definitive hosts become infected after ingestion of the second intermediate host harbouring encysted metacercariae. The metacercariae excyst in the duodenum and the juvenile parasites migrate to the small intestine where they attach to the mucosa by the ventral sucker and mature to the adult stage. The release of eggs from the definitive host starts at 10–16 days post-infection.

Humans become infected when they eat raw or undercooked freshwater fish or brackish water molluscs, fishes, snakes, crustaceans and amphibians (tadpoles or frogs).[4,5,16] Table 1 shows the source of infection of the major species of echinostomes infecting humans. Infections are thus most prevalent in areas where traditional cultural practices encourage ingestion of raw or undercooked fish, frogs, snakes, or snails and bivalves, or drinking untreated water. In the Philippines, human echinostome infections have been related to eating raw fish or snails dipped in a salt and vinegar mixture (kinilaw), and other methods of local fish preparations such as tinola (boiled), ginataan (stewed in coconut milk), kukhol and kiambu-ay (raw in coconut milk) or sinugba (charcoal-grilled).[17] In Cambodia, similar types of local food are commonly eaten, which determine the prevalence observed in these areas.[18,19] In the Republic of Korea, local fish dishes include sashimi (sliced raw fish) or sushi (fish roll with rice). In China, it is common to eat raw fish dishes. In Thailand, raw grass carp or raw fish (*lab-pla* and *plasom*) is also consumed.[1,20,21] In Indonesia, human infections appeared to be due to the habit of eating raw or insufficiently cooked lake molluscs, particularly bivalves (*Corbicula* spp.).[1]

Moreover, it has been suggested that drinking untreated water containing *Echinochasmus liliputanus* cercariae may be a source of human infection.[22] It was shown that the infection rate was only 1.5% in the individuals who did not drink untreated water, whereas it was 20.1% among those who drank untreated water.[22] An experimental study was performed to determine the mechanism of human oral infection with cercariae, and the phenomenon of cercarial encystment in the presence of human gastric juice was proposed as a possible mechanism.[23]

Epidemiology and geographical distribution

Transmission of echinostomiasis is limited to areas where both intermediate hosts live together in the same habitat and suitable climatic factors are present.[24] Hence, it is highly focal and strongly determined by dietary habits. Infections are most prevalent in areas such as Asia where traditional cultural practices encourage ingestion of raw or undercooked fish, frogs, snakes, or snails and bivalves, or drinking untreated water. Population growth, pollution, poverty, a lack of adequate sanitation due to declining socioeconomic conditions and man-made modifications have contributed to increased infection rates of foodborne trematode diseases in several parts of the world.[2–5] In contrast, social and economic advances, combined with health education campaigns and mass drug administration, have reduced prevalence in other settings.[21] Currently, it is not known how many people are infected or at risk of acquiring echinostomiasis and further efforts are required. WHO estimated the number of human infections for *Acanthoparyphium tyosenense* (1000 people), *Echinoschasmus japonicus* (5000), *Echinostoma cinetorchis* (1000) and *Echinostoma hortense* (50 000).[25]

It is known that echinostome infections in humans have occurred in human history. Paleoparasitological studies have demonstrated the presence of *Echinostoma* eggs in coprolites from a mummified human body in Brazil about 600 years ago.[26] The eggs could not be identified specifically, though the ITS1 and cox1 sequences showed similarity with *E. paraensei* and *E. luisreyi*. Similarly, eggs of *Echinostoma* spp. were detected in coprolites from humans from Cueva de los Muertos Chiquitos in Durango, Mexico.[27] The coprolites were dated to be approximately 1400-years-old. The first cases of human echinostomiasis in living populations were reported in Manila (the Philippines). Eggs of an echinostome were found in the faeces of five prisoners and adult worms were recovered from one patient after treatment.[28]

The number and identity of the species currently causing human echinostomiasis is uncertain due to the absence of systematic surveys; most of the available information is based on occasional case reports. Furthermore, the problematical taxonomy of the group complicates the situation. The number of echinostome species involved in human infections varies from 20–25 in the most recent reviews.[2–5] Table 1 summarises the species of echinostomes, belonging to 10 genera, infecting humans and their geographical distribution. The highest incidence of echinostomiasis occurs in Asia, mainly in Southeast Asia.

Human infections with *Acanthoparyphium tyosenense* have only been recorded in the Republic of Korea and Japan.[29] A total of 10 cases in Korea were associated with the practice of eating undercooked bivalves and gastropods.[3] Human infections with *Artyfechinostomum malayanum* (under the name *Echinostoma malayanum*) were first found in Malaysia. Thereafter, it has been reported in individuals from Singapore, Thailand, Indonesia, Lao PDR and the Philippines.[3,17,30–33] Several authors have considered *A. sufrartyfex* synonymous with *A. malayanum. A. sufrartyfex* human infections have only been recorded in India and Thailand, though its distribution may be higher due to its confusion with *A. malayanum*. Similarly, human infection with *A. oraoni* has only been described in India. A total of 20 cases were reported in a tribal community near Calcutta.[34,35]

Echinochasmus fujianensis in humans has only been recorded in Hubei and Fujian in China, where prevalence was estimated to range from 1.6 to 7.8%.[36] Human infections with *E. japonicus* have been found in China,[37,38] the Republic of Korea[39] and Lao PDR.[33,40] Prevalence in some counties of Guandong and Fujian in China reached 4.9%.[31] Human infections with *E. liliputanus* were first discovered in Anhui province in China.[41] A prevalence rate of 13.4% among 2426 people studied was reported. Since then, more than 2500 human infections have been reported in this province.[23] Drinking untreated water was suggested to be the cause of the elevated incidence. The prevalence of *Echinochasmus perfoliatus* in Guandong and Fujian (China) was 1.8%.[38]

Echinostoma cinetorchis was first described in Japan[42] and, later, in the Republic of Korea.[43–45] Recently, a new human case was diagnosed by colonoscopy.[46] A high prevalence of *Echinostoma echinatum* was detected from 1937–1956 in Lake Lindu Valley (Sulawesi, Indonesia). The average prevalence was 43%, but it reached 96% in some parts.[47] Human infections appeared to be due to the habit of eating raw or insufficiently cooked lake molluscs, particularly bivalves (*Corbicula* spp.). Recently a prevalence of 6% was found in southern Lao PDR.[48] In China, six patients with hepatitis were found to be infected with *E. hortense* in Liaoning province.[49] An endemicity of *E. hortense* has been reported among residents of Cheongsong-gun(Republic of Korea) with a prevalence of 22.4%.[50] *Echinostoma ilocanum* was first recorded in individuals from the Philippines.[51] The prevalence among the Ilocano population in Northern Luzón was 7–17%. Moreover, this species has been recorded in Indonesia (on the island of Java), China, Thailand and India.[38,52,53] *Echinostoma revolutum* is a cosmopolitan species that commonly parasitises birds and mammals. Although human cases have been described in Africa and Europe, most of the cases occur in Asia. The first human cases were detected in Taiwan and the estimated prevalence in this country varied from 2.8–6.5%.[16,54] In Cambodia, a prevalence of 2.4–7.5% was reported in children from Pursat province.[18] Recently, this species has also been reported in Lao PDR.[33] *Hypoderaeum conoideum* has only been reported in Thailand, where 55% of 254 residents in northern parts of the country were found to be infected.[55]

Apart from those described above, there are a number of species (*Echinochasmus jiufoensis, Echinostoma angustitestis, Echinostoma. macrorchis, Episthmium caninum, Euparyphium* sp. and *Isthmiophora melis*) which have been sporadically reported in people from Asia, mainly from China, Japan, Lao PDR, Taiwan and Thailand, and no further data are known.[38,56–61] Moreover, it should be noted that there are a large number of surveys in which no specific diagnosis was reported. Several cases of humans infected with *Echinostoma* spp. have been described in Cambodia,[62,63] Lao PDR[64] or Thailand.[65]

The number of known reports outside Asia is very limited.[2] A human infection with *I. melis* was detected in 1916 in a diarrhoeic patient in Romania and *E. recurvatum* and *E. revolutum* have been sporadically reported in Egypt and Russia, respectively.[3] Imported cases in the USA also have been reported. *H. muehlensi* was detected in a German patient who lived in Colombia and travelled to New York where he had eaten raw clams.[3] DeGirolami and Kimber[66] recorded *Echinostoma* sp. from Asian refugees in the USA. Poland and colleagues[67] reported 18 cases of imported echinostomiasis among a total of 20 American tourists to Kenya.

Clinical features

The clinical symptoms of human echinostomiasis may be more severe than those produced by other intestinal trematodes, though the clinical features depend on the parasite load.[1] Epigastric and abdominal pain, fatigue, diarrhoea and weight loss are the most common symptoms in human echinostomiasis.[1,68] Moreover, other symptoms can also occur. *E. hortense*-infected patients suffer acid belching, anorexia, headache, nausea and vomiting, and urinary incontinence.[69] Peripheral blood eosinophilia is common but the values may range from 2–24%.[68]

Intestinal pathology induced by echinostomes in humans has been poorly studied and most of the known data were obtained by gastroduodenal endoscopies in patients infected with *E. hortense*. 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.[69] Interestingly, stage IIc or stage III of early gastric cancer was determined by gastroduodenal fiberscopy. After biopsy, three spots were detected and a living worm was observed penetrating into one of the ulcers. Histopathological examination proved the lesion as adenocarcinoma.[70]

Diagnosis

Human echinostomiasis infections may remain unapparent or become symptomatic depending on the worm burden. The symptoms are often unspecific making clinical diagnosis difficult.

Laboratory diagnosis of echinostomiasis is based on the recovery of eggs in faeces (Figure 3B), usually being oval in shape, variable in size (but more than 50 μm), yellow, dark brown or silver-white in colour, with a thin and refractory shell, unembryonated when laid, and with a small, inconspicuous operculum and roughening or slight thickening of their shell at the abopercular end.[71] Although specific diagnosis can be made through careful observations and measurements of the eggs, the recovery and identification of the adult fluke is strongly required for a definitive diagnosis.[3] Nevertheless, the genus *Echinostoma* comprises the largest number of species producing eggs between 77–82 × 52–55 μm for *E. angustitestis* and 115–130×68–80 μm for *E. hortense*.[3] Echinostome eggs are similar to those of fasciolids and even to those of gastrodiscids, making differentiation difficult. The presence of wrinkles or a thickening at the abopercular end of the shell of echinostome eggs may facilitate their differentiation.[71]

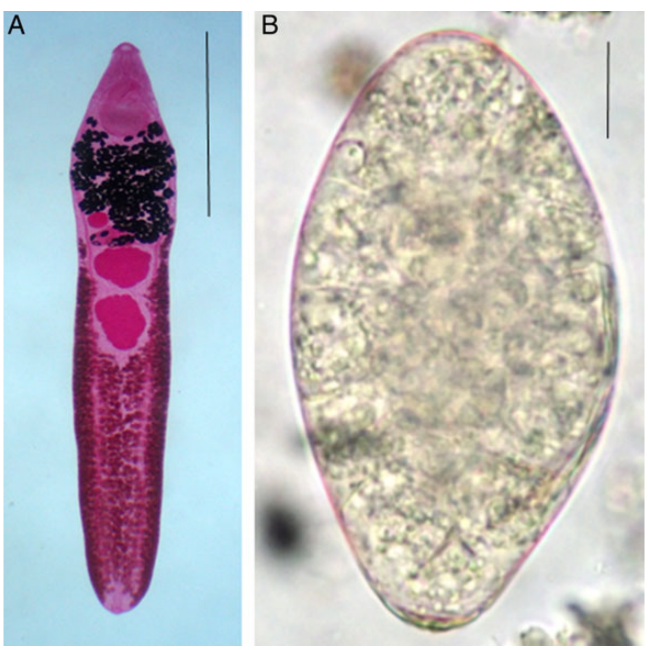

Figure 3. *Echinostoma hortense*. (A) Adult specimen (photomicrograph courtesy of Woon-Mok Sohn) (scale bar: 2 mm), (B) Eggs detected in formalin-preserved human fecal material (scale bar: 20 μm). This figure is available in black and white in print and colour at Transactions online.

Occasionally, human echinostomiasis has been revealed by gastroduodenal endoscopy performed in relation to severe gastrointestinal symptoms or colonoscopy.[46,69,70–74] In Japan, several cases of *E. hortense* infections were reported based on worm recoveries by upper endoscopy. Recently, a case of human echinostomiasis caused by *E. cinetorchis* was detected by colonoscopy. Two adult worms were detected in the ascending colon of a 68-year-old Korean man with complaint of intermittent right lower quadrant abdominal pain.[46]

Immunological and molecular methods for the diagnosis of human echinostomiasis have not been developed.

Treatment

Praziquantel is the drug of choice for echinostomiasis although it is not included in the US product labelling for these infections. Although a single dose of 25 mg/kg of praziquantel is the recommended dose for treatment of intestinal fluke infection, echinostome infections can be treated with a slightly lower, single, oral dose of 10–20 mg/kg praziquantel.[3] Side-effects are minimal and include abdominal pain, nausea, headache and dizziness; moreover, praziquantel is probably safe in pregnancy. The manufacturer stopped making the drug in the United States for human consumption in 2000 because it was not profitable but then resumed production. In this context, options for alternative therapy are limited. Some patients may opt to use a veterinary preparation of praziquantel. Alternatively, mebendazole can be used. In one study, about 50 patients infected with *E. fujianensis* were given table salt over 10 days medicated with either 400 or 800 mg of mebendazole. The cure rates were 71% and 85%, respectively, with egg reduction rates of 71–85%.[75] Albendazole has been reported to show an effect against infections with echinostomes, but no details with regard to dose or cure rates have been reported.[3]

The reduced number of available drugs has encouraged the search for alternative treatments. Several drugs were examined for their echinostomicidal effect several decades ago in vitro, such as brotianide, niclofolan, nitroxynil, oxyclozanide, rafoxanide, hilomid, niclosamide, hexachlorofen, bithionol and hetol.[76] In recent years, several compounds have been tested using *Echinostoma caproni* as a model. In one study, the activity of praziquantel, artemisinin, artemether, artemether, artesunate and dihydroartemsinin was analysed in vitro and all drugs showed activities against adult *E. caproni*. Praziquantel was the fastest acting compound and, among artemisinins, dihydroartemisinin showed the promptest in vitro activity.[77] Artemether and artesunate have also been screened in hamsters, showing significant reductions in worm burdens.[78] Due to the problems that artemisinins have in relation to their bioavailability, preparation and pharmacokinetics, many synthetic peroxide analogues have been investigated. Ozonide OZ78 has been shown to be an effective echinostomicidal in the rodent model.[79] Several studies assessed the echinostomicidal effect of benzimidazoles by inhibiting the secretion of glycoconjugates that protect the surface of *E. caproni*.[80] Multiple treatment courses with mebendazole achieved complete worm burden reductions. Single doses of clorsulon 100 mg/kg and rafoxanide 50 mg/kg eliminated 100% of worms in mice within one day.[81] Finally, recent studies revealed high worm burden reductions when the Chinese anthelminthic drug tribendimidine and artemisinins were administered as single oral doses to *E. caproni* infected mice.[77,79] More recently, two dixolanes showed in vitro activity against *E. caproni* down to concentrations of 5 mg/L, but none of the compounds revealed in vivo activity.[82]

The increasing interest in medicinal plants as new sources of antiparasitic drugs has led to the study of several extracts as flukicides. Ferreira and colleagues[83] showed that ethanolic extracts of *Artemisia annua, A. absinthium* and *Asimina triloba* kill *E. caproni*. Mangostin and mangostin diacetate, isolated from *Garcinia mangostana*, showed promising activities (IC_{50} of 2.9–15.6 μg/ml) against *E. caproni*. Single oral doses (400 mg/kg and 800 mg/kg) of the drugs achieved worm burden reductions ranging from 11–54% in vivo.[84] Active extracts from 50 medicinal plants used in Cote d'Ivoire were evaluated and several of them revealed activity in vivo against *E. caproni* in mice.[85]

Control

The control of echinostomiasis should be focused on a reduction or elimination of the transmission of the disease. The control measures in endemic regions can include a reduction of the sources of infection through effective treatment; the protection of fish ponds and aquaculture systems from contamination with faeces from definitive hosts; the treatment or sterilisation of faeces; the control of snail host populations; and the implementation of education campaigns. Since infection of the definitive host is only contracted through the ingestion of metaceracariae harboured by the second intermediate hosts, the most practical measure for preventing and controlling human infection is to eliminate the consumption of raw, undercooked or freshly pickled fish and shrimp flesh. In Indonesia, ecological changes due to the introduction of *Tilapia mossambica,* which led to the elimination of the *Corbicula* succeeded in reducing the prevalence of *E. echinatum*.[47]

There is evidence that various types of marinades and food preparations commonly used may not affect the viability of echinostome metacercaria. In a local market in Thailand, it was found that freshly killed freshwater fish (*Cyclocheilichthys armatus*) were infected with large numbers of echinostome metacercariae. These infected fish were used to evaluate the effect of traditional food preparations on the viability of the metacercariae using the following parameters: 1) left to dry at room temperature; 2) frozen; 3) refrigerated; 4) marinated in saline; and 5) marinated in 5% acetic acid solution. Degeneration of the metacercariae was slowed by cooling: degeneration of all metacercariae took approximately 10 hours in the refrigerated or frozen fish, compared with 4 hours in all other dishes left at room temperature and in the marinades.[86]

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 salt 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 cysts in snail tissues.[87]

In order to control the transmission of echinostomaiasis, analysis of foods for encysted metacercariae can be an adequate alternative. Examination of echinostome metacercariae in the second intermediate host, mainly in fish, can be performed by two methods, i.e. muscle compression and pepsin-HCl artificial digestion.[88] In the compression technique a sample of flesh from different parts of the fish (e.g. head, gill, muscle, fin, scale, intestine, other viscera) or any other host is compressed between two glass slides and examined under the stereomicroscope with 20- to 100-fold magnification. The artificial digestion method is more complicated. Small pieces of flesh are minced and mixed into a beaker with artificial gastric juice (conc. HCl 8 ml + pepsin 1:10 000 6 g + distilled water 1000 ml) and incubated at 37°C for 2 h with occasional stirring. After removal of the larger particles by filtration (1×1 mm of mesh), 0.85% saline is added and left to stand. The supernatant is then discarded and the sediment kept. This procedure is repeated until the supernantant becomes clear. Finally, the sediment is analysed in a Petri dish containing physiological saline under the stereomicroscope.

A decreasing pattern in some foodborne trematode diseases along with industrialisation, health education and alteration of the environment has been observed in certain areas of Southeast Asia. However, the prevalence of echinostomiasis has not changed, due to several reasons, such as expansion of aquaculture for production of freshwater fish and crustaceans, improved transportation of these products to local and international markets, and the existence of extensive zoonotic reservoirs of echinostomes.[1,21] Elimination of *Corbicula* clams from lake Lindoe disrupted the human life-cycle, though worm extinction did not occur as the sylvatic cycle was not affected. [47]

Concluding remarks

Although it is common to find people infected with echinostomes in surveys conducted in rural areas of Southeast Asia, echinostomiasis is among the most neglected intestinal trematode infections. Human echinostomiasis has been considered as a minor disease mainly confined to some areas of Asia and, in fact, it is not known the number of people infected or living at risk of infection. Further efforts to determine the real impact of human echinostomiasis are necessary and a more detailed follow-up of the disease is also required. Moreover, and as occurs with other foodborne trematodiases, the expansion of populations at risk cannot be discarded. Several factors such as growing international markets, new eating habits in developed countries or demographic changes are expanding the risk of infection with foodborne trematodes to areas where these diseases were not previously known. In this context, further studies are required to elaborate new maps of risk and a more detailed follow-up could be useful to gain a better understanding of the current incidence of the disease. Moreover, additional efforts to raise awareness of the risks of certain eating habits should be carried out.

Authors' contributions: RT and JGE conceived, designed, drafted, wrote and revised the manuscript. Both authors read and approved the final manuscript. RT is the guarantor of the paper.

Funding: This work was supported by the Projects BFU2013-41040-P from Ministerio de Economía y Competitividad (Madrid, Spain), PROMETEO2014-083 Fase II from Conselleria d'Educació, Generalitat Valenciana (Valencia, Spain), and No. RD12/0018/0013, Red de Investigación Cooperativa en Enfermedades Tropicales–RICET, IV National Program of I+D+I 2008–2011, ISCIII–Subdirección General de Redes y Centros de Investigación Cooperativa and FEDER from the Ministerio de Sanidad y Consumo (Madrid, Spain).

Competing interests: None declared.

Ethical approval: Not required.

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