Immunology and pathology of echinostomes and other intestinal trematodes

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Contents

Advances in Parasitology
ISSN 0065-308X, https://doi.org/10.1016/bs.apar.2024.02.002

Abstract

Intestinal trematodes constitute a major group of helminths that parasitize humans and animals with relevant morbidity and mortality. Despite the importance of the intestinal trematodes in medical and veterinary sciences, immunology and pathology of these helminth infections have been neglected for years. Apart from the work focused on the members of the family Echnistomatidae, there are only very isolated and sporadic studies on the representatives of other families of digeneans, which makes a compilation of all these studies necessary. In the present review, the most salient literature on the immunology and pathology of intestinal trematodes in their definitive hosts in examined. Emphasis will be placed on members of the echinostomatidae family, since it is the group in which the most work has been carried out. However, we also review the information on selected species of the families Brachylaimidae, Diplostomidae, Gymnophallidae, and Heterophyidae. For most of these families, coverage is considered under the following headings: (i) Background; (ii) Pathology of the infection; (iii) Immunology of the infection; and (iv) Human infections.

1. Introduction

Most studies on the immunology and pathology of intestinal helminth infections have been carried out using nematodes as experimental models. Despite the intestinal trematodes are the commonest parasitic infections in humans and animals, these infections have been neglected and little studied, both in the field and in laboratory conditions. It is estimated that more than one billion people are at risk of infection (Fürst et al., 2012). Almost 100 species belonging to 14 families have been recorded infecting humans (Table 1) (Toledo et al., 2019). Moreover, the impact of intestinal trematodes in wildlife is significant and these parasites play an important role as causative agents of disease and even death in wildfowl and other animals. Intestinal trematode infections have been often correlated with death of animals in the wild (Roscoe and Huffman, 1982, 1983; Bolek et al., 2019; Toledo et al., 2006a).

Apart from the interest of intestinal trematodes in medical and veterinary sciences, several trematodes may serve as experimental models for the study of the host-parasite relationships, with emphasis on the immunology

Table 1 Families of intestinal trematodes reported in intestinal humans and the number of species involved, sources of infection and geographical distribution.

Family	Number of species cited in humans	Source of infection	Geopgraphical distribution of human cases
Brachylaimidae	1	Terrestrial snails	Oceania
Cathaemaciidae	1	Not known	Asia
Diplostomidae	1	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	30	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

and pathology of intestinal infections. The use of these helminths as experimental models may serve to new studies to gain further insight in the responses that determine the susceptibility or the resistance to intestinal helminth infections (Toledo and Fried, 2005).

The purpose of this review is to examine the significant literature on pathological and immunological effects of intestinal trematodes in their definitive hosts. Herein, we update the data from a previous review (Toledo et al., 2006a). The main goal of our review is to acquaint and update readers on the published information on these topics in recent years.

For this reason, most of the review is focused on the representatives of the Echinostomatidae family and, more specifically, on one species, *Echinostoma caproni*. Most of the studies published in the last 20 years have been carried out using this species of echinostomatid as an experimental model. These studies have allowed us to deepen our knowledge of the regulation of the immune response against intestinal helminths and the determining factors of susceptibility or, on the contrary, resistance to these parasites.

2. Family Echinostomatidae

2.1 Background

The family Echinostomatidae includes digeneans characterized by the presence of a prominent cephalic collar of spines. 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 (Fig. 1).

Echinostomatidae is a heterogeneous group with a long history of controversy in relation to its systematics and taxonomy (Kostadinova and Gibson, 2000; Kostadinova, 2005; Esteban and Muñoz-Antolí, 2009). Adult echinostomatids are predominantly found in birds, but also parasitize mammals, including humans, and occasionally reptiles and fishes. Members

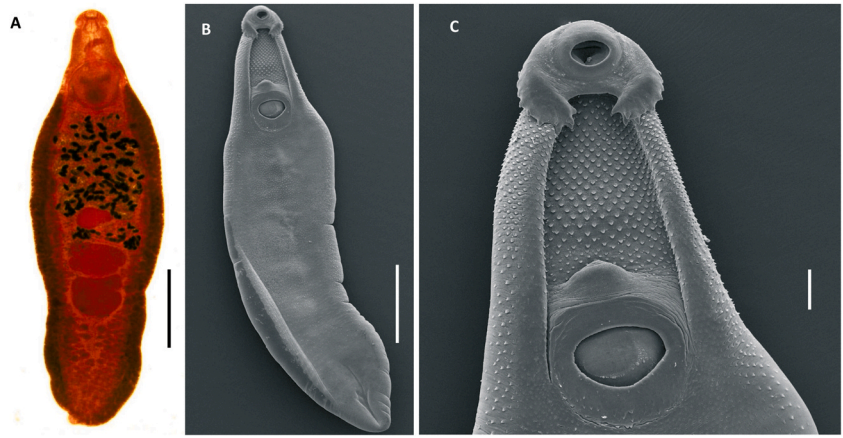

Fig. 1 Adult worm of *Echinostoma caproni* from an experimental infected mouse at 28 days post-infection: (A) Stained with Grenacher's Borax Carmine; (B) Low-Magnification scanning electron microphotograph; (C) Low-Magnification sacaning electron micro-photograph of the forebody. Scale bars: 200 μm.

of the Echinostomatidae follow a 3-host life cycle. The first intermediate hosts are aquatic snails in which a sporocyst, two generations of rediae and cercariae develop. Emerged cercariae infect the second intermediate host, which may be several species of snails, clams, frogs and even fishes. The definitive host becomes infected after ingestion of the second intermediate host harboring the encysted metacercariae (Toledo et al., 2006a, 2009). Human echinostomiasis is endemic in South-east Asia and the Far East, but human infections have been reported worldwide. There are about 23 species belonging to eight genera of Echinostomatidae involved in human disease (Toledo and Esteban, 2016; Toledo et al., 2019).

Immunology and pathology of echinostome infections have been reviewed in several papers (Toledo et al., 2006a; Toledo, 2009; Toledo et al., 2009). In the present review we will examine the most salient literature published after those revisions. Most of the studies have been performed using *Echinostoma caproni* as an experimental model (Toledo and Fried, 2005). We are going to focus the present review on this species, although we will comment on aspects of other species of echinostomatids when required.

2.2 Host parasite relationships

Immunology and pathology of echinostome infections have been extensively studied in laboratory rodents. Rodents are able to express various types of resistance against echinostomes. Hosts of high compatibility or susceptible hosts, such as mice or hamsters, are characterized by a high worm establishment and growth, elevated parasite egg output and fecundity and, moreover, the infection becomes chronic in these hosts. In contrast, the infections in hosts of low compatibility, such as rats, is characterized by lower values in all these biological features and a rapid rejection of the worms (Toledo and Fried, 2005; Toledo, 2009; Toledo et al., 2009). Infection in hosts of high compatibility is characterized by a high worm establishment and egg output and a long survival of the worms. In contrast, rat as a low compatible or resistant host is able to rapidly reject the parasites (Hansen et al., 1991; Toledo et al., 2004a; Trelis et al., 2011; Sotillo et al., 2014). Comparative studies on the development of infection in each type of definitive host have contributed to understanding the determining factors of the expulsion of parasites or the development of chronic infections.

Furthermore, it has been shown that primary infection primary infection with the echinostome *E. caproni* in ICR mice induces acquired

immunity against subsequent homologous infection which is manifested in reduced infection rate, worm recovery and growth (Muñoz-Antoli et al., 2016a, b), allowing comparisons between each condition in the same host. This fact has converted the *E. caproni*/ICR mouse system an excellent model for the study of the mechanisms generating acquired immunity against intestinal helminths.

2.3 Regulation of the immune response in echinostome infections

Echinostoma/rodent, especially *E. caproni*, model constitutes an excellent model to analyze the regulation of the immune response in intestinal helminth infections since resistance or susceptibility are each associated with a different process of the regulation of the immune response:

i. Hosts of high compatibility or susceptible are characterized by the development of chronic infections with high worm establishment and growth, elevated parasite egg output and fecundity in relation to a Th1 response. In contrast, the infections in hosts of low compatibility or resistant to infection is characterized by lower values in all these biological features and a rapid rejection of the worms in association with a Th2/Th17 immune response (Toledo and Fried, 2005; Muñoz-Antoli et al., 2007; Toledo, 2009; Toledo et al., 2004a, 2006a,b; Sotillo et al., 2011; Trelis et al., 2011).

ii. Unlike other intestinal helminths, *E. caproni* primary infections in ICR mice induce a local Th1 response with increased expression of IFN-γ in association with the development of chronic infections. However, primary infection induces partial resistance against subsequent homologous infections in mice associated to a Th2 response with elevated levels of IL-13 (Muñoz-Antoli et al., 2016a).

2.3.1 Immune response to echinostome infections

Resistance to intestinal helminths is based on the generation of Th2 responses in a complex process that involves the interaction between innate and adaptative mechanisms (Grencis, 2015; Cortés et al., 2017a; Harris, 2017). Protective Th2 immunity against intestinal helminths is initiated and amplified by the epithelial-derived alarmin cytokines including IL-25, IL-33 and thymic stromal lymphopoietin (TSLP), though the immune mechanisms behind the development of these responses are poorly understood (Cortés et al., 2017a; Smith et al., 2018).

In the case of echinostome infections, resistance or susceptibility appears to be strongly associated with the process of Th differentiation in response

to the infection (Toledo and Fried, 2005). The establishment of chronic *E. caproni* infections in hosts of high compatibility such as mice is dependent upon a local Th1 response with elevated production of IFN-γ (Trelis et al., 2011). In contrast, the resistance to infection in hosts of low compatibility is associated with the development of a local Th2 phenotype with high levels of IL-13 and IL-5, (Brunet et al., 2000; Sotillo et al., 2011; Trelis et al., 2011). Brunet et al. (2000) showed that cultured mouse splenocytes failed to respond to *E. caproni* antigens and mesenteric lymph nodes (MLN) produced IFN-γ and to a lesser extent IL-4 and IL-5. Moreover, the worm burden was reduced in mice infected with anti- IFN-γ antibodies, suggesting that this cytokine is essential for the development of chronic infections. In the related species *Isthmiophora hortensis* (designated as *Echinostoma hortense* in the original studies), the rejection of worms appeared to be related with the development of a Th2 phenotype with increase in the expression of IL-4 and IL-5 in cultured spleen cells after antigenic stimulation (Cho et al., 2007; Ryang et al., 2007).

At the local level, Trelis et al. (2011) studied in vivo cytokine profile in response to *E. caproni* infection through the analysis of mRNA expression of a large group of cytokines primarily involved in both Th1- and Th2-type responses. The early rejection of *E. caproni* adults in the host of low compatibility was associated with augmented expression of Th2-type cytokines, basically IL-13 and IL-4 at Peyer's patches and MLNs, whereas in the intestine host protection was related to marked increases of IL-13, IL-5 and IL-6. Sotillo et al. (2011) reported that protection against *E. caproni* was mediated by the expansion of Th2 lineage, concomitantly with Th17 lineage at the expense of Th1. Moreover, up-regulation of TGF-β mRNA was noticed in Peyer's patches and, especially, in the intestinal tissue. This fact, together with previously reported IL-10 up-regulation in rats (Trelis et al., 2011), suggests an important role of the Treg lineages in the generation of these responses. Therefore, *E. caproni* infection in rats induces an early biased Th17/Th2 response that results in the subsequent worm expulsion.

The fact that susceptibility of mice to *E. caproni* primary infections is due to their inability to respond with IL-25 leading to the development of a Th1 phenotype was confirmed by studies of challenge infections. Primary infection of mice with *E. caproni* does not elicit IL-25 upregulation. However, pharmacological curation of the primary infection induced a marked overexpression of IL-25. Due to these elevated levels of IL-25, mice became resistant to a challenge infection at 2 wppt (weeks post praziquantel treatment), concomitantly with the development of a robust

Th2 response (Muñoz-Antoli et al., 2016a,b; Álvarez-Izquierdo et al., 2020a). In resistant secondary infections against *E. caproni* there were an expansion of the populations of tuft cells and GATA3+ cells. The effector mechanisms activated in the resistance response against *E. caproni* appeared to be dependent on the expansion of the tuft cells, thereby promoting the overexpression of IL-25 and activation of group 2 innate lymphoid cells (ILC2) and Th2 cells. In an environment of helminth infection, IL-25 acts as a mediator of the activation of ILC2s promoting the polarization of the immune response towards a Th2 phenotype with elevated levels of IL-4 and IL-13 (Muñoz-Antoli et al., 2016a; Álvarez-Izquierdo et al., 2020a).

The crucial role of tuft cells and IL-25 in the generation of Th2 responses in *E. caproni* infections was confirmed by Muñoz-Antoli et al. (2016a), Treatment of mice with rIL-25 resulted in complete resistance against the primary infections and all rIL-25-treated mice were refractory to infection. Exogenous IL-25 induced tuft cell expansion together with a strongly polarized Th2 environment with elevated levels of IL-13. It is well known that IL-25 inhibits Th1 differentiation facilitating Th2 differentiation and protecting against intestinal helminth infections (Muñoz-Antoli et al., 2016a). In the absence of IL-25, the early production of both subunits of IL-12 (IL-12p35 and IL-12p40) may be essential for the determination of the susceptibility to infection. IL-12 (or IL-12p70) is a key pro-inflammatory cytokine that promotes the differentiation toward a Th1 phenotype and stimulates IFN-γ production inhibiting the expression of IL-13 leading to susceptibility (Muñoz-Antoli et al., 2016a).

2.3.2 The role of IL-25 in resistance to echinostome infections

Traditionally, the role of IL-25 in resistance against intestinal helminth infections has been exclusively attributed to its immune regulatory activity. It was accepted that IL-25 promotes Th2 immunity with production of IL-4 and/or IL-13 which, in turn, induce STAT6-mediated intestinal alterations determining parasite rejection (Fallon et al., 2006; Owyang et al., 2006; Zhao et al., 2010; Angkasekwinai et al., 2013, 2017). Upon helminth establishment, intestinal tuft cell populations expand and non-specific release of IL-25 that activate a variety of immune cells to initiate type 2 responses and promote Th2-cell-mediated immunity. In response to IL-25 and other alarmins, ILC2 produce large amounts of IL-13 that polarize naïve CD4+ T cells into Th2. Antigen-presenting cells, such as basophils and dendritic cells, are also activated and induce Th2 polarization through different mechanisms (Gerbe et al., 2016; von Moltke et al., 2016;

Cortés et al., 2017a). However, studies with the *E. caproni*/mice model have suggested that the regulatory role of IL-25 may be secondary and this cytokine operates autonomously from Th2 response in the generation of resistance against *E. caproni* (Álvarez-Izquierdo et al., 2020a).

It was observed that the involvement of IL-25 in intestinal helminth infections could be more complex than previously expected. Commonly, it was accepted that the role of IL-25 in resistance against intestinal helminths was due to its regulatory ability leading the response to Th2 and associated with IL-4-independent mechanisms and based on IL-13 activity and STAT6 activation. IL-4 and IL-13 share a common receptor, the IL-4R chain, but IL-13 also uses IL-13Rα1 for signaling via JAK1 and JAK2. IL-13 binds 13Rα1 which complexes with IL-4Rα to form the type 1 receptor signaling, but IL-13 also binds the cell surface and soluble forms of the monomeric type 2 receptor (IL-13Rαs2 chain). However, IL-13Rαs2 has a decoy effect, lacking signal transduction machinery and limiting the activity of IL-13 since binds the cytokine making it unavailable for activating type 1 receptor (Andrews et al., 2006; McCormick and Heller, 2015; Giuffrida et al., 2019; Bieber, 2020).

Álvarez-Izquierdo et al. (2020a) showed that:

i. IL-25 is not required for the development of Th2 responses in secondary infections and the differentiation toward this phenotype could be related to a memory response. Blocking of IL-25 reverted resistance to *E. caproni* challenge infection in mice despite the development of a Th2 phenotype;

ii. despite the development of a type 2 response, mice are susceptible to secondary infection in absence of IL-25, indicating that IL-25 acts autonomously from Th2 response in the parasite clearance. Blocking of the IL-13 receptors induced a significant reduction of STAT6 phosphorylation, though both control animals and those with the blocked IL-13 receptors were refractory to primary infection if they were treated with exogenous IL-25. Furthermore, Treatment of mice with rIL-4 or rIL-13 did not provide of resistance to a primary *E. caproni* infection in relation to the lack of IL-25, despite the development of a Th2 response.

2.3.3 Origin of the IL-25 in echinostome infections

It is well established that IL-25 is produces by the intestinal tuft cells in response to the damage induced by a helminth (Gerbe et al., 2016; von Moltke et al., 2016; Cortés et al., 2017a). However, a striking feature of

E. caproni infections in mice is that tuft cell hyperplasia and the subsequent IL-25 overexpression exclusively occurs as a consequence of the healing of the infection. Howitt et al. (2016) suggested that IL-25 upregulation in intestinal helminth infection is initiated during colonization by the recognition of parasite compounds by tuft cells via taste chemosensory pathways. To this purpose, tuft cells possess multiple taste-chemosensory G protein coupled receptors and many of them require the G protein subunit gustducin and the transient receptor potential cation channel subfamily M member 5 (TRMP5) to transduce the signals (Huang et al., 2015). The lack of IL-25 expression in both *E. caproni* primary and challenge infections suggests that parasite components do not activate taste chemosensory pathways in tuft cells of mice which explain the susceptibility to both type of infections. A striking fact observed in infections of mice with *E. caproni* was that the primary infection induced hyperplasia of tuft cells, but this was not accompanied by the production of IL-25. This cytokine only was produced as a consequence pharmacological curation of the primary infections and before secondary infection, suggesting that IL-25 production and, consequently, mechanisms of resistance to infection are initiated by parasite-independent signals (Muñoz-Antoli et al., 2016a; Álvarez-Izquierdo et al., 2022).

Álvarez-Izquierdo et al. (2022) showed that treatment of mice with a cocktail of antibiotics abrogated the IL-25 response after the curation of the primary *E. caproni* infection concomitantly with a decrease in bacterial abundance in feces and susceptibility to challenge infection, suggesting that resident microbiota could be involved in the of IL-25. These authors detected important changes in the resident microbiota of mice in relation to the primary *E. caproni* infection, pharmacological healing and the subsequent challenge infection. Both primary and challenge infection depleted the presence of members of Verrucomicrobia that only appeared in response to the curation of the primary infection, in parallel with the upregulation of IL-25 and the subsequent resistance to infection.

2.4 Effector mechanisms

Although the relationship between IL-25 and resistance to echinostome infections seems well established, the effector mechanisms through which the parasites are rejected are not well determined and, possibly, expulsion is the result of a complex interaction of different mechanisms.

2.4.1 Antibody responses

It is well known that echinostomes elicit robust antibody responses in their hosts (Toledo et al., 2006a). However, they do not appear to be determinant in the course of the infection.

Agger et al. (1993) analyzed the kinetics of IgM, IgA and IgG in the serum and intestinal wall of *E. caproni*-infected mice. Elevated levels of IgM, IgA and IgG were detected in the serum and intestinal wall, and only an increased level of IgA was detected in the intestinal luminal content. Regarding the IgG subclasses, Brunet et al. (2000) analyzed the profiles of IgG1 and IgG2a in the serum of mice experimentally infected with *E. caproni* and a weak increase of IgG2a was noted.

Sotillo et al. (2007) showed that both host species, mice and rats, developed specific antibody responses against *E. caproni*. Moreover, the absence of detectable levels of albumin in the intestinal fluids of infected rats and mice indicated that antibodies detected in these samples were produced locally. In general, mice developed earlier and more intense reactions than rats in both serum and intestinal samples.

The early response in serum was of the IgM class in both host species, showing the maximum IgM values at 1 and 2 weeks post-infection in rats and mice, respectively. This agreed with the study of Agger et al. (1993) in which a significant increase of IgM levels was detected at 2 weeks post-infection in *E. caproni*-infected mice. The rapid and intense IgM response, together with the low levels of IgG detected in rats, suggested the presence of thymus independent antigens in *E. caproni* adult worms. This was supported by the intense IgG3 response observed in mice. Responses mediated by IgG3 are related to carbohydrate epitopes (Sotillo et al., 2007, 2008, 2014). Considering that *E. caproni* infections in mice are characterized by a high expression of IFN-γ, the intense IgG3 response observed in mice could be related to carbohydrate antigens released from adult worms and adsorbed throughout the intestinal mucosa (Andresen et al., 1989; Toledo et al., 2005, 2006b; Sotillo et al., 2007; Trelis et al., 2011).

In this sense, Sotillo et al. (2014) analyzed the effect of glycosylation in *E. caproni* antigens in antibody responses against the parasite in experimentally infected mice. IgG subclass responses generated in mice against *E. caproni* were essentially due to glycoproteins, being the IgG1 the most affected subclass. The authors suggested that these features could be implicated in the Th1/Th2 biasing. The reactivity significantly decreased after any of the deglycosylation treatments and the N-glycans appears to be of greater importance than O-glycans. Interestingly, the IgM response

increased after N-deglycosylation suggesting that carbohydrates may mask peptide antigens.

Although IgM can be produced locally, intestinal responses were slower and less intense than those in the serum. Agger et al. (1993) did not detect IgM in the intestinal content of *E. caproni*-infected mice at 4 weeks post-infection. The late rise of the intestinal IgM observed in the present study might be an explanation of the negative results obtained by Agger et al. (1993). However, the lack of intestinal IgM responses in rats and the earlier worm rejection in this host indicate that the role of local IgM response in parasite clearance could be considered only as secondary and other effector mechanisms should be implicated.

The serum IgG response was markedly greater in mice than in rats, probably in relation to a greater intestinal antigen uptake in relation to a higher local inflammation. Sotillo et al. (2007) found significant total IgG responses concurrently with high circulating antigen levels in mice. In contrast, the infection in rats were characterized by a weak systemic IgG response and low levels of circulating *E. caproni* antigens. The presence of circulating *E. caproni* antigens has been associated with the intestinal inflammatory responses induced by the parasite (Toledo et al., 2004b, 2006a,b). The results obtained by Sotillo et al. (2007) showed a markedly greater local inflammation response in mice than in rats, and this could be responsible for the greater IgG systemic response observed in mice. Under inflammatory conditions, the maintenance of the epithelial barrier is disrupted resulting in increased passage of worm antigens through the intestinal mucosa. Toledo et al. (2006b) demonstrated in an immunohistochemical study a greater passage of *E. caproni* antigens through the intestinal mucosa of hamsters (susceptible host) than in rats (resistant host) concomitantly with a higher local inflammation. Thus, the higher IgG systemic appeared to be related to the higher local inflammation observed in this host species.

The kinetics of serum IgG1 and IgG2a were also different between both host species. The results on IgG subclasses obtained suggest a markedly biased response toward Th2 phenotype in mice and characterized by a strong production of IgG1 from 3 weeks post-infection. In rats, the responses were markedly weaker. However, an initial dominance of IgG2a response was observed. Moreover, slight increase of IgG1 was detected from 7 weeks post-infection suggesting that early expulsion of *E. caproni* could be associated with balanced Th1/Th2 responses. Brunet et al. (2000)

analyzed the IgG1 and IgG2a profiles in the serum of *E. caproni*-infected mice and found a weak dominance of IgG2a from 3 weeks post-infection.

The IgG1 and IgG2a intestinal responses in mice and rats were slower and of lesser intensity than in serum. In mice, increases in both IgG1 and IgG2a subclasses were observed which could reflect a balanced Th1/Th2 local response. In contrast, rats only developed IgG2a responses, which suggests that local cellular responses could be of importance in the parasite expulsion. Interestingly, the worm expulsion coincides with the pike of IgG2a observed in rats at 7–8 weeks post-infection (Sotillo et al., 2007; Trelis et al., 2011).

Sotillo et al. (2008) confirmed the antigenicity nature of the excretory/secretory products (ESP) of *E. caproni* using an immunoproteomic approach to identify the *E. caproni* major antigenic proteins recognized by IgM, IgA, total IgG, IgG1 and/or IgG2a. A total of four proteins (enolase, 70 kDa heat-shock protein (HSP-70), actin and aldolase) were accurately identified. Enolases appear to be the most antigenic proteins in ESP of *E. caproni*. A total of 8 spots were identified as enolase. Strikingly, one of the enolase isoforms was highly glycosylated. Strikingly, the glycosylation of enolase also affected the antibody response against *E. caproni*. The non-glycosylated enolase was recognized by IgG, IgG/IgG1 and IgA, suggesting that these isoforms could be involved in the responses during chronic phases of the infection. In contrast, the glycosylated form of enolase only generated IgM responses, confirming that the glycosylation processes can be responsible for the early IgM responses against *E. caproni* observed. The authors suggested that glycosylated forms of *E. caproni* enolase may be involved in the early thymus-independent responses against the parasite. The early IgM responses were also generated by actin and the 70 kDa heat-shock protein which suggested that these proteins were exposed early to the host and may be of importance in parasite establishment. The IgA responses also appeared to be mediated by the 70 kDa heat-shock protein and aldolase which could be related to close contact of these proteins with the host mucosal surface (Sotillo et al., 2008).

Despite the strong antibody responses generated by echinostome infections, their role in the course on the infections is unclear. Antibody responses appear to be simply a collateral consequence of the inflammation and damage caused by the parasite in the intestinal mucosa. Weaker antibody responses and higher seroantigen levels were observed in those host species in which the parasite was rejected earlier and associated with lesser intestinal damage (Toledo et al., 2004b, 2005). Toledo et al. (2004b, 2005)

associated the different levels of *E. caproni* seroantigens detected in susceptible and resistant hosts with different intestinal responses to the parasite that may be involved in the dynamics of worm rejection observed in each host species. In fact, Toledo et al. (2006b) attributed the greater levels of seroantigens to an increased worm antigen uptake due to a greater local inflammatory response. Under inflammatory conditions, the maintenance of the epithelial barrier is disrupted resulting in increased passage of worm antigens through the intestinal mucosa. Thus, the antibody production to *E. caproni* infection in each host species appears to be related to the kinetics of seroantigens. The intestinal absorption of *E. caproni* antigens induces systemic antibody responses by B-cell stimulation (Toledo et al., 2004b).

2.4.2 Cellular responses

The cellular changes induced by echinostome infections that may be related to resistance can be summarized as follow: (i) eosinophilic infiltration; (ii) increase in the number of mucosal neutrophils and mononuclear inflammatory cells in the mesentery (iii) mastocytosis and increasing in goblet cell numbers; (iv) the subsequent alteration of goblet cell function by modification of the mucin's terminal sugar; and (v) activation of macrophages.

The role of eosinophils in gastrointestinal helminth infections is controversial. Eosinophilic infiltration is a common feature in mice infected with *E. caproni* (Bindseil and Christensen, 1984; Muñoz-Antoli et al., 2007) and *E. trivolvis* (Fujino et al., 1993), though the life span of each echinostome markedly differs in the mice. Ryang et al. (2007) showed that immunosuppressant treatment of *I. hortensis* inhibited the local eosinophilia but this only resulted in slight changes in the worm recovery. Herein, similar kinetics in the populations of local eosinophils in mice and rats has been observed, though the survival of *E. caproni* in each host was markedly different (Toledo et al., 2006b; Muñoz-Antoli et al., 2007). Thus, the role of local esoinophils does not seem to be relevant for the expulsion of *E. caproni* adult worms.

Local inflammatory infiltrations rich in neutrophils is a common feature in susceptible host to *E. caproni* such as mice or hamsters. Neutrophils are an important source of proinflammatory cytokines such as IL-12 and TNF-α as well a free radicals and several chemokines. This raises the possibility that neutrophil infiltration and the consequences in the inflammatory response are essential in the longer survival of *E. caproni*. However, these facts appear to be more related to the pathology caused by

the infection than to the resistance to infection (Cortés et al., 2014, 2016a; Muñoz-Antoli-et al., 2016a).

Mucosal goblet and mast cell, together with augmented expression of mucins, have been often involved in resistance against intestinal helminths (Toledo et al., 2006a,b; McGuckin et al., 2011). In the case of echinostomes, available data are somewhat confused. Several studies showed that elevated levels of N-acetyl-D-glucosamine (GlcNAc) and N-acetyl-D-galactosamine (GalNAc) residues induced by *E. caproni* primary infection prevented heterologous or superimposed infection. In C3H mice, the expulsion of *E. trivolvis* was associated with goblet cell hyperplasia together with strong expression of GalNAc and α-L-Fucose (Fuc) residues on the surface of the intestinal villi and GalNAc, α-L-Fuc and GlcNAc in the goblet cells content. Moreover, it was suggested that these changes may mediate the rapid expulsion of *E. caproni* in a heterologous secondary infection (Fujino and Fried, 1993b; Fujino et al., 1996a,b). In contrast, Cortés et al. (2015a) studied the goblet cells and extracellular mucus (gel-forming mucins) was investigated in susceptible (mice) and resistant (rats) hosts. The results showed altered glycosylation patterns in the intestine of *E. caproni*-infected mice and rats. In both host species, enhanced expression of GlcNAc and GalNAc residues were detected in the glycocalyx of intestinal epithelial cells, goblet cells vesicles and extracellular mucus, suggesting a limited involvement in parasite rejection.

The development of chronic infections in mice has been associated with the up-regulation of several mucin genes, whereas the early expulsion of worms in rats occurs concomitantly with low levels of mucins expression (Cortés et al., 2015a). Gene expression of most of the mucins analyzed were found to be down-regulated in the ileum of infected rats (i.e. Muc1, Muc2, Muc5ac, Muc13, Muc16 and Muc 20), whereas the remainder ones (i.e. Muc3, Muc, 4 and Muc15) were unaltered (Cortés et al., 2015a). This suggests that, *E. caproni* is able to alter mucin expression in low-compatible hosts as described for other infectious agents.

Cortés et al. (2015a) observed marked differences in the staining kinetics with SNA lectin, which binds α-2,6-sialic acid. The intensity of the staining was trivial during the complete experiment in mice, but a marked decrease in the signal intensity was observed at 2 weeks post-infection in rats. The staining level in rats was recovered at 4 weeks post-infection. The authors suggested that this alteration could affect the rheological properties of the mucus barrier at early infection in rats. Sialic acids occupying terminal positions on mucus glycoproteins have been seen

to elevate the viscosity of mucus and desylation of mucins may lead to the degradation of mucus matrix (Yusuf et al., 2005). The decrease of SNA staining observed in the ileum of *E. caproni*-infected rats at 2 weeks post-infection might be induced by the parasites themselves in an attempt to facilitate their establishment in a hostile environment with goblet cell hyperplasia and a Th2/Th17 immune profile.

Recent studies have shown that activation of macrophages may play an important role in the susceptibility or resistance to echinostome infections. Primary infections of mice with *E. caproni* (associated with susceptibility) are characterized by the development of a Th1 environment with elevated levels of IFN-γ and markers of classical activation of macrophages (iNOS). In contrast, pharmacological healing of the primary infection changed this picture to a Th2 milieu with elevated production of IL-25 and markers of alternative activation of macrophages such as ArgI and Ym-1, concomitantly with resistance to homologous secondary infections (Muñoz-Antoli et al., 2016a; Álvarez-Izquierdo et al., 2020a,b).

IL-25 induces alternative activation of macrophages and that this way of activation may be an important mechanism for parasite rejection (Smith et al., 2018; Li et al., 2019). It was suggested that upregulation of ArgI in *E. caproni* secondary infections was protective by enhancing the generation of polyamines in addition to competitive inhibition of iNOS. Modulation of the balance between iNOS and ArgI, and the arginase-ornithine decarboxylase metabolic pathway may represent a strategy for regulating intestinal inflammation. Moreover, polyamines and Ym-I are useful for the repair of tissue damage. In this context, The healing and regeneration processes of the intestinal mucosa that begin after the primary infection has been cured may be decisive in resistance to subsequent infections (Muñoz-Antoli et al., 2016a; Álvarez-Izquierdo et al., 2020a,b).

2.4.3 Mucosal responses

Several studies have analyzed the mucosal changes induced by *E. caproni* in primary infection in relation to susceptibility (in mice) and resistance (in rats) and the results suggest that processes related to mucosal healing and regeneration activated by the infection are involved in resistance to infection (Muñoz-Antoli et al., 2014; Cortés et al., 2015b,c; Álvarez-Izquierdo et al., 2020b).

Cortés et al. (2015c), using a bromodeoxyuridine pulse-chase approach, showed that primary infection induced in both, rat and mice, alterations in the small intestine epithelial cell turnover. However, the

magnitude and kinetics in each host was markedly different. In mice, the susceptibility was associated with tissue hyperplasia related to dysfunction on normal epithelial renewal caused by the parasite and characterized by high levels of crypt-cell proliferation and a slow rate of epithelial turnover leading to alterations in the tissue homeostasis. In contrast, cell migration rate in rats was about two times higher in the ileum of infected rats compared with controls, with no changes in tissue structure and the maintenance of the epithelial homeostasis. In view of this results, the authors concluded that an accelerated cell turnover can be an effective mechanism for the expulsion of intestinal helminths by enhancing the maintenance of tissue homeostasis.

The kinetics of cell turnover and, consequently, was related to the different pathology and mucosal changes induced by *E. caproni* in each host. The greater inflammation and tissue damage caused by the parasite in mice would be the cause of the dysregulation of the intestinal homeostasis leading to alteration of balance and tissue architecture associated with susceptibility. In *E. caproni*-infected mice the slow cell turnover and tissue hyperplasia would enable more stable interactions, thereby favoring the development of chronic infections. Conversely, Th2 response in resistant hosts, and especially IL-13, was suggested as a key immune mediator due to role in regulating the epithelial cell turnover. an accelerated epithelial cell turnover may hinder the establishment of proper host-parasite interactions at molecular level, impairing the development of long-lasting infections. However, the role of IL-25 appears to be more relevant.

Álvarez-Izquierdo et al. (2020b) studied the effect of rIL-25 in the ileum and its potential relation to resistance to infection in sensibilized mice. To this purpose, they analyzed the ileal proteomic changes in IL-25-trated mice. Al the treated mice were refractory to infection, concomitantly with alteration were those involved in the preservation and healing of the epithelial architecture enhancing the maintenance of the epithelium.

All the above exposed suggest that mechanisms enhancing the maintenance of the intestinal homeostasis seems to be essential for resistance to infection, being probably the most important element in this resistance by making the establishment of the parasite difficult.

2.5 Pathology of the infection

Pathological features associated with echinostome infections are difficult to analyze since they depend on a variety of factors including the parasite and the host species (Toledo and Fried, 2005; Toledo et al., 2006b Muñoz-

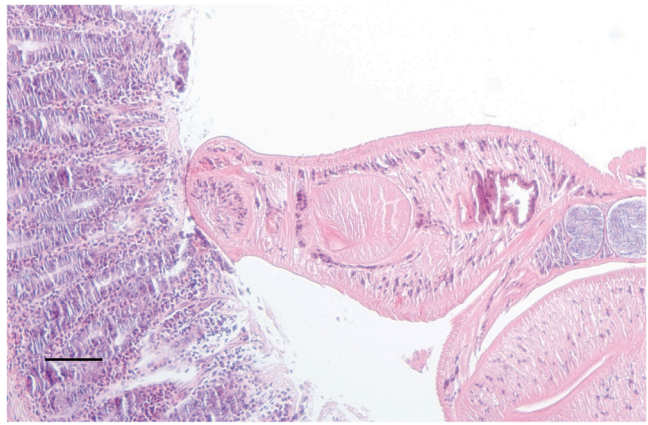

Fig. 2 Adult worm of *Echinostoma caproni* attached by oral sucker at the intestinal mucosa of a mouse at 28 days post-infection; stained with Giemsa. Scale bar: 100 μm.

Antoli et al., 2007). Gross pathology observed in echinostome infections is mainly due to mechanical effects due to the fixation of adult worms to the intestinal mucosa. The attachment sites consist of a plug of grasped mucosa occupying the cavity of the ventral sucker (Toledo et al., 2006a,b) (Fig. 2).

Microvilli atrophy, including fusion, destruction and/or erosion of microvilli, is a fact described in mice infected with *E. caproni* (Weinstein and Fried, 1991; Fujino and Fried, 1993a,b; Toledo et al., 2006b). Moreover, infection with *E. caproni* of susceptible hosts, such as the mouse, is characterized by hyperplasia and increased mitosis in intestinal crypts (Fujino and Fried, 1993a,b). Strikingly, this damage is regenerated more quickly in susceptible host than in hosts resistant to infection, probably in relation to the rapid activation of tissue regeneration processes activated by the greater damage induced in the mucosa, as mentioned above (Toledo et al., 2006b; Cortés et al., 2015a) (Fig. 3).

An intense infiltration of inflammatory cells was observed along with elevated neutrophil counts in the intestinal mucosa in susceptible hosts. However, in rats, no evidence of a local inflammatory response was observed. This fact suggests that the development of intense local inflammatory responses is associated with the establishment of chronic infections with *E. caproni*.

The elevated level of IFN-γ expression, and the subsequent NO production, in susceptible hosts has been regarded as the main responsible for the inflammation and tissues damage (Toledo et al., 2006b; Trelis et al.,

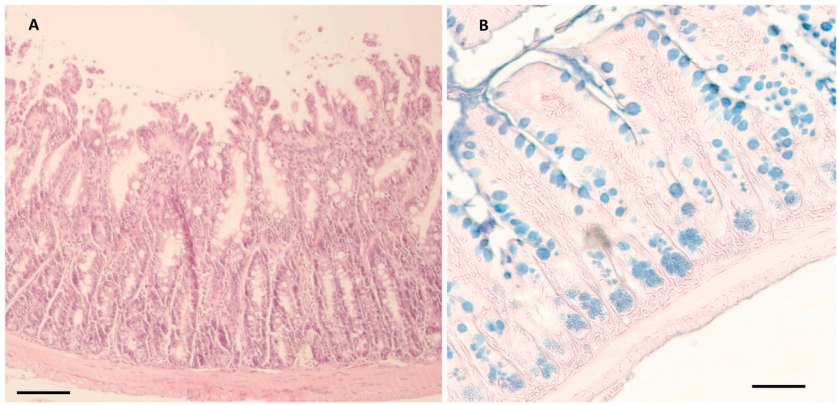

Fig. 3 (A) Eroded villi of a mice infected with *Echinostoma caproni* at 28 days post-infection (stained with Giemsa); (B) goblet cell hyperplasia in a mice infected with *Echinostoma caproni* at 28 days post-infection (stained with Alcian blue). Scale bars: 100 μm.

2011). However, Cortes et al. (2014) suggested that IFN-γ can 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. The authors studied the role of IFN-γ in *E. caproni* infections in several strains of mice including ICR (a susceptible host for *E. caproni*), BALB/c (a prototypical Th2 strain), and BALB/c deficient for IFN-γ mice (IFN-γ-/-). Worm recovery was similar in all the strains, though marked differences were observed in relation to the clinical signs induced by the infection. ICR mice did not show evidences of illness after infection, and the general aspect was similar to that of the controls. In contrast, evident signs of disease were reported in BALB/c mice and, especially, in the mice of the IFN- γ deficient strain concomitantly with the low levels of IFN-γ production. It was finally suggested that IFN-γ may participate in the preservation of the mucosal architecture preventing a severe form of disease as. In contrast, the lack of IFN-γ could led to a lethal outcome due to a disruption of the mucosal homeostasis.

Studies on the intestinal proteomic changes induced by the infection have contributed to better understanding the pathological processes in echinostome infections. Cortés et al. (2015 Plos) analyzed the proteomic changes induced by a primary *E. caproni* infection in a susceptible host, such as the mice. The results confirmed that the establishment of chronic infections was associated with the development of early and strong inflammatory responses and tissue damaged together with elevated production of IFN-γ. This response was characterized by a rapid and intense

mitochondrial dysfunction and a shift of cell metabolism to an anaerobic use of glucose, limited respiration, ATP production and b-oxidation, leading to accumulation of reactive of oxygen and nitrogen species and fatty acids causing injury and cell death. Moreover, several proteins implicated in the epithelial restitution were observed to be also over-expressed. These facts, together, with the alterations in cell deaths processes may contribute to the homeostatic dysregulation in the intestinal epithelium that characterize the development of chronic infections.

In contrast, changes associated with primary infections in a resistant host (rats) appear to be related to the regeneration of the ileal epithelium throughout an augmented proliferation and differentiation of enterocytes. The alterations observed in the ileum of rats enabled the preservation of the functional integrity of epithelial barrier, thereby limiting the generation of tissue injury in this host. This may be crucial for parasite expulsion, as this physical barrier constitutes a major defense line against intestinal helminths. In this sense, the elevated levels of IL-13 expression generated in the ileum of rats after *E. caproni* infection could be responsible for the induction of effector mechanisms such as changes in intestinal epithelial cell function and accelerated epithelial renewal, which could lead to the early expulsion of this parasite from the rat intestine (Muñoz-Antoli et al., 2014).

2.6 Antigenic characterization

The first attempts of antigenic characterization of echinostomatids were based on SDS-PAGE and western blot analysis. Andresen et al. (1989) identified four tegumental antigens of *E. caproni*, weighing 26, 66, 78 and 88 kDa, that were suggested as the main responsible for the immune response. Toledo et al. (2004c) also defined by western-blot a total of 11 and 7 polypeptides in the crude and excretory-secretory extracts of *E. caproni* and demonstrated that some of these polypeptides were specifically produced in the early stages of the infection. Carpena et al. (2007) reported that host species may affect the protein expression of *Echinostoma friedi*. Several common and specific bands were detected in excretory-secretory products of *E. friedi* adult worms collected from hamster and rat. Higón et al. (2008) demonstrated a higher level of transcription and secretion of a 70 kDa heat-shock protein from *E. caproni* in a susceptible (hamster) than in a resistant host (rat). Since no differences were observed in the amount of protein either in somatic lysates or surface materials, the increase in transcription in *E. caproni* from hamsters could be related to a

higher secretion of the molecule, which in turn could facilitate the parasite establishment in the host, and/or modulate the host response.

Bernal et al. (2006) identified structural actin-like proteins, tropomyosin, and paramyosin; glycolytic enzymes like enolase, glyceraldehyde 3 P dehydrogenase (GAPDH), and aldolase; detoxifying enzymes like GSTs; and the 70 kDa heat-shock protein in the secretome of *E. friedi*. Moreover, these authors also showed that actin and the 70 kDa heat-shock protein exhibited differential expression patterns depending on the definitive host (susceptible or resistant), suggesting that these proteins may play a role in the survival within the host (Bernal et al., 2006). Moreover, Marcilla et al. (2004) showed post-translational modifications in the protein pattern of echinostomes proteins including tyrosine phosphorylation in response to different stimulus.

A total of 39 parasite proteins were accurately identified by shot-gun liquid chromatography/tandem mass spectrometry (Sotillo et al., 2010). Metabolic enzymes, and particularly glycolytic enzymes, were the most represented protein families in the secretome of *E. caproni* adult worms. Moreover, proteins involved in parasite structure, response against stress, chaperones, calcium-binding, and signal transduction were also identified.

Garg et al. (2013) described the first transcriptome of the adult stage of *E. caproni*. 557,236 raw sequence reads were assembled into 28,577 contiguous sequences and 23,296 putative proteins were characterized based on homology, gene ontology and/or biochemical pathways. Comparisons of this transcriptome with those of other trematodes revealed similarities in the transcription pattern of molecules inferred to have key roles in parasite-host interactions. Enzymatic proteins like kinases and peptidases were the most abundant.

2.7 Immune evasion mechanisms

Several mechanisms to evade the host immune response have been described in echinostome infection. For example, it was shown that antibodies bound to the surface of *E. caproni* are rapidly lost probably in relation to a rapid rate of surface antigens turnover (Simonsen and Andersen, 1986; Andresen et al., 1989; Simonsen et al., 1990).

The effect of the antibodies bound to the surface of *E. caproni* was studied by Cortés et al., (2017b). They showed that, instead of being shed from the surface of the parasite as previously suggested, the antibodies become trapped and covered within a layer of excretory/secretory products that covers the parasite (Fig. 4). Furthermore, antibodies were subsequently degraded by parasite-derived peptidases. Cortés et al. (2019) observed that cathepsin L-like peptidases active in the excretory-secretory products of

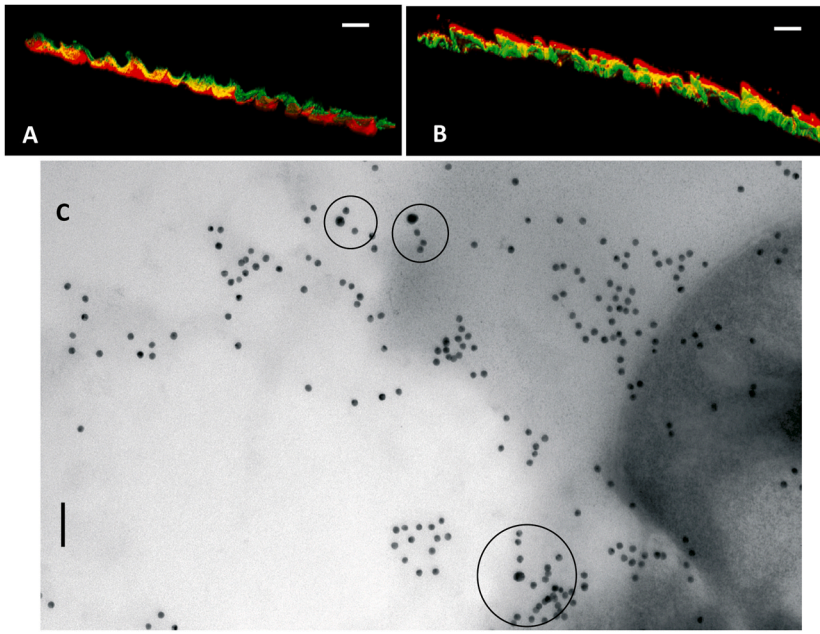

Fig. 4 Antibody trapping: (A) 3D surface modeling of the *Echinostoma caproni* adult worms showing antibodies (green) covering o the surface and the excretory/secretory products of the parasite (red) and the merger area (yellow); (B) after incubation, excretory/secretory products have trapped the bound antibodies (scale bars = 20 μm); (C) Transmission electron micrographs of the extracellular secretions of *E. caproni* adult worms with double immunogold staining using rabbit sera against *E. caproni* excretory/secretory products (detected by a secondary antibody coupled to 12 nm colloidal gold) and an anti-mouse IgG conjugated with 18 nm colloidal gold. Images show antibody trapping by secreted products on the worm surface (encircled). Scale bars = 100 nm.

E. caproni were involved in the degradation of surface-trapped antibodies, suggesting that cysteine peptidases are not only crucial for tissue-invading trematodes, but they can be equally relevant at the parasite-host interface in gut-dwelling flukes. Antibody trapping was suggested as a potential mechanism for parasite immune evasion, alleviating the deleterious effects of antibodies and promoting parasite survival (Cortés et al., 2017b).

Recent studies showed that *E. caproni* adult worms to modulate their proteome and secretome to enhance their survival in a hostile environment. Cortés et al. (2016b) reported that *E. caproni* is able to modify its proteome in response to the milieu to enhance its survival. The results suggested a better adaptation of the parasite to the Th1-type environment developed in the intestine of susceptible hosts, thereby enabling its chronic

establishment. Furthermore, Cortés et al. (2018) compared the secretome of *E. caproni* in mice in primary infections exposed to a Th1 milieu with that of in challenge infections in a Th2 environment. Adult worms exposed to a primary infection upregulated several proteins involved in detoxification processes, response to stress, and enhancement of parasite survival. In contrast, Th2 milieu did not induce significant upregulation of any protein in the secretome of *E. caproni*, concomitantly with an early rejection of worms. This ability to modulate the secretome in a hostile Th1 milieu in primary infections in mice may serve to withstand this environment, with inflammatory response and oxidative stress, resulting in the development of chronic infections.

2.8 Human infections

Human infections with echinostomes have been reviewed by Toledo and Esteban (2016) and Toledo et al. (2019, 2022), among others. Human echinostomiasis is endemic in Southeastern and Notheastern Asia, though occasional cases may occur worldwide (Toledo et al., 2022). The number of species that can cause infections in humans is confusing due to the problematics regarding the taxonomy of the group. Toledo et al. (2022) compiled a total of 23 species of Echinostomatidae causing human infections (Table 2).

Geographical distribution of human echinostomiasis is strongly determined by eating habits. Humans become infected by eating raw or undercooked freshwater or brackish water molluscs, fishes, snakes crustaceans or amphibians harboring the metacercariae. Traditional eating habits in Asia facilitate infections.

The current incidence of human echinostomiasis is not known. Most of the available information is based on sporadic reports with scarce information and, in many cases, lacking specific identification as mentioned above. Moreover, microscopists may misinterpret echinostome eggs, particularly considering that eggs of different echinostome species markedly resemble making difficult specific diagnosis (Esteban et al., 2019; Chai et al., 2020). The only estimate available is the one made by WHO (2004) for *I. hortensis* (50,000 people infected), *Echinoschasmus japonicus* (about 5000 cases) and *Echinostoma cinetorchis* and *Acanthoparyphium tyosenense* (with about 1000 cases each).

Knowledge on the immunology and pathology of human echinostome infections is scarce and based on erratic reports. The clinical symptoms may depend on the worm burden, but they can cause more severe symptoms

Table 2 Species of Echinostomatidae involved in human infections, main source of infection and geographical distribution.

Species	Source of infection	Geographical distribution
Acanthoparyphium tyosenense	Brackish snails	Korea
Artyfechinostomum malayanum[a]	Freshwater snails	Cambodia, India, Indonesia, Lao PDR, Malaysia, Philippines, Singapore, Thailand
Artyfechinostomum oraoni	Freshwater fishes	India
Artyfechinostomum sufrartyfex	Freshwater fishes	India
Echinochasmus caninus[b]	Freshwater fishes	Lao PDR, Thailand
Echinochasmus fujianensis	Freshwater fishes	China
Echinochasmus japonicus	Freshwater fishes	China, Korea, Lao PDR
Echinochasmus jiufoensis	Freshwater fishes	China
Echinochasmus liliputanus	Freshwater fishes	China
Echinochasmus perfoliatus	Freshwater fishes	China, Japan, Korea
Echinoparyphium recurvatum	Freshwater snails	Egypt, Indonesia, Korea, Taiwan
Echinostoma aegyptica	Not known	China
Echinostoma angustitestis	Freshwater fishes	China
Echinostoma cinetorchis	Freshwater fishes/snails	China, Japan, Korea, Taiwan
Echinostoma ilocanum	Freshwater snails	Cambodia, China, India, Indonesia, Malaysia, Philippines, Thailand
Echinoastoma lindoense[c]	Freshwater snails	Indonesia, Lao PDR
Echinostoma macrorchis	Freshwater snails	Japan, Korea, Lao PDR
Echinostoma mekongi	Freshwater snails	Cambodia

Echinostoma revolutum	Freshwater snails	Cambodia, China, Egypt, Indonesia, Lao PDR, Russia, Europe
Himasthla muehlensi	Brackish bivalves	USA[d]
Hypoderaeum conoideum	Freshwater snails	Thailand
Isthmiophora hortensis[e]	Freshwater fishes/tadpoles	China, Japan, Korea
Isthmiophora melis	Freshwater fishes/tadpoles	China, Rumania, Taiwan

[a]Syn. *Echinostoma malayanum*
[b]Syn. *Episthmium caninum*
[c]Syn. *Echinostoma echinatum*
[d]Imported infection
[e]Syn. *Echinostoma hortense*
Source: Adapted from Toledo, R., Álvarez-Izquierdo, M., Esteban, J.G., Muñoz-Antoli, C. (2022). Neglected food-borne trematodiases: echinostomiasis and gastrodiscoidiasis. Parasitology 149, 1319–1326.

than other intestinal trematodes. The most common signs are unspecific symptoms such as diarrhea, loss of body weight, epigastric and abdominal pain or easy fatigue. However, additional symptoms may include local eosinophilia, anemia, edema and anorexia (Graczyk and Fried, 1998; Toledo et al., 2006a, 2019, 2022; Toledo and Esteban, 2016).

Severe anemia has also been associated with echinostomiasis in a coinfection of a child with *Echinostoma* sp., *Trichuris trichiura* and *Ascaris lumbricoides* in India (Khanna et al., 2019). In *I. hortensis*-infected patients also appeared nausea and vomiting, headache, acid belching and urinary incontinence (Chang et al., 2005). Mucosal damage may include signs of chronic gastritis, infiltration of inflammatory cells, mucosal erosion and bleeding and lesions similar to adenocarcinoma (Chang et al., 2005).

3. Family Brachylaimidae

3.1 Background

Brachylaimidae is a complex family of terrestrial digeneans with a long history of confusion regarding its systematics and taxonomy (Pojmansha, 2002). Brachylaimids commonly infects mammals, birds and reptiles

Fig. 5 Adult worm of *Brachylaima* sp. from a naturally infected mouse, *Mus musculus*; the worm was stained in Grenacher's Borax Carmine. Scale bar: 50 μm. *Reproduced with permission from Toledo, R., Esteban, J.G., Fried, B., 2006a. Immunology and pathology of intestinal trematodes in their definitive hosts. Adv. Parasitol. 63, 285–365.*

(Gibson and Bray, 1994). The most representative genus within this family is *Brachylaima*. However, this is a problematic genus and includes a large number of species, but many of them are poorly known. This problem is aggravated by the fact that many species have been described simply on the basis of the morphology of the adult worm and the similarity between different species is great (Fig. 5). Species of *Brachylaima* follow a 3-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. Humans have also been reported as an incidental definitive host for one species in the genus, *Brachylaima cribbi* (Butcher et al., 1996, 1998).

3.2 Immunology of the infection

Currently there are very few studies on the immunology of *Brachylaima* infections and focused on *B. cribbi* infections in mice. The main evidences on the relevance of the immune response in *B. cribbi* infections comes from the fact that the course of the infection varies depending on the strain of infected mouse and a partial degree of resistance to secondary infections. Moreover, it is well known that *B. cribbi* infections elicit significant antibody responses. Using an indirect ELISA based on crude antigens from *B. cribbi* adult worms, Butcher et al. (2003) demonstrated that primary infections in mice provoked 4- to 7-fold increases in antibody levels in the serum of experimentally infected mice.

Butcher et al. (2002a) showed that CB57BL/6J is the most susceptible strain of *Mus musculus* to *B. cribbi* infection. Although, there was variability in susceptibility in relation to gender and maturity of mice (Butcher et al., 2002b), this strain exhibited the highest levels of egg release and the longest duration of infection. Mature female CB57BL/6J mice were significantly more resistant to *B. cribbi* infection that older mature females and adolescent females in terms of reduced worm burden, fecundity and egg fertility. These changes were attributed to physiological factors, suggesting that sex hormones provided a significant level of protection to *B. cribbi* infection (Butcher et al., 2002b).

The course of the infection in immunocompetent CB57BL/6J and immunodeficient NOD SCID mice re-infected with *B. cribbi* was assessed by comparing faecal egg release of the re-infected mice with age- and sex-matched mice receiving only a primary infection. 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 (Butcher et al., 2003).

There are no studies on the pathology of infections with brachylaimid species either in natural or experimental infections.

3.3 Human infections

Only *B. cribbi* has been reported infecting humans and all the cases (three) were reported in South Australia, where the life cycle of *B. cribbi* is maintained between mice and helicid and hygromiid snails (Butcher et al., 1996). Two of the cases were in 21-month-old children who used to eat raw snails in which eggs were detected in feces (Butcher et al., 1996). The third case corresponds to a 78-year-old woman who had a history of intermittent diarrhea for 18 months. Examination of her stools revealed the presence of brachylaimid eggs. She ate raw vegetables which had been contaminated with helicid snails. After treatment with praziquantel a degenerate adult *Brachylaima* species was recovered in her stools (Butcher et al., 1998). The parasite was identified as B. cribbi (Butcher and Grove, 2001).

4. Family Diplostomidae

4.1 Background

The family Diplostomidae includes a group of digeneans that parasitize numerous orders of birds and mammals, including humans. This family comprises a total of 41 genera grouped into four subfamilies (Alariinae, Codonocephalinae, Crassiphialinae and Diplostominae) that are mainly differentiated on the basis of the host specificity, forebody shape, extension of the vitellarium and type of metacercariae (Dubois, 1970; Yamaguti, 1971; Gibson, 1996; Niewiadomska, 2002).

Most of the species of Diplostomidae have a three-host life cycle. Fork-tailed cercariae, produced in sporocysts in the snail first intermediate host, emerge from the gastropod and penetrate amphibians, molluscs, fishes or annelids, in which it forms the metacercariae. Definitive host becomes infected after eating the second intermediate host harboring the metacercariae. Adult worms usually inhabit the duodenum but may extend to the jejunum and ileum in heavy infection. Eggs shed with feces of the definitive host hatch and penetrate the first intermediate host (Hong, 1982). In some cases, the life cycle in expanded by the incorporation of a

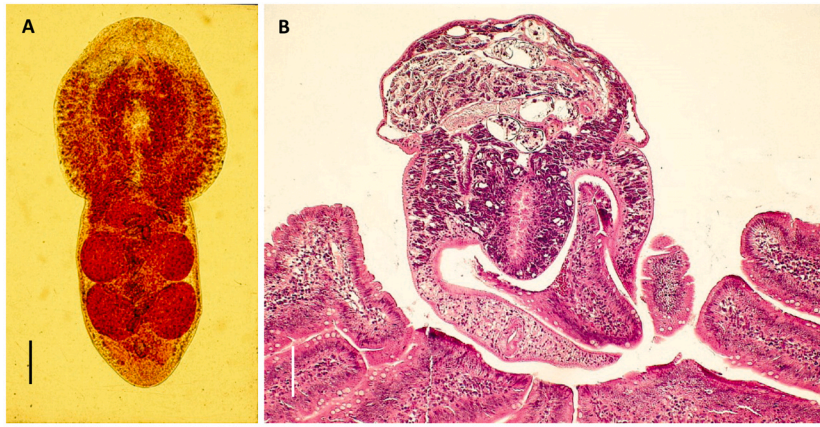

Fig. 6 (A) *Neodiplostomum seoulense*: Adult worm from an experimentally infected rat necropsied at 7 days post-infection; the worm was stained in acetocarmine; (B) Small intestine of a rat experimentally infected with *Neodiplostomum seoulense* and necropsied at 28 days post-infection; the preparation was stained with hematoxylin and eosin. Scale bars: 100 μm. *Photomicrographs courtesy Jong-Yil Chai. Reproduced with permission from Toledo, R., Esteban, J.G., Fried, B., 2006a. Immunology and pathology of intestinal trematodes in their definitive hosts. Adv. Parasitol. 63, 285–365.*

fourth (paratenic) host, usually snakes, that harbors an unencysted larval stage called mesocercaria, infective for the definitive host (Cribb, 2003).

Studies on the immunology of Diplostomidae only have been performed on a single species (*Neodiplostomum seoulense*) belonging to the Diplostominae (Fig. 6A). This species has been frequently reported in humans in Korea (Chai and Lee, 2002; Chai and Jung, 2020; Toledo et al., 2019), which have stimulated the work on this topic.

4.2 Immunology of the infection

Immune response against *N. seoulense* has been mainly studied on laboratory rodents. This response in BLAB/c mice is characterized by a mixed Th1/Th2 balance resulting in the induction of humoral and cellular reactions against the parasite (Shin et al., 2007). Infection of BALB/c mice caused a continuous increase of TCD4+ and TCD8+ cells both in the spleen and MLN during the course of the infection. However, the response of Th1 cytokines appeared to be stronger than that of Th2 cytokines. Production of IFN-γ and IL-12 was significantly greater than that of IL-4 or IL-10 from 7 to 28 days post-infection (Shin et al., 2007).

Antibody response in mice and rats is characterized by the production of elevated serum levels of IgA, IgG and IgM from 7 days post-infection

(Kho et al., 1990; Shin et al., 2007; Han et al., 2008). Similar levels of IgG1 and IgG2a were observed, reflecting the mixed response that induces *N. seoulense* (Shin et al., 2007). At local level, increased levels of IgA were also detected in the lamina propria and submucosa but these responses were non-specific. These antibody responses were not related to worm expulsion since significant decreases in worm recoveries were not observed during the experiment (Huh et al., 1995).

Regarding the cellular responses, most of the studies have been focused on mucosal mast cells and goblet cells. Although several experiments have shown that *N. seoulense* infection in rats and mice induces mucosal mast cell hyperplasia, no relationships with neither worm expulsion nor resistance to infection has been observed. Worm expulsion concomitantly occurred with intestinal mastocytosis (mainly in the duodenum) in Sprague-Dawley rats (Kho et al., 1990; Shin et al., 2003) and mice (Chai et al., 1998). However, the kinetics and degree of mastocytosis were different in each host species, suggesting that the role of mucosal mast cells is different in mice and rats. The highly susceptible strain of BALB/c mice showed a greater mucosal mast cell reaction than C3H mice at 7 days post-infection but they did not expel the worms until 28 days post-infection. Therefore, proliferation of mucosal mast cell in the two strains of mice was regarded as local host responses rather than a responsible effector mechanism for worm expulsion (Chai et al., 1998). These observations were confirmed by Kook et al. (1998) by infecting mast cell-deficient W/Wv mice and their normal littermate +/+ mice with 200 metacercariae of *N. seoulense*. The parasite was highly lethal for both strains of mice, but +/+ mice showed a significantly greater level of worm recovery than W/Wv mice, suggesting that mucosal mast cells are not involved in the protective mechanisms.

Depletion of mastocytosis using antiallergic drugs (hydroxyzine, cimetidine, cyclosporin-A, and prednisolone) in Sprague-Dawley rats infected with *N. seoulense* did not change the kinetics of worm rejection. Only in hydroxyzine- and cimetidine-treated mice (a histamine receptor H1 and H2 blockers, respectively) slightly delayed worm expulsion were observed. This was surprising since mucosal mast cells are the main source of histamine and, thus, intestinal smooth muscle contraction was suggested to play a major role in *N. seoulense* expulsion (Shin et al., 2003). Hydroxyzine and cimetidine inhibit the smooth muscle contraction by blocking the histamine receptors and, consequently, could delay the worm expulsion regardless of the level of mastocytosis.

The intestinal goblet cell hyperplasia in *N. seoulense* infections was studied in BALB/c and C3H mice by Chai et al. (1998). Goblet cell hyperplasia was observed in the duodenum of BALB/c mice during the course of the infection but was not seen in C3H mice. Mucin activation also was noted in both strains of mice, especially in BALB/c mice, bat no relationship with the worm recovery rate kinetics in each strain of mice was noted.

Macrophages could be of greater interest in possible resistance to infections. Significant increase in the number of splenic macrophages and heavy infiltration of macrophages in the small intestine mucosa have been observed in BALB/c mice infected with *N. seoulense*. Furthermore, it was shown that macrophages were able to in vitro kill *N. seoulense* adult worms via antibody-dependent cell-mediated cytotoxicity (Shin et al., 2007).

4.3 Pathology of the infection

The pathology of *N. seoulense* human infections has been poorly studied and most of the data proceeds from studies in laboratory rodents. *N. seoulense* infections appear to be more harmful than other intestinal flukes. In fact, *N. seoulense* is highly pathogenic, producing severe anatomical and functional damage, and lethal to mice (Kook et al., 1998; Chai et al., 2000). However, pathology appears to be related with the worm burden and the genetic background of the host (Huh et al., 1988; Kook et al., 1998).

In the intestine of the definitive host, adult worms of *N. seoulense* entrap the host villi using their ventral curvature of the anterior forebody, with the tribocytic organ piercing the villous stroma (Fig. 6B). Villous changes can be observed from the first week of infection. They are characterized by shortening, widening and fusion of the villi. There was also a reduction of the goblet cell numbers adjacent to the worms, hemorrhage, capillary congestion, lymphatic dilation and inflammatory cell infiltration. The inflammatory cells involved were lymphocytes, plasma cells, eosinophils and occasional giant cells (Lee et al., 1985). In other Diplostomidae, such as *Pharyngostomum cordatum*, pass of the metacercaria through the intestinal wall to the peritoneal cavity of rodents and migration to the diaphragm, intercostal muscles and other organs such as the lung, heart and brain have been observed (Shin et al., 2001).

However, pathology appears to be related with the worm burden and the genetic background of the host (Huh et al., 1988; Kook et al., 1998). Huh et al. (1988) compared the course of the infections in ICR mice with 500 and 1000 metacercariae per host. Despite the histopathological findings were similar, cyst inoculum of 500 did not affected mouse survival. In contrast,

infections with 1000 metacercariae induced a severe course of infection with diarrhea, occult blood and a gradual weight loss and, finally, a fatal outcome. Death of the infected mice began at 11 days post-infection, and at 16 days post-infection all the mice were dead. Intestinal malabsorption induced by mucosal changes and intestinal bleeding were suggested as the main causes of the earlier death in the heavily infected mice. Similarly, Kook et al., (1998) showed that survival of C3H/HeJ mice was inversely related to the dose of metacercariae. In heavier infections, length of the intestine was significantly shortened and charcoal meal transit was significantly quicker after 14 days post-infection.

Genetic background of the host may also contribute to the pathology and resistance to infection. Kook et al. (1998) experimentally infected two strains of mice (BALB/cA and C3H/HeJ) and their F_1 hybrid. BALB/cA mice showed the greatest worm intensities, but mice of this strain were resistant to pathogenesis and mortality due to the infection. In contrast, C3H/HeJ and F_1 mice showed marked intestinal paralysis. Moreover, the survival of BALB/cA mice was significantly higher than that observed in the other mouse strains. Chai et al. (2000) observed that a greater proportion of C3H/He (H-2k) succumbed to *N. seoulense* infection despite having fewer worms and shorter duration of the infection than BALB/c (H-2a) and C57BL/6 (H-2b) mice. It was suggested that resistance to *N. seoulense* infection was dependent upon H-2 gene product differences.

Apart from the intestinal damage, Pyo et al. (2012) showed that *N. seoulense* can downregulate the production of the neuronal growth associated protein (GAP)-43 in mice suggestin that neuronal damage can be elicited. Moreover, Shin et al. (2016) reported that *N. seoulense* may reduce the fecundity of mice in relation to destruction of gonad tissues. This damage was expressed by reductions in the fertility rate, birth time and number of litters. Gonadal tissue destruction was associated with a predominance of Th1 responses to the infection.

4.4 Antigenic characterization

Little is known about the antigenic composition of *N. seoulense*. Han et al. (2008) analyzed the antigen composition of crude antigens of *N. seoulense* by western-blot using rea from ICR, BALB/c and C3H experimentally infected mice. About 14 protein bands, from 30 kDa to 100 kDa in size, were recognize by IgG from infected mice. However, nothing is known about the identity and characteristics of these antigens. Previous studies using thin sections of *N. seoulense* adult worms treated with sera from immunized mice had shown a high antibody concentration over the rough endoplasmic

reticulum of the cells of the tribocytic organ, spermatozoa in the seminal vesicle, microvilli of the caecum, and vitelline follicles (Lee et al., 1997a,b). Using serum from experimentally infected mice, the most reactive structure was the vitelline follicle, suggesting that the most antigenic components in infections were the excretory/secretory products originated from the vitelline follicles as described for other intestinal trematodes (Ahn et al., 1991).

Choi et al. (1999) purified a 54 kDa protease from crude antigens of *N. seoulense* adult worms. This enzyme was able to degrade extracellular matrix proteins such as type I collagen and fibronectin with different cleaving activities, suggesting that this protein play an important role in parasite survival and pathogenesis in vivo. Moreover, Choi et al. (1999) suggested a possible role in nutrient uptake by degrading host tissue proteins.

4.5 Human infections

Members of 3 genera of Diplostomidae (*Alaria, Neodiplostomum,* and *Fibricol*a) have beenrecorded infecting humans. In the case of *Alaria* spp., humans only serve as paratenic hosts harboring mesocercariae in different tissues (Fernandes et al., 1976; Freeman et al., 1976; Beaver et al., 1977; McDonald et al., 1994; Kramer et al., 1996). At the intestinal level, only *N. seoulense* and *F. cratera* have been found parasitizing humans.

Human infection with *F. cratera*, a trematode species indigenous to North America can be considered as anecdotal since the only one record is an experimental infection of a human volunteer with 100 metacercariae (Shoop, 1989). Experimental infection produced a patent infection that lasted for 40 months with periods of epigastric discomfort, loose stools and flatulence, especially during the first year of the infection.

More common are the human infections with *N. seoulense*. in Korea (Chai and Jung, 2020). This species was first implicated when a 25-year old male suddenly suffered severe gastrointestinal symptoms (Seo et al., 1982). Another 26 cases were reported among Korean soldiers, probably infected during their survival training. In the infections were associated with consumption of 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. Commonly, symptoms of the infections were mild which was attributed to relatively low number of adults worms in the intestine (Hong et al., 1984a). However, (Seo et al., 1982) reported the case of a patient with epigastric discomfort, fullness and pain, anorexia, diarrhea, fever and tenderness. After treatment and purgation, a total of 79 worms were collected.

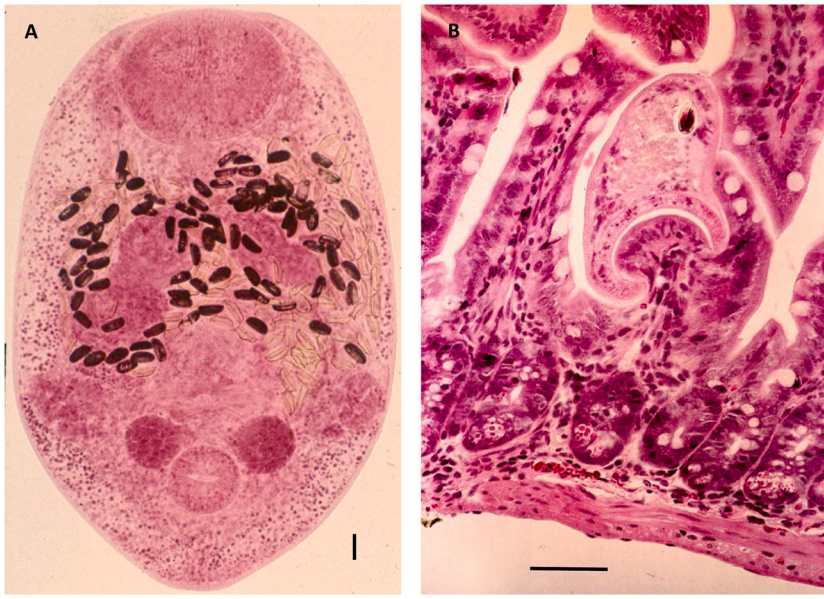

Fig. 7 (A) Adult worm of *Gymnophalloides seoi* collected from a naturally infected human and stained in acetocarmine (scale bar: 50 μm); (B) Small intestine of a mouse experimentally infected with *Gymnophalloides seoi* and necropsied at 5 days post-infection stained with hematoxylin and eosin (scale bar: 100 μm). *Photomicrographs courtesy Jong-Yil Chai. Reproduced with permission from Toledo, R., Esteban, J.G., Fried, B., 2006a. Immunology and pathology of intestinal trematodes in their definitive hosts. Adv. Parasitol. 63, 285–365.*

5. Family Gymnophallidae

5.1 Background

Gymnophallidae is a family that includes a small group of digeneans that may occur in the intestine, gall-bladder and bursa Frabricii of marine birds and the intestine of mammals (Scholz, 2002). Furcocercous cercariae and metacercariae that never encyst are characteristics of the group. Tipically, bivalves act as the first and second intermediate host. Moreover, polichaetes, gastropods and brachiopods also can act as the second intermediate host. Definitive host becomes infected after ingestion of the second intermediate host harboring the unencysted metacercariae (Cremonte et al., 2013).

Although five genera are accepted in the family, only one species, *Gymnophalloides seoi* (Fig. 7A), has been found infecting humans and all the studies in relation to the immunology of the infection have been referred to this species (Toledo et al., 2019).

5.2 Immunology and pathology of the infection

Apparently, *G. seoi* induces less severe damage than other intestinal trematodes, though it depends on the host species (Chai et al., 2001). Adult worms inhabit the duodenum and jejunum where they are fixed by grabbing or pinching the epithelial surface with the oral suction cup. 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). Moreover, inflammatory responses in reactins in the villous stroma and the crypt have been described (Chai et al., 2001) (Fig. 7B).

Interestingly, histopathology is similar in immunocompetent and immunosuppressed C3H/HeN mice, although much more accentuated in the case of immunosuppressed mice, suggesting a relevant role of the host immune response (Lee et al., 1997a,b; Chai et al., 1999; Seo et al., 2003). Mucosal changes in immunocompetent mice were characterized by marked goblet cell hyperplasia, especially in the jejunum. the destruction of the villi adjacent to the worms was more severe than in immunocompetent mice. Moreover, no remarkable hyperplasia was observed in immunosuppressed mice (Chai et al., 2001).

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., 1997a,b; Chai et al., 1999) indicating a genetic background in the resistance to infection. C3H/HeN is the most susceptible strain in terms of greater worm recovery rates, worm growth, and uterine egg counts. In contrast, C57BL/6 mice rejected the worms more rapidly than the other strains (Lee et al., 1997a,b; Seo et al., 2003).

Resistance to *G. seoi* infection appears to be mediated by both T-cell dependent and independent mechanisms including goblet cell hyperplasia and changes in mucin glycosylation. Worm expulsion was associated with high numbers of mucosal goblet cells in C57BL/6 mice together with changes in the terminal sugar chain of mucins and high levels of IL-4 and IL-5 mRNA expression in mesenteric lymph nodes. CD4 T-helper cells play an important role as a mediator of goblet cell hyperplasia, but not for functional activation of these cells (Guk et al., 2009). Strong goblet cell responses were observed at 3–7 days post-infection in immunocompetent C3H/HeN mice and most of the worms were expelled at 7–14 days post-infection. In immunosuppressed C3H/HeN mice, only relatively low

levels of goblet cell hyperplasia were observed concomitantly with a higher survival of the worms (Chai et al., 2001). Seo et al. (2003) studied the goblet cell hyperplasia and the secretion of mucus in relation to worm recovery in immunocompetent and immunosuppressed C56BL/6 mice. A significant reduction in goblet cell hyperplasia and mucins together with a greater worm recovery rate was observed in immunosuppressed mice with respect to immunocompetent mice.

Furthermore, Lee et al. (2014) showed that *G. seoi* infection in C57BL/6 mice induces overexpression of STAT6 and IL-13 enhancing goblet cell hyperplasia responsible for the worm expulsion. Increased intestinal epithelial cell turnover, and increased intestinal motility should be important mucosal defense mechanisms in *G. seoi*-infected mice (Lee et al., 2014). Products released from the parasite could be essential for the development of protective responses. Lee et al. (2010) showed that *G. seoi* antigen significantly induced expression of *TLR2* and *MUC2* in cultured HT29 cells. In this context, exosomes from the metacercariae appeared to be the responsible for the overexpression of those genes (Song et al., 2018).

5.3 Antigenic characterization

Studies on the antigenic composition of *G. seoi* are limited to the characterization of two cysteine proteases. Choi et al. (1998a) identified a 16 kDa cysteine protease from *G. seoi* metacercariae that showed the ability to degrade human immunoglobulins. The protease only degraded partially IgG2a after incubation overnight. However, heavy chains of IgA were completely degraded and a significant degradation of light chains was also observed. Moreover, this protease also degraded extracellular matrix proteins such as collagen or fibronectin. It was suggested that this protein may also be involved in the nutrition of *G. seoi* by degrading extracellular matrix proteins. Choi et al. (1998b) described another cysteine protease weighing 40 kDa from adult crude extracts of *G. seoi* that showed similar enzymatic activity.

5.4 Human infections

Human infections with *G. seoi* only have been recorded in the Republic of Korea (Fried et al., 2004; Chai et al., 2009; Toledo et al., 2019; Chai and Jung, 2020). This fluke was first discovered in 1988 in a Korean woman suffering pancreatitis and gastric discomfort (Lee et al. 1993a; Chai et al., 2003). Currently, it is now known to be distributed in 25 seashore villages of western and southern coastal islands and 3 coastal inlands of South Korea

(Chai et al., 2009). Oysters, *Crassostrea gigas* were verified to be the second intermediate hosts (Chai et al., 2003). Consumption of raw oysters is the main mode of human infection Chai and Jung (2020).

Although symptomatology induced by *G. seoi* in humans may vary greatly between patients, it appears to be considerably greater than in laboratory rodents. 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 *G. seoi* infection increases the levels of amylase in the serum and urine. In two patients, the infection was accompanied by diabetes mellitus suggesting a relationship between both diseases (Lee et al., 1995). Several cases of severe gastroenteritis and signs of acute pancreatitis have been reported and medical attention can be required in relation to pancreatic problems (Lee et al., 1993a; Lee and Chai, 2001; Chai et al., 2003). It has been also shown that *G. seoi* adult worms can invade gut lymphoid tissue (Seo et al., 2006).

6. Family Heteropyiidae

6.1 Background

Heterophyiasis is an intestinal trematode infection caused by several members of the family Heterophyidae. Heterophyids are small egg-shaped flukes possessing a gonotyle or genital sucker (Yamaguti, 1971) (Fig. 8). There is a lot of controversy regarding the classification of the family. Yamaguti (1971) accepted 13 subfamilies but, in contrast, Pearson (2008) did not include any subfamily and accepts a total of 32 genera and several *incertae sedis*. In the present review we will be focused on those genera for which there are studies on immunology and pathology in the definitive host, specifically the genera *Centrocestus, Haplorchis, Heterophyes, Metagonimus* and *Pygidiopsis*.

The definitive host becomes infected by eating raw or poorly cooked fish harboring metacercariae. The adult worms inhabit between the villi of the anterior region of the small intestine and release embryonated eggs into water. The eggs are then ingested often by littorine snails hatching within the snail's intestine. Intramolluscan development comprises sporocyst and redial stages and cercariae are released into the water where they tipically 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, 2019).

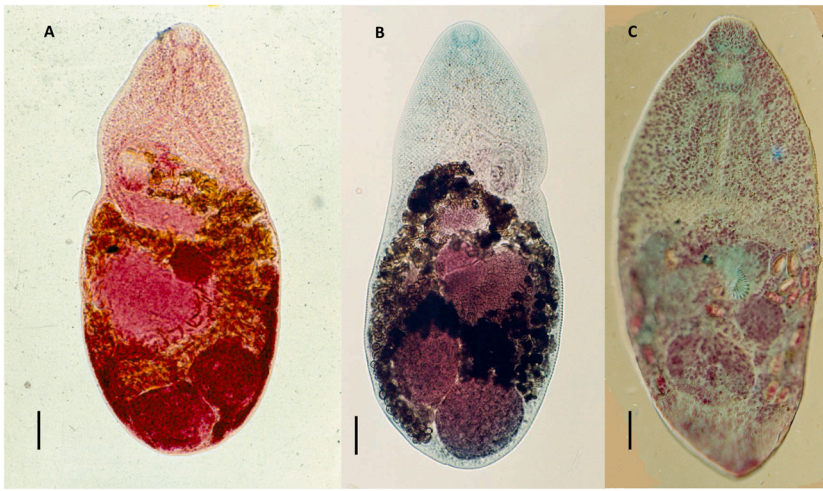

Fig. 8 (A) Adult worm of *Metagonimus yokogawai* from an experimentally infected dog necropsied at 14 days post-infection; the worm was stained in acetocarmine. (B) Adult worm of *Heterophyes heterophyes* from a human infection. The worm was stained in acetocarmine; (C) Adult worm of *Haplorchis taichui* from a human infection. The worm was stained in acetocarmine. Scale bars: 50 μm. *(A) Photomicrograph courtesy Jong-Yil Chai. (A and C) Reproduced with permission from Toledo, R., Esteban, J.G., Fried, B., 2006a. Immunology and pathology of intestinal trematodes in their definitive hosts. Adv. Parasitol. 63, 285–365.*

6.2 Pathology of the infection

Heterophyiasis normally presents with mild symptoms, except in the case of heavy infections or in immunocompromised patients (Chai and Lee, 2002). The most common symptoms are are malabsorption, abdominal pain, mucus-rich feces, dyspepsia, anorexia, nausea and vomiting and diarrhea (Lee et al., 1981; Kang et al., 1983; Cho et al., 1985; Snyder et al., 1989; Hong et al., 1990; Yu et al., 1997; Toledo et al., 2019). The pathological features of heterophyiid infections have been studied in several hosts, including cats, mice, dogs, rats, and humans.

Typically, adult worms live in the duodenum and proximal jejunum (Fig. 9), though in heavy infections the location of the worms tends to extend more distally (Lee et al., 1981). In early stages of the infection, the worms are located in the glandular lumens of the crypts of Lieberkühn, whereas in the later phases they are found between the villi (Jang et al., 1985; Chai et al., 1995). The main pathological features are villous atrophy, characterized by fusion, thickening and shortening of the villi, hypertrophy

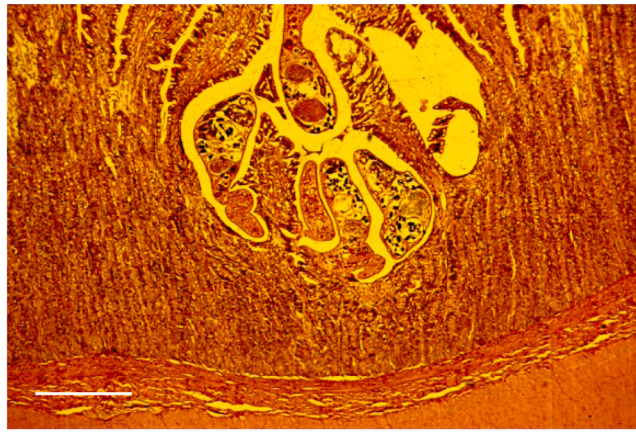

Fig. 9 Small intestine of a dog experimentally infected with *Metagonimus yokogawai* and necropsied at 7 days post-infection; the preparation was stained with hematoxylin and eosin. Scale bar: 50 μm. *Photomicrograph courtesy Jong-Yil Chai. Reproduced with permission from Toledo, R., Esteban, J.G., Fried, B., 2006a. Immunology and pathology of intestinal trematodes in their definitive hosts. Adv. Parasitol. 63, 285–365.*

of the crypts of Lieberkühn, resulting in a decreasing of the villous/crypt ratio, enlargement of mesenteric lymph nodes, and inflammatory responses with lymphocytic infiltration (Chai, 1979; Chai et al., 1994, 1995; Lee et al., 1981; Kang et al., 1983; Rho et al., 1984; Hong et al., 1997; Yu et al., 1997; Saenphet et al., 2008; Chai et al., 2014; Nada et al., 2017). Ashour et al. (2014) showed that atrophic villi covered by poorly differentiated epithelial cells were observed in the 2nd week post-infection in mice with *Heterophyes 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-κB showed positive staining in the intestinal epithelial cells with varying grades of intensity over the length of infection.

In addition to the mechanical pressure exerted by the parasite itself, it seems that there are other factors responsible for villous atrophy. In the case of *Centrocestus armatus*, the circumoral spines present in this parasite was suggested as the cause for mechanical damage (Hong et al., 1997). Moreover, bulky foamy intestinal content and intraluminal pressure by gas and mucous content caused villous atrophy in *M. yokogawai*-infected rats or the increased number of intraepithelial lymphocytes can also be the cause (Lee et al., 1981; Chai et al., 1994). Decreased levels of proliferating cell and the subsequent reduction in the population of proliferative cells may be

implicated. Yu et al. (1997) suggested that *M. yokogawai* induces inhibition of cell proliferation in the intestinal crypts in the early stages of the infection.

Probably, the villous atrophy is the main cause of the malabsortion and watery diarrhea that characterize metagonimiasis. The distribution of watery content along the intestine of dogs experimentally infected with *M. yokogawai* suggested that it was the product of poor digestion due to villous atrophy (Cho et al., 1985). This is supported by the changes observed in the brush border enzyme activities in *M. yokogawai*-infected mice (Hong et al., 1990). The nature of these changes was different in each intestinal segment. The activities of disaccharidases, alkaline phosphatase, and L-leucine aminopeptidase were decreased in the duodenum and proximal jejunum, but increased in the distal jejunum. The reduced activity in the areas which constitutes the main habitat of the parasite was explained by the damage of the enterocytes at the upper layer of the crypts and the reduction of the mucosal surface area due to villous changes. In contrast, the greater activity in the distal jejunum could be a compensatory mechanism due to increased concentrations of substrates in relation to the malabsorption in the more proximal areas of the small intestine (Hong et al., 1990).

6.3 Immunology of the infection

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).

Inflammatory cell infiltration is a common feature in infections with *M. yokogawai* (Lee et al., 1981; Kang et al., 1983; Chai et al., 1995; Hong et al., 1997). In dogs, the cell infitration was mainly composed of eosinophils, lymphocytes, and plasma cells (Kang et al., 1983), whereas neutrophils were the main cellular component in mice (Chai et al., 1995). Interestingly, the cell infiltration remained after the restoration of the villi in *M. yokogawai*-infected cats (Lee et al., 1981). *Haplorchis taichui* infection in rats is characterized by mastocytosis and eosinophilia. In fact, mucosal mast cells were thought to be the most relevant effector mechanism against infection since levels of local mastocytosis were positively correlated with worm recovery over the course of the infection. Mastocytosis was also observed in *Pygidiopsis summa*-infected mice, accompanied with goblet cell hyperplasia (Chai et al., 2014). Increases in intraepithelial lymphocytes also

appear to be a common feature in heterophiid infections (Chai et al., 2014; Nada et al., 2017). In fact, Chai et al. (2014) suggested that T cells play a major role in the response against these infections.

Ectopic locations have been also described by spreading beyond the intestine in heavy infections (Elsheikha, 2007). Ectopic infections commonly involve the brain, spinal cord and heart (Africa and Garcia, 1935; Africa et al., 1936a; Africa et al., 1936b; Gallais et al., 1955; Collomb and Bert, 1959; Tantachamrun and Kliks, 1978; Chai et al., 1984; Zhang and Fan, 1990).

It is well known that both systemic and local antibody responses occur in infections with heterophyids. The specific IgG levels in the serum of *M. yokogawai*-infected cats rise from 7 days post-infection and the maximum levels were observed at 2–4 weeks post-infection with both antigens (Cho et al., 1987). Moreover, marked increases of IgA and IgE were observed in *P. summa*-infected mice and *H. taichui*-infected rats, respectively (Saenphet et al., 2008; Chai et al., 2014). There is some controversy on the potential role on the antibody response in the course of heterophyid infections. Cho et al. (1987) detected that specific IgG levels were directly related to the number of *M. yokogawai* adult worms in experimentally infected cats. In contrast, 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). Saenphet et al. (2008) considered the IgE response in *H. taichui*-infected rats as a secondary response.

6.4 Antigenic characterization

Antigens of heterophyids have been poorly studied. Ahn et al. (1991) determined that the antigenic localization in *M. yokogawai* adult parasite was determined using the serum of cats experimentally infected with the metacercariae. It was suggested that the major antigenic protein was specifically concentrated at the tegumental syncytium as well as in the cytoplasm of tegumental cells and epithelial lamella of the cecum.

Han et al. (2014) analyzed the crude extracts of *M. yokogawai* adult worms. This crude antigen consisted of major 100, 67, 46, 28–35, and 17 kDa bands. In western blot analysis, several antigenic proteins reacted with the sera of human patient at 100, 67, 46, 30, and 20 kDa. No special reactive bands were visible in healthy controls. Among these proteins bands, 100 kDa and 67 kDa were those who reacted most frequently against sera of infected humans. Furthermore, they described a 100 kDa

antigen that was recognized by sera from M. yokogawai-infected humans, but also by sera from patients of other helminth infections such as *G. seoi, Paragonimus westermani, Clonorchis sinensis* and *Fasciola hepatica*.

Using indirect immunoperoxidase staining was performed with monoclonal antibodies developed against this protein, a strong positive reaction was observed in the tegumental and subtegumental layers of adult *M. yokogawai* and *C. sinensis*. The authors suggested that the 100 kDa somatic protein of *M. yokogawai* is a common antigen which recognizes a target epitope present over the tegumental layer of different trematode species (Han et al., 2014).

6.5 Human infections

A total of 30 species of heterophyids have been reported infecting humans (Table 3), probably being *H. heterophyes* and *M.yokogawai* the most relevant species involved in human disease (Toledo et al., 2019). Humans become infected by eating fish or shrimps harboring viable metacercariae, which mature into adults in 5–10 days. Adult heterophyids attach and live embedded the intestinal mucosa producing embryonated eggs that reach water reservoir allowing for the maintenance of the life cycle.

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).

Anaphylaxis was developed by a woman after infection with *H. heterophyes* after eating raw fish in Spain (Martínez-Alonso et al., 1999). Occasionally worm eggs may enter the circulatory system via the crypts of Lieberkhün which may be fatal (Marty and Andersen, 2000; Sripa et al., 2010). Eggs can be transported to other organs and they have been found in cardiac muscles in autopsies, spinal cord or brain, where they become trapped and elicit granulomatous lesions and fibrosis. Signs of heterophyid myocarditis may include subepicardial edema, epicardium hemorrhage, fragmentation of muscle fibers, cardiac enlargement, embolism of the capillaries, cough, dyspnea, cyanosis, fatigue, edema and ascites, palpitation, loss of reflexes and abnormal heart sounds. Eggs or worms in the spinal cord or brain may cause neurological disease, transverse myelitis and loss of sensory and motor function (Sripa et al., 2010). Recently, *H. taichui* has been identified as a possible etiologic causative agent of irritable Bowel syndrome-like symptoms

Table 3 Species of Heterophyidae involved in human infections.

Species	Geographical distribution of human cases
Acanthotrema felis	Korea
Apophallus. donicus	USA
Ascocotyle longa	Brazil
Centrocestus. armatus	Japan, Korea
Centrocestus caninus	Thailand
Centrocestus cuspidatus	Egypt
Centrocestus. formosanus	Japan, Taiwan, Vietnam, Lao PDR
Centrocestus kurokawai	Japan
Cryptocotyle lingua	Greenland
Haplorchis pleurolophocerca	Egypt
Haplorchis pumilio	China, Egypt, Iran, Korea, Lao PDR, Philippines, Taiwan, Thailand, Vietnam
Haplorchis taichui	China, Iran, Lao PDR, Philippines, Thailand, Vietnam
Haplorchis vanissimus	Philippines
Haplorchis yokogawai	China, Egypt, India, Indonesia, Lao PDR, Malaysia, Philippines, Taiwan, Thailand
Heterophyes dispar	Korea
Heterophyes heterophyes	Egypt, India, Iran, Korea, Sudan
Heterophyes nocens	China, Japan, Korea
Heterophyopsis continua	Japan, Korea
Metagonimus katsuradai	Japan, Russia
Metagonimus minutus	Taiwan
Metagonimus miyatai	Japan, Korea
Metagonimus pusills	Russia

(*continued*)

Table 3 Species of Heterophyidae involved in human infections. (*cont'd*)

Species	Geographical distribution of human cases
Metagonimus suifunensis	Russia
Metagonimus takahashii	Korea
Metagonimus yokogawai	All Far East, India, Balkan states, Israel, Iran, Korea, Siberia, Spain
Procerovum calderoni	Africa, China, Philippines
Procerovum varium	India, Lao PDR
Pygidiopsis genata	Egypt
Pygidiopsis summa	Japan, Korea
Stellanchasmus falcatus	Hawaii, Japan, Korea, Palestine, Philippines, Thailand, Vietnam
Stellanchasmus pseudocirratus	Hawaii, Philippines
Stictodora fuscata	Korea
Stictodora lari	Korea

The source of infection for all the species is commonly freshwater snails or brackish water fishes. Adapted from Toledo, R., Alvárez-Izquierdo, M., Muñoz-Antoli, C., Esteban, J.G., 2019. Intestinal trematode infections. Adv. Exp. Med. Biol. 1154, 181–213.

(Watthanakulpanich et al., 2010). Arya et al. (2016) described pediatric granulomatous eye disease caused by *Pocerovum varium* cercariae in 42 children with a history of exposure to village ponds and river waters in South India.

7. Concluding remarks

This review introduces the reader to the pathological and immunological aspects of intestinal trematodes in their definitive hosts. In this sense, special emphasis has been placed on members of the family Echinostomatidae, since it is the group of intestinal trematodes in which the most studies have been done. Information on the topics covered in the review is relatively sparse compared with that on other helminths, particularly the intestinal nematodes. It is difficult to ascertain the reasons

behind the lack of research on these topics. Many of the species are not considered of clinical importance and are only locally endemic. This may explain in part the minimal attention given to these helminths. However, many of the species considered in the review cause significant pathology and elicit an immune response.

Moreover, recent studies have shown that intestinal flukes can be a great model for the study of immune reactions in the intestinal mucosa and their influence on the course of infections at this level. These studies have contributed to increasing our knowledge about aspects such as the role of IL-25 in generating resistance to infections, the regulation of these responses, the actual involvement of antibodies in the infections or, even, the interactions between the trinomial parasite/host immune system/resident microbiota. and their influence on the infection. Therefore, studies using intestinal trematodes as experimental models, may be useful to analyze the immune mechanisms involved in the generation of resistance or susceptibility to intestinal pathogens.

Acknowledgments

The present work was supported by Universitat de València (Valencia, Spain) (grant number: UV-INV_AE-2637588). PC was supported by a María Zambrano fellowship from of Ministerio de Universidades (Madrid, Spain), European Union-Next generation EU and EF (Universitat de València). EF was supported by a fellowship from the ACIF-Grisolía program from the Generalitat Valencian (Spain).

References

Africa, C.M., Garcia, E.Y., 1935. Heterophyid trematodes of man and dog in the Philippines with descriptions of three new species. Philippine J. Sci. 57, 253.

Africa, C.M., De Leon, W., Garcia, E.Y., 1936a. Heterophyidiasis: IV. Lesions found in the myocardium of eleven infested hearts including three cases valvular involvement. *The Philippine*. J. Public Health 3, 1–2.

Africa, C.M., De Leon, W., Garcia, E.Y., 1936b. Heterophyidiasis: VI. Two more cases of heart failure associated with the presence of eggs in sclerosed veins. J. Philippine Isl. Med. Assoc. 17, 605–610.

Agger, M.K., Simonsen, P.E., Vennervald, B.J., 1993. The antibody response in serum, intestinal wall and intestinal lumen of NMRI mice infected with *Echinostoma caproni*. J. Helminthol. 67, 169–178.

Ahn, H., Rim, H.J., Kim, S.J., 1991. Antigenic localities in the tissues of *Metagonimus yokogawai* observed by immunogoldlabeling method. Korean J. Parasitol. 29, 245–257 (in Korean).

Álvarez-Izquierdo, M., Pérez-Crespo, M., Esteban, J.G., Muñoz-Antoli, C., Toledo, R., 2020a. Interleukin-25-mediated resistance against intestinal trematodes does not depend on the generation of Th2 responses. Parasites Vectors 13, 1–13.

Álvarez-Izquierdo, M., Guillermo Esteban, J., Muñoz-Antoli, C., Toledo, R., 2020b. Ileal proteomic changes associated with IL-25-mediated resistance against intestinal trematode infections. Parasites Vectors 13, 336.

Álvarez-Izquierdo, M., Fiallos, E., Cociancic, P., Esteban, J.G., Muñoz-Antoli, C., Toledo, R., 2022. Changes in resident microbiota associated with mice susceptibility or resistance to the intestinal trematode *Echinostoma caproni*. Parasitology 149, 1781–1793.

Andresen, K., Simonsen, P.E., Andersen, B.J., Birch-Andersen, A., 1989. *Echinostoma caproni* in mice, shedding of antigens from the surface of an intestinal trematode. Int. J. Parasitol. 19, 111–118.

Andrews, A., Holloway, J.W., Holgate, S.T., Davies, D.E., 2006. IL-4 receptor α is an important modulator of IL-4 and IL-13 receptor binding: implications for the development of therapeutic targets. J. Immunol. 176, 7456–7461.

Angkasekwinai, P., Sodthawon, W., Jeerawattanawart, S., Hansakon, A., Pattanapanyasat, K., Wang, Y., 2017. ILC2s activated by IL-25 promote antigen-specific Th2 and Th9 functions that contribute to the control of *Trichinella spiralis* infection. PLoS One 12, e0184684.

Angkasekwinai, P., Srimanote, P., Wang, Y., Pootong, A., Sakolvaree, Y., Pattanapanyasat, K., et al., 2013. Interleukin-25 (IL-25) promotes efficient protective immunity against *Trichinella spiralis* infection by enhancing the antigen-specific IL-9 response. Infect. Immun. 81, 3731–3741.

Arya, L.K., Rathinam, S.R., Lalitha, P., Kim, U.R., Ghatani, S., Tandon, V., 2016. Trematode fluke *Procerovum varium* as cause of ocular inflammation in children, South India. Emerg. Infect. Dis. 22, 192–200.

Ashour, D.S., Othman, A.A., Radi, D.A., 2014. Insights into regulatory molecules of intestinal epithelial cell turnover during experimental infection by *Heterophyes heterophyes*. Exp. Parasitol. 143, 48–54.

Beaver, P.C., Little, M.D., Tucker, C.F., Reed, R.J., 1977. Mesocercaria in the skin of man in Louisiana. Am. J. Tropical Med. Hyg. 26, 422–426.

Bernal, D., Carpena, I., Espert, A.M., De la Rubia, J.E., Esteban, J.G., Toledo, R., et al., 2006. Identification of proteins in excretory/secretory extracts of *Echinostoma friedi* (Trematoda) from chronic and acute infections. Proteomics 6, 2835–2843.

Bieber, T., 2020. Interleukin-13: Targeting an underestimated cytokine in atopic dermatitis. Allergy 75, 54–62.

Bindseil, E., Christensen, N.Ø, 1984. Thymus-independent crypt hyperplasia and villous atrophy in the small intestine of mice infected with the trematode *Echinostoma revolutum*. Parasitology 88, 431–438.

Bolek, M.G., Detwiler, J.T., Stigge, H.A., 2019. Selected wildlife trematodes. Adv. Exp. Med. Biol. 1154, 321–355.

Brunet, L.R., Joseph, S., Dunne, D.W., Fried, B., 2000. Immune responses during the acute stages of infection with the intestinal trematode *Echinostoma caproni*. Parasitology 120, 565–571.

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

Butcher, A.R., Palethorpe, H.M., Grove, D.I., 2002a. The susceptibility of inbred mice to infection with *Brachylaima cribbi* (Digenea: Brachylaimidae). Parasitol. Int. 51, 109–115.

Butcher, A.R., Palethorpe, H.M., Grove, D.I., 2002b. Effects of sex and age on the susceptibility of C57BL/6J mice to infection with *Brachylaima cribbi* and the course of the infection in NOD SCID mice. Parasitol. Res. 88, 668–674.

Butcher, A.R., Palethorpe, H.M., Grove, D.I., 2003. Response to re-infection with *Brachylaima cribbi* in immunocompetent and immunodeficient mice. Parasitol. Int. 52, 219–228.

Butcher, A.R., Parasuramar, P.S., Thompson, C.S., Grove, D.I., 1998. First report of the isolation of an adult worm of the genus *Brachylaima* (Digenea: Brachylaimidae), from the gastrointestinal tract of a human. Int. J. Parasitol. 28, 607–610.

Butcher, A.R., Talbot, G.A., Norton, R.E., Kirk, M.D., Cribb, T.H., Forsyth, J.R.L., et al., 1996. Locally acquired *Brachylaima* sp. (Digenea: Brachylaimidae) intestinal fluke infection in two South Australian infants. Med. J. Aust. 164, 475–478.

Carpena, I., Espert, A., Muñoz-Antoli, C., Marcilla, A., Esteban, J.G., Toledo, R., 2007. Antigenic characterization of *Echinostoma friedi* (Trematoda) in hamsters and rats experimentally infected. Rev. Ibérica de. Parasitología 67, 127–134.

Chai, J.Y., 1979. Study on *Metagonimus yokogawai* (Katsurada, 1912) in Korea. V. Intestinal pathology in experimentally infected albino rats. Seoul J. Med. 20, 104–117.

Chai, J.Y., Choi, M.H., Yu, J.R., Lee, S.H., 2003. *Gymnophalloides seoi*, a new human intestinal trematode. Trends Parasitol. 19, 109–112.

Chai, J.Y., Jung, B.K., 2020. Foodborne intestinal flukes: a brief review of epidemiology and geographical distribution. Acta Tropica 201, 105210.

Chai, J., Cho, J., Chang, T., Jung, B., Sohn, W., 2020. Taxonomy of *Echinostoma revolutum* and 37-collar-spined *Echinostoma* spp.: a historical review. Korean J. Parasitol. 58, 343.

Chai, J.Y., Chung, W.J., Kook, J., Seo, M., Park, Y.K., Guk, S.M., et al., 1999. Growth and development of *Gymnophalloides seoi* in immunocompetent and immunosuppressed C3H/HeN mice. Korean J. Parasitol. 37, 21–26.

Chai, J.Y., Kim, T.K., Cho, W.H., Seo, M., Kook, J., Guk, S.M., et al., 1998. Intestinal mastocytosis and goblet cell hyperplasia in BALB/c and C3H mice infected with *Neodiplostomum seoulense*. Korean J. Parasitol. 36, 109–119.

Chai, J.Y., Kim, J., Lee, S.H., 1995. Invasion of *Metagonimus yokogawai* into the submucosal layer of the small intestine of immunosuppressed mice. Korean J. Parasitol. 33, 313–321.

Chai, J.Y., Lee, S.H., 2002. Food-borne intestinal trematode infections in the Republic of Korea. Parasitol. Int. 51, 129–154.

Chai, J.Y., Lee, H.S., Hong, S.J., Yoo, J.H., Guk, S.M., Seo, M., et al., 2001. Intestinal histopathology and in situ postures of *Gymnophalloides seoi* in experimentally infected mice. Korean J. Parasitol. 39, 31–41.

Chai, J.Y., Park, Y.J., Park, J.H., Jung, B.K., Shin, E.H., 2014. Mucosal immune responses of mice experimentally infected with *Pygidiopsis summa* (Trematoda: Heterophyidae). Korean J. Parasitol. 52, 27–33.

Chai, J.Y., Seo, B.S., Lee, S.H., 1984. Study on *Metagonimus yokogawai* (Katsurada, 1912) In Korea. VII. Susceptibility of various strains f mice to *Metagonimus* infection and effect of prednisolone. Korean J. Parasitol. 22, 153–160.

Chai, J.Y., Shin, E.H., Han, E.T., Guk, S.M., Choi, M.H., Lee, S.H., 2000. Genetic difference in susceptibility and fatality of three strains of mice experimentally infected with *Neodiplostomum seoulense*. J. Parasitol. 86, 1140–1144.

Chai, J.Y., Shin, E.H., Lee, S.H., Rim, H.J., 2009. Foodborne intestinal flukes in Southeast Asia. Korean J. Parasitol. 47 (Suppl), S69–S102.

Chai, J.Y., Yun, T.Y., Kim, J., Huh, S., Choi, M.H., Lee, S.H., 1994. Chronological observation on intestinal histopathology and intraepithelial lymphocytes in the intestine of rats infected with *Metagonimus yokogawai*. Korean J. Parasitol. 32, 215–221 (in Korean).

Chang, Y.D., Sohn, W.M., Ryu, J.H., Kang, S.Y., Hong, S.J., 2005. A human infection of *Echinostoma hortense* in duodenal bulb diagnosed by endoscopy. Korean J. Parasitol. 43, 57–60.

Choi, M.H., Chai, J.Y., Lee, S.H., 1998a. Purification and characterization of a 16-kDa cysteine proteinase of *Gymnophalloides seoi* (Gymnophallidae) metacercariae. J. Parasitol. 84, 350–535.

Choi, M.H., Park, W.J., Park, Y.K., Chai, J.Y., Lee, S.H., 1998b. Isolation and characterization of a 40 kDa cysteine protease from *Gymnophalloides seoi* adult worms. Korean J. Parasitol. 36, 133–141.

Choi, M.H., Choe, S.C., Lee, S.H., 1999. A 54 kDa cysteine protease purified from the crude extract of *Neodiplostomum seoulense* adult worms. Korean J. Parasitol. 37, 39–46.

Cho, S.Y., Kim, S.I., Earm, Y.E., Ho, W.K., 1985. A preliminary observation on watery content of small intestine in *Metagonimus yokogawai* infected dog. Korean J. Parasitol. 23, 175–177.

Cho, S.Y., Kim, S.I., Kang, S.Y., 1987. Specific IgG responses in experimental cat metagonimiasis. Korean J. Parasitol. 25, 149–153.

Cho, Y.K., Ryang, Y.S., Kim, I.S., Park, S.K., Im, J.A., Lee, K.J., 2007. Differential immune profiles following experimental *Echinostoma hortense infection* in BALB/c and C3H/HeN mice. Parasitol. Res. 100, 1053–1061.

Collomb, H., Bert, J., 1959. Parasitic epilepsy of unusual origin: distomiasis (due to *Heterophyes heterophyes*). Neurochirurgie 5, 330–333.

Cortés, A., Mikeš, L., Muñoz-Antolí, C., Álvarez-Izquierdo, M., Esteban, J.G., Horák, P., et al., 2019. Secreted cathepsin L-like peptidases are involved in the degradation of trapped antibodies on the surface of *Echinostoma caproni*. Parasitol. Res. 118, 3377–3386.

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

Cortés, A., Muñoz-Antoli, C., Esteban, J.G., Toledo, R., 2017a. Th2 and Th1 responses: clear and hidden sides of immunity against intestinal helminths. Trends Parasitol. 33, 678–693.

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

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

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

Cortés, A., Muñoz-Antoli, C., Martín-Grau, C., Esteban, J.G., Grencis, R.K., Toledo, R., 2015c. Differential alterations in the small intestine epithelial cell turnover during acute and chronic infection with *Echinostoma caproni* (Trematoda). Parasites Vectors 8, 334.

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

Cortés, A., Sotillo, J., Muñoz-Antolí, C., Martín-Grau, C., Esteban, J.G., Toledo, R., 2016a. Resistance against *Echinostoma caproni* (Trematoda) secondary infections in mice is not dependent on the ileal protein production. J. Proteom. 140, 37–47.

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

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

Ditrich, O., Kopacek, P., Giboda, M., Gutvirth, J., Scholz, T., 1991. Serological differentiation of human small fluke infections using *Opisthorchis viverrini* and *Haplorchis taichui* antigens. Southeast. Asian J. Tropical Med. Public Health 22, 174–178.

Dubois, G., 1970. Synopsis des Strigeidae et Diplostomatidae (Trematoda). Mémoires de. la. Société Neuchâteloise des. Sci. Naturelles 10, 259–727.

El-Ganayni, G.A., Youssef, M.E., Handousa, A.E., Abou-Zakham, A.A., Hegazi, M.M., 1989. Serum and intestinal immunoglobulins in heterophyiasis. J. Egypt. Soc. Parasitol. 19, 219–223.

Elsheikha, H.M., 2007. Heterophyosis: risk of ectopic infection. Vet. Parasitol. 147, 341–342.

Esteban, J.G., Muñoz-Antolí, C., 2009. Echinostomes: systematics and life cycles. In: Fried, B., Toledo, R. (Eds.), The Biology of Echinostomes. From the Molecule to the Community. Springer, New York, pp. 1–34.

Esteban, J.G., Muñoz-Antoli, C., Toledo, R., Ash, L.R., 2019. Diagnosis of human trematode infections. Adv. Exp. Med. Biol. 1154, 437–471.

Fallon, P.G., Ballantyne, S.J., Mangan, N.E., Barlow, J.L., Dasvarma, A., Hewett, D.R., et al., 2006. Identification of an interleukin (IL)-25–dependent cell population that provides IL-4, IL-5, and IL-13 at the onset of helminth expulsion. J. Exp. Med. 203, 1105–1116.

Fernandes, B.J., Cooper, J.D., Cullen, J.B., Freeman, R.S., Ritchie, A.C., Scott, A.A., et al., 1976. Systemic infection with *Alaria americana* (Trematoda). Can. Med. Assoc. J. 115, 1111–1114.

Freeman, R.S., Stuart, P.F., Cullen, J.B., Ritchie, A.C., Mildon, A., Fernandes, B.J., et al., 1976. Fatal human infection with mesocercariae of the trematode *Alaria americana*. Am. J. Tropical Med. Hyg. 25, 803–807.

Fried, B., Graczyk, T.K., Tamang, L., 2004. Food-borne intestinal trematodiases in humans. Parasitol. Res. 93, 159–170.

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

Fujino, T., Fried, B., 1993b. *Echinostoma caproni* and *E. trivolvis* alter the binding of glycoconjugates in the intestinal mucosa of C3H mice as determined by lectin histochemistry. J. Helminthol. 67, 179–188.

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

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

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

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

Gallais, P., Paillas, P., Collomb, H., Luigi, D.M., Demarchi, J., Deschiens, R., 1955. [Anatomo-pathological study of a parasitic cerebral cyst in man. Bull. de. la. Societe de Pathologie Exotique 48, 830–832.

Garg, G., Bernal, D., Trelis, M., Forment, J., Ortiz, J., Valero, M.L., et al., 2013. The transcriptome of *Echinostoma caproni* adults: further characterization of the secretome and identification of new potential drug targets. J. Proteom. 89, 202–214.

Gerbe, F., Sidot, E., Smyth, D.J., Ohmoto, M., Matsumoto, I., Dardalhon, V., et al., 2016. Intestinal epithelial tuft cells initiate type 2 mucosal immunity to helminth parasites. Nature 529, 226–230.

Gibson, D.I., Bray, R.A., 1994. The evolutionary expansion and host-parasite relationship of the Digenea. Int. J. Parasitol. 24, 1213–1226.

Gibson, D.I., 1996. Trematoda. In: Margolis, L., Kabata, Z. (Eds.), *Guide to the Parasites of Fishes of Canada. Part IV. Canadian Special Publication of Fisheries and Aquatic Sciences* 124. NRC Press, Ottawa, pp. 1–373.

Giuffrida, P., Caprioli, F., Facciotti, F., Di Sabatino, A., 2019. The role of interleukin-13 in chronic inflammatory intestinal disorders. Autoimmun. Rev. 18, 549–555.

Graczyk, T.K., Fried, B., 1998. Echinostomiasis: a common but forgotten food-borne disease. Am. J. Tropical Med. Hyg. 58, 501–504.

Grencis, R.K., 2015. Immunity to helminths: resistance, regulation, and susceptibility to gastrointestinal nematodes. Annu. Rev. Immunol. 33, 201–225.

Guk, S.M., Lee, J.H., Kim, H.J., Kim, W.H., Shin, E.H., Chai, J.Y., 2009. CD4+ T-cell-dependent goblet cell proliferation and expulsion of *Gymnophalloides seoi* from the intestine of C57BL/6 mice. J. Parasitol. 95, 581–590.

Hansen, K., Nielsen, J.W., Hindsbo, O., Christensen, N. Ø, 1991. *Echinostoma caproni* in rats: worm population dynamics and host blood eosinophilia during primary infections with 6, 25 and 50 metacercariae and resistance to secondary and superimposed infections. Parasitol. Res. 77, 686–690.

Han, E.T., Chen, J.H., Chai, J.Y., 2008. Antibody responses in sera of different mouse strains experimentally infected with *Neodiplostomum seoulense*. Korean J. Parasitol. 46, 279–283.

Han, E.T., Yang, H.J., Park, Y.J., Park, J.H., Chai, J.Y., 2014. *Metagonimus yokogawai*: a 100-kDa somatic antigen commonly reacting with other trematodes. Korean J. Parasitol. 52, 201–204.

Harris, N.L., 2017. Recent advances in type-2-cell-mediated immunity: insights from helminth infection. Immunity 47, 1024–1036.

Higón, M., Monteagudo, C., Fried, B., Esteban, J.G., Toledo, R., Marcilla, A., 2008. Molecular cloning and characterization of *Echinostoma caproni* heat shock protein-70 and differential expression in the parasite derived from low- and high-compatible hosts. Parasitology 135, 1469–1477.

Hong, S.T., Chai, J.Y., Lee, S.H., 1984a. Ten human cases of *Fibricola seoulensis* infection and mixed one with *Stellantchasmus* and *Metagonimus*. Korean J. Parasitol. 24, 95–97.

Hong, S.T., Cho, T.K., Hong, S.J., Chai, J.Y., Lee, S.H., Seo, B.S., 1984b. Fifteen human cases of *Fibricola seoulensis* infection in Korea. Korean J. Parasitol. 22, 61–65.

Hong, S.J., Han, J.H., Park, C.K., Kang, S.Y., 1997. Intestinal pathologic findings at early stage infection by *Centrocestus armatus* in albino rats. Korean J. Parasitol. 35, 135–138.

Hong, S.T., 1982. Studies on intestinal trematodes in Korea VII. Growth, development and recovery of *Fibricola seoulensis* from experimentally infected rats and mice. Korean J. Parasitol. 20, 112–121.

Hong, S.T., Yu, J.R., Myong, N.H., Chai, J.Y., Lee, S.H., 1990. Activities of brush border membrane bound enzymes of the small intestine in *Metagonimus yokogawai* infection in mice. Korean J. Parasitol. 29, 9–20.

Howitt, M.R., Lavoie, S., Michaud, M., Blum, A.M., Tran, S.V., Weinstock, J.V., et al., 2016. Tuft cells, taste-chemosensory cells, orchestrate parasite type 2 immunity in the gut. Science 351, 1329–1333.

Huang, Y., Guo, L., Qiu, J., Chen, X., Hu-Li, J., Siebenlist, U., et al., 2015. IL-25-responsive, lineage-negative KLRG1 hi cells are multipotential 'inflammatory'type 2 innate lymphoid cells. Nat. Immunol. 16, 161–169.

Huh, S., Chai, J.Y., Hong, S.T. and, Lee, S.H., 1988. Clinical and histopathological findings in mice heavily infected with *Fibricola seoulensis*. Korean J. Parasitol. 26, 45–53.

Huh, A., Lee, S.U., Won, M.H., Jeong, Y.G., Kwon, Y.H., Choi, C.S., 1995. IgA response in mice infected with *Neodiplostomum seoulensis*. Korean J. Parasitol. 33, 55–60.

Jang, Y.K., Kang, S.Y., Kim, S.I., Cho, S.Y., 1985. In situ posture of anterior body of *Metagonimus yokogawai* in experimentally infected dog. Korean J. Parasitol. 23, 203–213.

Kang, S.Y., Cho, S.Y., Chai, J.Y., Lee, J.B., Jang, D.H., 1983. A study on intestinal lesions of experimentally reinfected dogs with Metagonimus yokogawai. The Korean Journal of Parasitology 21, 53–73.

Khanna, V., Ashraf, A.A., Khanna, R., 2019. Echinostomiasis in a child with severe anemia. Tropical Parasitol. 9, 54–56.

Kho, W.G., Chai, J.Y., Chun, C.H., Lee, S.H., 1990. Mucosal mast cell responses to experimental *Fibricola seoulensis* infection in rats. Seoul J. Med. 31, 191–199.

Kook, J., Nawa, Y., Lee, S.H., Chai, J.Y., 1998. Pathogenicity and lethality of a minute intestinal fluke, *Neodiplostomum seoulense*, to various strains of mice. J. Parasitol. 84, 1178–1183.

Kostadinova, A., Gibson, D., 2000. The systematics of the Echinostomes. In: Fried, B., Graczyk, T.K. (Eds.), Echinostomes as Experimental Models for Biological Research. Kluwer Academic Publishers, Dordrecht, pp. 31–57.

Kostadinova, A., 2005. Family Echinostomatidae Looss, 1899. In: Gibson, D.I., Jones, A., Bray, R.A. (Eds.), Keys to the Trematoda 2. CABI Publishing, Wallingford, pp. 9–64.

Kramer, M.H., Eberhard, M.L., Blankenberg, T.A., 1996. Respiratory symptoms and subcutaneous granuloma caused by mesocercariae: a case report. Am. J. Tropical Med. Hyg. 55, 447–448.

Lee, S.H., Chai, J.Y., 2001. A review of *Gymnophalloides seoi* (Digenea: Gymnophallidae) and human infections in the Republic of Korea. Korean J. Parasitol. 39, 85–118.

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

Lee, S.C., Chung, Y.B., Kong, Y., Kang, S.Y., Cho, S.Y., 1993b. Antigenic protein fractions of *Metagonimus yokogawai* reacting with patient sera. Korean J. Parasitol. 31, 43–48.

Lee, S.H., Chai, J.Y., Seo, M., Choi, M.H., Kim, D.C., Lee, S.K., 1995. Two cases of *Gymnophalloides seoi* infection accompanied by diabetes mellitus. Korean J. Parasitol. 33, 61–64.

Lee, J.B., Chi, J.G., Lee, S.K., Cho, S.Y., 1981. Study on the pathology of metagonimiasis in experimentally infected cat intestine. Korean J. Parasitol. 19, 109–129.

Lee, K.D., Guk, S.M., Chai, J.Y., 2010. Toll-like receptor 2 and Muc2 expression on human intestinal epithelial cells by *Gymnophalloides seoi* adult antigen. J. Parasitol. 96, 58–66.

Lee, S.H., Jung, B.K., Park, J.H., Shin, E.H., Chai, J.Y., 2014. Increased intestinal epithelial cell turnover and intestinal motility in *Gymnophalloides seoi*-infected C57BL/6 mice. Korean J. Parasitol. 52, 273–280.

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

Lee, S.H., Park, S.K., Seo, M., Guk, S.M., Choi, M.H., Chai, J.Y., 1997b. Susceptibility of various species of animals and strains of mice to *Gymnophalloides seoi* infection and the effects of immunosuppression in C3H/HeN mice. J. Parasitol. 83, 883–886.

Lee, S.H., Yoo, B.H., Hong, S.T., Chai, J.Y., Seo, B.S., Chi, J.G., 1985. A histopathological study on the intestine of mice and rats experimentally infected by *Fibricola seoulensis*. Korean J. Parasitol. 23, 58–72.

Li, Y., Qiu, Y., Zeng, M., Diao, P., Chang, Q., Gao, Y., et al., 2019. The complete mitochondrial genome of *Echinostoma miyagawai*: comparisons with closely related species and phylogenetic implications. Infect. Genet. Evol. 75, 103961.

Marcilla, A., Rubia, J.E., Espert, A., Carpena, I., Esteban, J.G., Toledo, R., 2004. Specific tyrosine phosphorylation in response to bile in *Fasciola hepatica* and *Echinostoma friedi*. Exp. Parasitol. 106, 56–58.

Martínez-Alonso, J.C., Armentia, A., Vega, J.M., Callejo, A., Gomez, A., 1999. Anaphylactic reaction concurrent with *Heterophyes heterophyes* infestation. Rev. Española de. Alergología e Inmunología Clínica 14, 37–39.

Marty, A.M., Andersen, E.M., 2000. Fasciolopsiasis and other intestinal trematodiasis. In: Meyers, W.M., Neafie, R.C., Marty, A.M., Wear, D.J. (Eds.), *Pathology of Infectious Diseases* I. Armed Forces Institute of pathology, American Registry of Pathology, Washington, DC, pp. 93–105.

McCormick, S.M., Heller, N.M., 2015. Commentary: IL-4 and IL-13 receptors and signaling. Cytokine 75, 38–50.

McDonald, H.R., Kazakos, K.R., Schatz, H., Johnson, R.N., 1994. Two cases of intraocular infection with *Alaria* mesocercariae (Trematoda). Am. J. Ophtalmol. 117, 447–455.

McGuckin, M.A., Lindén, S.K., Sutton, P., Florin, T.H., 2011. Mucin dynamics and enteric pathogens. Nat. Rev. Microbiol. 9, 265–278.

Muñoz-Antoli, C., Cortés, A., Santano, R., Sotillo, J., Esteban, J.G., Toledo, R., 2016a. Interleukin-25 induces resistance against intestinal trematodes. Sci. Rep. 6 (1), 12.

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

Muñoz-Antoli, C., Cortés, A., Sotillo, J., Fried, B., Esteban, J.G., Toledo, R., 2014. Differential expression and glycosylation of proteins in the rat ileal epithelium in response to *Echinostoma caproni* infection. J. Proteom. 101, 169–178.

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

Nada, S.M.M., Taha, A.A., Fathy, G.M., Mohammed, F.A., Abdel-Aziz, M.O., Rashed, H.H., 2017. Histopathological and immunohistochemical studies in experimental heterophyiasis and the role of praziquantel and aminoguanidine. J. Egypt. Soc. Parasitol. 47, 81–92.

Niewiadomska, K., 2002. Family Diplostomidae Poirier, 1886. In: Gibson, D.I., Jones, A., Bray, R.A. (Eds.), Keys to the Trematoda 1. CABI Publishing, Wallingford, pp. 167–196.

Owyang, A.M., Zaph, C., Wilson, E.H., Guild, K.J., McClanahan, T., Miller, H.R., et al., 2006. Interleukin 25 regulates type 2 cytokine-dependent immunity and limits chronic inflammation in the gastrointestinal tract. J. Exp. Med. 203, 843–849.

Pearson, J., 2008. Family Heterophyidae Leiper, 1909. In: Bray, R.A., Gibson, D.I., Jones, A. (Eds.), *Keys to the Trematoda* 3. CABI and Natural History Museum, Wallingford, pp. 113–141.

Pica, R., Castellano, C., Cilia, C., Errico, F.P., 2003. Intestinal fluke infections: the heterophyids. La. Clinica Terapeutica 154, 61–63.

Pojmansha, T., 2002. Family Brachylaimidae Joyeux & Foley, 1930. In: Gibson, D.I., Jones, A., Bray, R.A. (Eds.), Keys to the Trematoda 1. CABI Publishing, Wallingford, pp. 37–43.

Pyo, K.H., Kang, E.Y., Jung, B.K., Moon, J.H., Chai, J.Y., Shin, E.H., 2012. Depressed neuronal growth associated protein (GAP)-43 expression in the small intestines of mice experimentally infected with *Neodiplostomum seoulense*. Korean J. Parasitol. 50, 89–93.

Rho, I.H., Kim, S.I., Kang, S.Y., Cho, S.Y., 1984. [Observation on the pathogenesis of villous changes in early phase of experimental metagonimiasis]. Chung-Ang J. Med. 9, 67–77.

Roscoe, D.E., Huffman, J.E., 1982. Trematode (*Sphaeridotrema globules*)-induced ulcerative hemorrhagic enteritis in wild mute swans (*Cygnus olor*). Avian Dis. 26, 214–224.

Roscoe, D.E., Huffman, J.E., 1983. Fatal enteritis caused by *Sphaeridotrema globulus* (Trematoda: Psilostomidae) in whistling swan. J. Wildl. Dis. 19, 370–371.

Ryang, Y.S., Yang, E.J., Kim, J.L., Lee, K.J., Sung, H.J., Kim, J.B., et al., 2007. Immune response and inhibitory effect of ketotifen on the BALB/c and C3H/HeN mice infected with *Echinostoma hortense*. Parasitol. Res. 101, 1103–1110.

Saenphet, S., Wongsawad, C., Saenphet, K., Rojanapaibul, A., Vanittanakom, P., Chai, J.Y., 2008. *Haplorchis taichui*: worm recovery rate and immune responses in infected rats (*Rattus norvegicus*). Exp. Parasitol. 120, 175–179.

Scholz, T., 2002. Family Gymnophallidae Odhner, 1905. In: Gibson, D.I., Jones, A., Bray, R.A. (Eds.), Keys to the Trematoda 1. CABI Publishing, Wallingford, pp. 245–251.

Seo, M., Chun, H., Ahn, G., Jang, K.T., Guk, S.M., Chai, J.Y., 2006. A case of colonic lymphoid tissue invasion by *Gymnophalloides seoi* in a Korean man. Korean J. Parasitol. 44, 87–89.

Seo, M., Guk, S.M., Han, E.T., Chai, J.Y., 2003. Role of intestinal goblet cells in the expulsion of *Gymnophalloides seoi* from mice. J. Parasitol. 89, 1080–1082.

Seo, B.S., Lee, S.H., Hong, S.T., Hong, S.J., Kim, C.Y., Lee, H.Y., 1982. Studies on intestinal trematodes in Korea: V. A human case infected by *Fibricola seoulensis* (Trematoda: Diplostomatidae). Korean J. Parasitol. 20, 93–99.

Shin, E.H., Chai, J.Y., Lee, S.H., 2001. Extraintestinal migration of *Pharyngostomum cordatum* metacercariae in experimental rodents. J. Helminthol. 75, 285–290.

Shin, E.H., Im, T.K., Park, Y.K., Cho, J., Kim, J.L., Chai, J.Y., 2016. Fecundity reduction of BALB/c mice after survival from lethal *Neodiplostomum seoulense* infection. Parasitol. Res. 115, 2051–2059.

Shin, E.H., Kim, T.H., Hong, S.J., Park, J.H., Guk, S.M., Chai, J.Y., 2003. Effects of anti-allergic drugs on intestinal mastocytosis and worm expulsion of rats infected with *Neodiplostomum seoulense*. Korean J. Parasitol. 41, 81–87.

Shin, E.H., Lee, S.H., Kim, J.L., Chai, J.Y., 2007. T-helper-1 and T-helper-2 immune responses in mice infected with the intestinal fluke *Neodiplostomum seoulense*: their possible roles in worm expulsion and host fatality. J. Parasitol. 93, 1036–1045.

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

Simonsen, P.E., Andersen, B.J., 1986. *Echinostoma revolutum* in mice; dynamics of the antibody attack to the surface of an intestinal trematode. Int. J. Parasitol. 16, 475–482.

Simonsen, P.E., Vennervald, B.J., Birch-Andersen, A., 1990. *Echinostoma caproni* in mice: ultrastructural studies on the formation of immune complexes on the surface of an intestinal trematode. Int. J. Parasitol. 20, 935 -491.

Smith, K.A., Löser, S., Varyani, F., Harcus, Y., McSorley, H.J., McKenzie, A.N., et al., 2018. Concerted IL-25R and IL-4Rα signaling drive innate type 2 effector immunity for optimal helminth expulsion. eLife 7, e38269.

Snyder, D.E., Hamir, A.N., Hanlon, C.A., Rupprecht, C.E., 1989. *Phagicola angrense* (Digenea: Heterophyidae) as a cause of enteritis in a raccoon (*Procyon lotor*). J. Wildl. Dis. 25, 273–275.

Song, H., Jung, B.K., Cho, J., Chai, J.Y., 2018. Worm expulsion of *Gymnophalloides seoi* from C57BL/6 mice: role of metacercarial exosomes in upregulating TLR2 and MUC2 expression in intestinal tissues. Parasitol. Res. 117, 3309–3314.

Sotillo, J., Cortés, A., Muñoz-Antoli, C., Fried, B., Esteban, J.G., Toledo, R., 2014. The effect of glycosylation of antigens on the antibody responses against *Echinostoma caproni* (Trematoda: Echinostomatidae). Parasitology 141, 1333–1340.

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

Sotillo, J., Trelis, M., Cortes, A., Fried, B., Marcilla, A., Esteban, J.G., et al., 2011. Th17 responses in *Echinostoma caproni* infections in hosts of high and low compatibility. Exp. Parasitol. 129, 307–311.

Sotillo, J., Trudgett, A., Halferty, L., Marcilla, A., Esteban, J.G., Toledo, R., 2010. *Echinostoma caproni*: differential tegumental responses to growth in compatible and less compatible hosts. Exp. Parasitol. 125, 304–309.

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

Sripa, B., Kaewkes, S., Intapan, P.M., Maleewong, W., Brindley, P.J., 2010. Food-borne trematodiases in Southeast Asia epidemiology, pathology, clinical manifestation and control. *Adv. Parasitol.* 72, 305–350.

Tantachamrun, T., Kliks, M., 1978. Heterophyid infection in human ileum: report of three cases. Southeast. Asian J. Tropical Med. Public Health 9, 228–231.

Toledo, R., Álvarez-Izquierdo, M., Esteban, J.G., Muñoz-Antoli, C., 2022. Neglected food-borne trematodiases: echinostomiasis and gastrodiscoidiasis. Parasitology 149, 1319–1326.

Toledo, R., Alvárez-Izquierdo, M., Muñoz-Antoli, C., Esteban, J.G., 2019. Intestinal trematode infections. Adv. Exp. Med. Biol. 1154, 181–213.

Toledo, R., Espert, A., Carpena, I., Muñoz-Antoli, C., Fried, B., Esteban, J.G., 2004a. The comparative development of *Echinostoma caproni* (Trematoda: Echinostomatidae) adults in experimentally infected hamsters and rats. Parasitol. Res. 93, 439–444.

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

Toledo, R., Espert, A., Muñoz-Antoli, C., Fried, B., Esteban, J.G., 2004c. Immunological characterization of somatic and excretory/secretory antigens of *Echinostoma caproni* (Trematoda: Echinostomatidae) in experimentally infected rats. Comp. Parasitol. 71, 42–48.

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

Toledo, R., Esteban, J.G., 2016. An update on human echinostomiasis. Trans. R. Soc. Tropical Med. Hyg. 110, 37–45.

Toledo, R., Esteban, J.G., Fried, B., 2006a. Immunology and pathology of intestinal trematodes in their definitive hosts. *Adv. Parasitol.* 63, 285–365.

Toledo, R., Monteagudo, C., Espert, A., Fried, B., Esteban, J.G., Marcilla, A., 2006b. *Echinostoma caproni*: intestinal pathology in the golden hamster, a highly compatible host, and the Wistar rat, a less compatible host. Exp. Parasitol. 112, 164–171.

Toledo, R., 2009. Echinostomes in the definitive host: a model for the study of host-parasite relationships. In: Fried, B., Toledo, R. (Eds.), The Biology of Echinostomes. From the Molecule to the Community. Springer, New York, pp. 89–109.

Toledo, R., Esteban, J.G., Fried, B., 2009. Chapter 3. Recent advances in the biology of echinostomes. *Adv. Parasitol.* 69, 147–204.

Toledo, R., Fried, B., 2005. Echinostomes as experimental models in adult parasite-vertebrate host interactions. Trends Parasitol. 21, 251–254.

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

von Moltke, J., Ji, M., Liang, H., Locksley, R.M., 2016. Tuft-cell-derived IL-25 regulates an intestinal ILC2–epithelial response circuit. Nature 529, 221–225.

Watthanakulpanich, D., Waikagul, J., Maipanich, W., Nuamtanong, S., Sanguankiat, S., Pubampen, S., et al., 2010. *Haplorchis taichui* as a possible etiologic agent of irritable bowel syndrome-like symptoms. Korean J. Parasitol. 48, 225–229.

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

WHO, 2004. Food-Borne Trematode Infections in Asia. Manila, Philippines. World Health Organization Regional Office for the Western Pacific (WPRO-WHO), Manila.

Yamaguti, S., 1971. Synopsis of Digenetic Trematodes of Vertebrates. Keigaku Publishing Company, Tokyo.

Yamaguti, S., 1975. A Synoptical Review of the Life Histories of Digenetic Trematodes of Vertebrates: With Special Reference to the Morphology of their Larval Forms. Keigaku Publishing Company, Tokyo.

Yusuf, S., Nok, A.J., Ameh, D.A., Adelaiye, A.B., Balogun, E.O., 2005. Correlation of gastric mucosal damage with sialic acid profile in rats: effect of hydrochloric acid, pepsin and hypertonic saline. Cell Biochem. Funct. 23, 339–345.

Yu, J.R., Myong, N., Chai, J.Y., 1997. Expression patterns of proliferating cell nuclear antigen in the small intestine of mice infected with *Metagonimus yokogawai* and *Metagonimus* Miyata type. Korean J. Parasitol. 35, 239–244.

Zhang, S.L., Fan, G.H., 1990. [Case report: brain abscess caused by heterophyid eggs]. Chinese J. Parasitol. Parasitic Dis. 8, 178 (in Chinese).

Zhao, A., Urban, J.F., Sun, R., Stiltz, J., Morimoto, M., Notari, L., et al., 2010. Critical role of IL-25 in nematode infection-induced alterations in intestinal function. J. Immunol. 185, 6921–6929.