Acta Tropica 120S (2011) S158–S168

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Review

Opisthorchiasis and *Opisthorchis*-associated cholangiocarcinoma in Thailand and Laos

Banchob Sripa[a,*], Jeffrey M. Bethony[b], Paiboon Sithithaworn[c], Sasithorn Kaewkes[c], Eimorn Mairiang[d], Alex Loukas[e], Jason Mulvenna[e], Thewarach Laha[c], Peter J. Hotez[b], Paul J. Brindley[b,**]

[a] *Tropical Disease Research Laboratory (TDR), Department of Pathology, Khon Kaen University, Khon Kaen 40002, Thailand*
[b] *Department of Microbiology, Immunology & Tropical Medicine, George Washington University Medical Center, Washington, DC 20037, USA*
[c] *Department of Parasitology, Khon Kaen University, Khon Kaen 40002, Thailand*
[d] *Department of Radiology, Khon Kaen University, Khon Kaen 40002, Thailand*
[e] *Queensland Tropical Health Alliance, James Cook University, Cairns, Queensland 4878, Australia*

ARTICLE INFO

Article history:
Available online 23 July 2010

Keywords:
Opisthorchiasis
Opisthorchis
Clonorchis
Cholangiocarcinoma
Liver fluke
Liver cancer
Food-borne trematodiases
Anthelmintic
Praziquantel
Hepatobiliary disease

ABSTRACT

Liver fluke infection caused by *Opisthorchis viverrini* is a major public health problem in Thailand and the Lao People's Democratic Republic (Lao PDR; Laos). Currently, more than 600 million people are at risk of infection with these fish-borne trematodes and/or their close relatives. Opisthorchiasis has been studied extensively in Thailand, where about 8 million people are infected with the liver fluke. Here we review the pathogenesis, control and re-emergence of *O. viverrini* infection, in particular in Thailand and, to a lesser extent in Lao PDR given the contiguous geographical range of *O. viverrini* through these two regions. We also review the association of *O. viverrini* infection and cholangiocarcinoma, bile duct cancer, and highlight new findings on pathogenesis of liver fluke-induced cholangiocarcinogenesis. Last, we comment on national control strategies in Thailand for the control of *O. viverrini* infection aimed at reduction in the prevalence of *O. viverrini*-associated liver cancer in the longer term.

Contents

* Corresponding author at: Department of Microbiology, Immunology & Tropical Medicine, George Washington University Medical Center, Washington, DC 20037, USA. Fax: +1 202 994 2913.
** Corresponding author. Fax: +1 202 994 2913.
E-mail addresses: banchob@kku.ac.th (B. Sripa), paul.brindley@gwumc.edu (P.J. Brindley).

0001-706X/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.actatropica.2010.07.006

1. Opisthorchiasis is a neglected tropical disease

The neglected tropical diseases (NTDs) represent the most common parasitic infections affecting the world's poorest people (Hotez et al., 2007). In addition to their detrimental effects on the health, NTDs have a chronic debilitating effect by undermining the physical and cognitive development of individuals resident in areas endemic for NTDs, especially children and women of child bearing age. An especially deleterious effect has been shown on their educational performance and future economic productivity (Hotez et al., 2007, 2009). Hence, the NTDs are thought to contribute greatly to keeping the poor impoverished. Over the last 2 years, the major NTDs have been ranked according to their global prevalence (Hotez et al., 2007) and their disease burden, as measured in Disability Adjusted Life Years (DALYs) (Hotez et al., 2009). However, the impacts of lesser known NTDs have been overlooked; these include with liver fluke infection in East and Southeast Asia. New data from East Asia indicate that the disease burden brought about by infection with fish-borne liver flukes, *Opisthorchis viverrini* and the closely related *Clonorchis sinensis*, rivals the disease burden of the seven most common NTDs in their impact on this region (see Andrews et al., 2008; Shin et al., 2010). Indeed, it may be that because of the intense regional nature of the disease burden of this NTD, a region marked by sub-optimal or poor infrastructure and poverty, the true extent of the burden of disease of opisthorchiasis is considerably underestimated.

2. Life cycle of *O. viverrini*

The definitive hosts of *O. viverrini* are humans and other piscivorous mammals. The eggs, shed by adult worms, are deposited in the biliary tree of the infected person, enter the intestine, and are passed with the feces. On reaching water, the eggs are ingested by snails. Several species of the prosobranch gastropod genus *Bithynia* serve as the first intermediate host. Within the snail, the eggs metamorphose into sporocysts which reproduce asexually for 4–5 weeks, after which cercariae are shed into the water. These free-swimming forms penetrate the skin between the scales of freshwater fish. Numerous species of freshwater fish, mostly cyprinoids, serve as the second intermediate host (Fig. 1). After a few days in the fish muscle, cercariae encyst as metacercariae. Humans acquire the infection by ingesting raw or inadequately cooked infected fish. Influenced by digestive processes in the stomach and intestines, the metacercariae excyst in the duodenum and migrate though the ampulla of Vater into the bile duct, where they mature into adult worms over a period of a month. The adult fluke inhabits the biliary tract, generally localizing within the intrahepatic bile ducts. In humans, the adult fluke can survive for more than 20 years, which explains the persistent infection of long duration. Locations of endemic infection reflect the geographic distribution of the essential intermediate host snails (Sripa and Pairojkul, 2008).

The closely related liver flukes *Opisthorchis felineus* and *C. sinensis* have similar life cycles. Opisthorchiasis and clonorchiasis are prevalent in geographical regions where raw cyprinid fish (*O. viverrini*, *O. felineus* and *C. sinensis*) and/or shrimp (*C. sinensis*) are dietary staples. All three flukes establish in the bile ducts of the liver as well as extrahepatic ducts and the gall bladder of infected persons (Sripa et al., 2010).

3. Geographical distribution, prevalence and public health impact of opisthorchiasis

3.1. Geographical distribution of *O. viverrini* and relatives

The geographical distribution of opisthorchiasis is constrained by the presence of the requisite intermediate host snails and the second intermediate host fishes, and dietary preferences of local populations for uncooked or undercooked fish. *O. viverrini* infection is prevalent in Thailand and the Lao People's Democratic Republic (PDR), and also occurs in Cambodia and Vietnam. There is a marked geographical heterogeneity in the prevalence of *O. viverrini* infection among the four regions of Thailand; nowadays the highest prevalence occurs in the North and Northeast (below). In 1981, the Thailand nationwide prevalence of *O. viverrini* infection was 14%, but prevalence varied greatly by region – North (5.59%), Northeast (34.60%), Central (6.34%) and South (0.01%) (Harinasuta and Harinasuta, 1984). After intensive and continuous control activities over the subsequent two decades, prevalence had declined to 15.7% in the Northeast, leading to a corresponding decline in the national prevalence to 9.4% in 2001 (Jongsuksuntigul and Imsomboon, 2003).

At least 10 million people in Thailand and the Lao PDR remain infected with *O. viverrini*, with 80% of the Thai cases occurring in northern and northeastern Thailand. An unknown number of additional cases also occur in Cambodia and Vietnam (Shin et al., 2010). In the Lao PDR, *O. viverrini* infection occurs mainly in southern and central regions adjacent (i.e. on the opposite side of the Mekong River) to the endemic regions in northeastern Thailand (Parkin, 2006). Based on a nationwide survey of ~30,000 primary school children, in 17 provinces and the Municipality of Vientiane, the prevalence of *O. viverrini* infection the Lao PDR was estimated at 10.9%. Laotian provinces along the Mekong River including Khammuane, Saravane or Savannakhet showed even higher prevalence – 32.2, 21.5, and 25.9%, respectively (Rim et al., 2003). A recent survey in the southern province of Champasak revealed a prevalence of *O. viverrini* infection of 58.5%, among 814 persons sampled from 13 villages (Sayasone et al., 2007).

C. sinensis infection is common in rural areas of northern Vietnam, Korea, China and the Russian Far East. *O. felineus* occurs in Russia, some republics of the former USSR, Kazakhstan and nearby countries, and has been recorded recently in Germany, Greece and Italy (Lun et al., 2005; Keiser and Utzinger, 2005; Armignacco et al., 2008; Shekhovtsov et al., 2009; Shin et al., 2010). In China, the liver fluke *C. sinensis* was recently estimated by the Chinese Center for Disease Control to infect 12.5 million people (Ministry of Health, 2005) (Table 1).

Elevated prevalence of *O. viverrini* infection in the North and Northeast of Thailand and in the Lao PDR results from the pervasive habit by local people of eating raw or undercooked (wild caught) cyprinoid fish and fish-based dishes (Fig. 1). The fish are the second intermediate host of *O. viverrini* and natural populations of these fishes can be widely and heavily infected (Harinasuta

Fig. 1. Montage of images relating to transmission of *Opisthorchis viverrini* infection in Khon Kaen Province, Thailand. (Panel A) *Bithynia* species nails, the intermediate host of *O. viverrini*; Chonnabot District, Khon Kaen Province, Thailand. (Panel B) A fisherman in Chonnabot District, Khon Kaen Province, Thailand. (Panel C) Cyprinoid fish, about 10 cm in length, caught in natural water courses in Chonnabot District, Khon Kaen Province, Thailand (http://en.wikipedia.org/wiki/Amphoe_Chonnabot). (Panel D) A plate of *koi-pla*, a traditional, widely consumed dish prepared from uncooked cyprinoid fish (as shown in Panel C), salad vegetables and condiments. Koi-pla is frequently contaminated with viable, infectious metacercariae of *O. viverrini*.

and Harinasuta, 1984; Sithithaworn et al., 1997; Sayasone et al., 2007). The current therapy for opisthorchiasis is oral treatment with the anti-trematode drug, praziquantel. Despite widespread chemotherapy with praziquantel undertaken in the past, the prevalence of *O. viverrini* still approaches 70% in some districts (amphurs) (Sripa and Pairojkul, 2008; Sripa, unpublished). Like many neglected infections, opisthorchiasis is chronic and affects people for decades, with an insidious onset of disabling health effects and even death. Indeed, among the helminth parasitic infections none can rival the high mortality levels of opisthorchiasis (Bouvard et al., 2009; Shin et al., 2010).

3.2. Infection with O. viverrini – public health overview

Although the association between *Opisthorchis* and *Clonorchis* infections and bile duct cancer has been known for >50 years, and notwithstanding the long history of public health interactions in Thailand focused on this problem (below), there remains a disconcertingly high incidence of *O. viverrini*-associated cholangiocarcinoma in northeastern Thailand (Sripa et al., 2007), and likely also in adjacent provinces of the Lao PDR (Shin et al., 2010) (Table 1). Traditional dishes such as koi-pla and pla-som, prepared from freshwater cyprinoid fish, are the usual source of *O. viverrini* infection in northeastern Thailand and the Lao PDR. It has proven difficult to dissuade at risk populations from the risky behavior of consuming these dishes, including modifying preparation of the dishes by heating the constituent fish flesh sufficiently to kill the metacercariae (Sithithaworn and Haswell-Elkins, 2003; Andrews et al., 2008; Sripa and Pairojkul, 2008; Shin et al., 2010). Progress on this front, in tandem with improvements in reticulated sewage systems to block transmission from human feces to the *Bithynia* snails, and education of at risk populations of the dangers of ingestion of undercooked fish dishes, and/or to cook the fish, should eventually lower the incidence of liver fluke-induced liver cancer. Biomarkers of incipient cholangiocarcinoma are needed given that by the time diagnosis of cholangiocarcinoma, in a resource challenged setting such as the Mekong River provinces of Thailand and the Lao PDR, prognosis is almost universally poor. Encouragingly, inflammatory biomarkers such as urinary 8oxodG reported in opisthorchiasis and *Opisthorchis*-associated cholangiocarcinoma

Table 1
The disease burden of human *Opisthorchis viverrini* and *Clonorchis sinensis* infection and their most severe sequela, cholangiocarcinoma.

Country	Disease	Estimated numbers		References
		Infected	CCA	
Thailand	Opisthorchiasis	8 million	5000	Parkin (2006)
Lao PDR	Opisthorchiasis	2 million	>100[a]	Andrews et al. (2008)
Cambodia	Opisthorchiasis	*ND*	*ND*	
Vietnam	Clonorchiasis	*ND*	>100[a]	Shin et al. (2010)
China	Clonorchiasis	12.5 million	800[a]	Lun et al. (2005)
Korea	Clonorchiasis	*ND*	200	

[a] Probable underestimates. *ND*, not determined. CCA, cholangiocarcinoma.

are potentially useful for diagnosis, surveillance and control (see Andrews et al., 2008).

Meantime, the prevalence of infection occurs early in life, plateaus after the teens, and declines in old age. A small minority of the population harbors most of the worms while most people are lightly or not infected. In sites of heavy transmission of *O. viverrini*, high incidence and rapid re-infection after treatment with praziquantel are common. In northeastern Thailand, transmission to humans from the infected fish is seasonal, peaking in the cooler months when fish exhibit their highest burden of metacercariae. Although infection frequently is sub-clinical, hepatobiliary disease frequently is seen in residents of *O. viverrini* endemic regions. Whereas the hepatobiliary changes usually reverse after praziquantel chemotherapy, resolution of hepatobiliary disease does not occur in all persons after the flukes have been eliminated. Problematically, these individuals may be at increased risk of cholangiocarcinoma (Sripa et al., 2009). In any event, upon parasitological diagnosis of liver fluke infection (below) immediate treatment with praziquantel is indicated to eliminate these long-lived, carcinogenic parasites.

4. Diagnosis of opisthorchiasis

4.1. Parasitological (faecal) diagnosis

Demonstration of eggs in feces, bile, or duodenal fluid or the recovery of flukes during transhepatic stent implantation or from the liver post mortem is considered the "gold standard" for diagnosis *O. viverrini* (Sithithaworn et al., 1991). Faecal examination is the routine method for diagnosing *O. viverrini* infection due to ease, non-invasiveness and cost; techniques include the formalin-ether concentration technique (FECT), the modified quick Kato Katz thick smear, and Stoll's dilution egg count technique (Viyanant et al., 1983; Elkins et al., 1990; Hong et al., 2003). Sensitivity and specificity varies by the method of examination and experience of the microscopist. The diagnostic value of these methods lies in the ability to detect light infections, e.g. an individual treated recently with praziquantel. Repeated examinations are needed to improve diagnostic sensitivity of faecal exams. However, even with repeated stool examination using a standardized method like FECT, there can remain discrepancy between egg count and worm detection, i.e. sufficient probability of a false negative diagnosis. In an autopsy study (Sithithaworn et al., 1991), adult *O. viverrini* were directly recovered from 139 livers (the gold standard); fecal examinations of these same individuals determined that only 67% were positive for *O. viverrini* infection. Conventional stool examination methods may underestimate prevalence by ~20% (Sithithaworn et al., 1991).

Diagnostic specificity of faecal exams can be problematic. Under light microscopy, the eggs of *O. viverrini* are characterized by rough and thick egg shells, and are similar to several species of food-borne trematodes belonging to the families Opisthorchiidae, Heterophyidae and Lecithodendriidae endemic in the same areas. In Thailand and Laos, the latter two families of trematodes are collectively referred to as minute intestinal flukes (MIFs), because of their small size compared to *O. viverrini* (Kaewkes et al., 1991). The Lecithodendriidae include *Phaneropsolus bonnei* and *Prosto-dendrium molenkampi* while the Heterophyidae include *Haplorchis taichui*, *Haplorchis pumilio* and *Stellanchasmus falcatus* (Radomyos et al., 1984; Radomyos et al., 1994). Within the heterophyids, *Metagonimus* spp. and *Heterophyes* spp., *Haplorchis* spp. and *Pygidiopsis* spp. occur in Laos and Korea (Chai et al., 2005). The marked morphological resemblance of MIF eggs with those of *O. viverrini* increases the probability of a false positive diagnosis, with concomitant decrease in diagnostic specificity (Fig. 2). In areas where *O. viverrini* coexists with heterophyid flukes including *Haplorchis* spp., the term '*O. viverrini*-like egg' is sometimes used (Chai et al., 2007). This dilemma reflects the limitation of the fecal exam in which diagnosis is based solely on egg morphology; it also highlights the need for alternative diagnostic methods.

4.2. Serological diagnosis

Serological tests have been developed, with the aim of developing a diagnostic assay with greater sensitivity and specificity than fecal exams. These include the intradermal test (IDT), immunoelectrophoresis (IEP), indirect haemagglutination assay (IHA), indirect fluorescent antibody test (IFAT) and indirect enzyme-linked immunosorbent assay (indirect ELISA) (Wongratanacheewin et al., 2003). Indirect ELISA is favored for its ease and the increase in diagnostic sensitivity and specificity obtained with an antibody-based system. However, serological tests have failed to achieve superior diagnostic ability, because of the complex composition of the crude antigen extracts employed. Crude adult somatic extracts from *O. viverrini* have been used for IEP and ELISA, both of which are estimated to have a sensitivity of 76–100% for IgG and lower sensitivities for IgA [63%] and IgE [74%] (Poopyruchpong et al., 1990; Wongsaroj et al., 2001). Additionally, excretory secretory (ES) antigens from adult *O. viverrini* have been used. An 89 kDa protein, among the most prominent in the bands in ES antigen of *O. viverrini*, has shown immunodiagnostic potential, even though it cross-reacts with *C. sinensis* (Sirisinha et al., 1991). Sandwich ELISA tests have been developed for increased sensitivity and specificity for *O. viverrini* infection. Here, monoclonal antibody-based affinity-purified crude somatic egg antigen shows improved diagnostic specificity and sensitivity in the detection of serum antibody against *O. viverrini* by immunoblotting (Wongsaroj et al., 2001). Attempts to produce recombinant antigen for an IgG antibody detection kit in an ELISA, such as was tried with the recombinant *O. viverrini* egg shell protein (rOVESP), showed increased diagnostic sensitivity and specificity over faecal exams (Ruangsittichai et al., 2006). A drawback of antibody-based detection methods is the inability to differentiate past and present infections with *O. viverrini*, due to the long half-life of the antibody response to *O. viverrini*, which can persist in the infected and non-infected hosts for years after cure (Thammapalerd et al., 1988; Ruangsittichai et al., 2006).

Antigen-based detection systems have also proven to be informative for current infection (Chaicumpa et al., 1991; Sirisinha et al., 1991, 1995). Monoclonal antibody (mAb)-based systems offer an increase in diagnostic sensitivity, enough to detect secretory product from a few adult worms, even when eggs are not found in the stool. Recently, mAbs against ES products of *O. viverrini*, recognizing antigens of 45–110 kDa, were developed for the ELISA for faecal antigen (Sithithaworn, unpublished), exhibiting 69% sensitivity and 39% specificity.

4.3. Molecular diagnosis

Molecular genetic studies of *O. viverrini* have identified genes encoding various proteins that are stage specific, potential candidates in recombinant form for serodiagnosis (Laha et al., 2008; Suttiprapa et al., 2008). For DNA-based methods, a probe targeting repetitive DNA has been used for the detection of eggs (Sirisinha et al., 1991). A PCR-based approach based on a pair of primers complementary to the same target DNA has shown utility for detection of fecal eggs (Wongratanacheewin et al., 2001, 2002). The assay achieved a specificity of 98% and a sensitivity of 100% in moderate to severe infections (infections with stool epg levels of >1000) but was less sensitive (68%) in light infections (<200 epg). Application of this method to detect *O. viverrini* DNA in stool samples from Laos yielded sensitivity of ~50% in samples with egg count >1000 epg, with assay performance possible

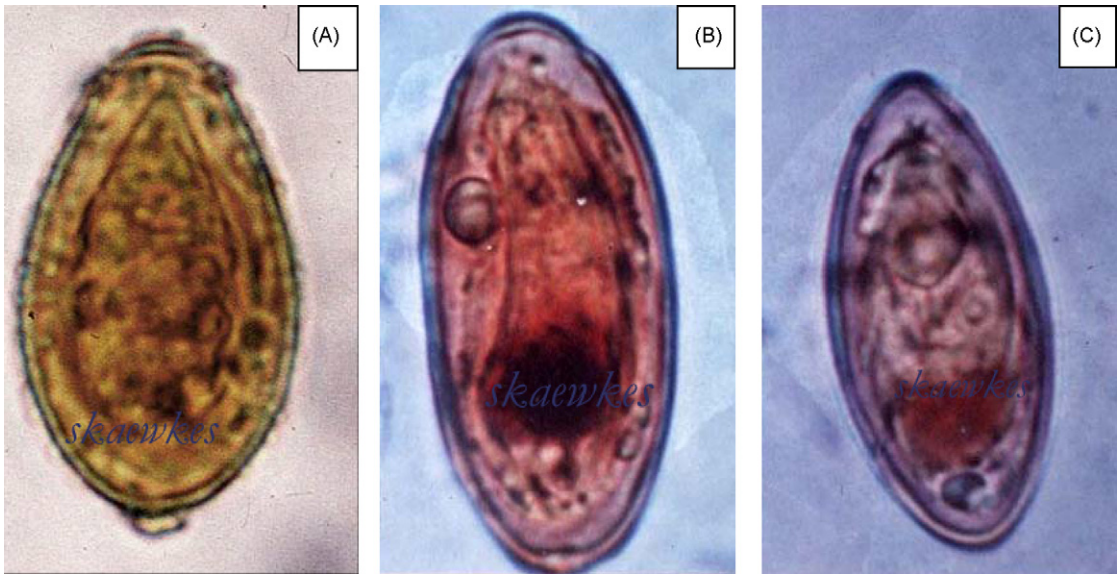

Fig. 2. Photomicrographs of an egg of *O. viverrini* (Panel A) and eggs from two microscopic intestinal flukes (MIF), *P. bonnei* (Panel B) and *Prosthodendrium molenkampi* (Panel C). All three eggs were passed in human feces. The average dimensions of these eggs are 27 μm × 15 μm (*O. viverrini*), 30 μm × 15 μm (*P. bonnei*) and 24 μm × 12 μm (*Prostodendrium molenkampi*) (Kaewkes et al., 1991). When stained with iodine, there is an iodophilic body in the miracidium of embryonated MIF (lecithodendrid) eggs, which is absent from *O. viverrini* eggs.

harmed by PCR inhibitors in faeces (Stensvold et al., 2006). Assay improvements using cetyltrimethylammonium bromide to remove inhibitors resulted in enhanced sensitivity (Duenngai et al., 2008), including capacity to detect ~10 ng of genomic DNA and as few as three metacercariae in fish samples (Parvathi et al., 2008). Species-specific PCR tests can discriminate among *O. viverrini* (Ando et al., 2001; Wongratanacheewin et al., 2001) and *O. viverrini*, *C. sinensis* (Le et al., 2006) and *O. felineus* (Pauly et al., 2003). These tests can be expected to play significant roles in the assessment of cure, re-infection, and characterizing the endemic range of these food-borne trematodes in East Asia (Touch et al., 2009; Traub et al., 2009). These approaches also are applicable to food safety inspection of fish products (Parvathi et al., 2007, 2008; Thaenkham et al., 2007; Sato et al., 2009).

5. Pathobiology and pathogenesis in opisthorchiasis

5.1. Periductal fibrosis due to O. viverrini infection

O. viverrini causes chronic inflammation around the biliary tree, with severe hyperplasia of the cholangiocytes that line the biliary tract proximal to the flukes. Metaplasia of the biliary epithelial cells into mucin-producing cells occurs early during the infection, as these cells proliferate to produce small gland-like structures in the mucosa, leading to persistent and excessive mucus in the bile (Glaser et al., 2009). Chronic and persistent *O. viverrini* infection also results in a gradual increase in fibrous tissues in the biliary tract, which eventually engulfs these proliferating glands (Sripa et al., 2003). Severity of this periductal fibrosis correlates with the duration of infection (Elkins et al., 1991, 1996), parasite burden (Haswell-Elkins et al., 1991, 1994; Sripa et al., 2003) and genetic susceptibility of the host.

The host immune response mediates much of the hepatobiliary damage in opisthorchiasis (Glaser et al., 2009). As with other chronic inflammation, persistent irritants sustain the simultaneous production of growth factors and fibrogenic cytokines, which in turn stimulate the deposition of connective tissue that progressively remodels and destroys normal tissue architecture of the biliary epithelium, resulting in the accumulation of fibrotic elements (Wynn, 2007, 2008). In opisthorchiasis, the persistent irritation can derive from several sources (Glaser et al., 2009) – mechanical irritation, due feeding and migration of flukes as oral and ventral suckers of flukes hook onto biliary epithelia and damage tissue, and metabolic products released from the tegument and excretory openings of parasites that come into contact with the bile duct epithelium. The metabolic products are thought to be immunogenic, mitogenic, and/or even toxic to the biliary epithelium (Sripa et al., 2007). Notably, the process of fibrotic deposition during opisthorchiasis differs from the well-known phenomenon of *Schistosoma mansoni* liver fibrosis. Advanced periductal fibrosis (APF) in *O. viverrini* infection (diagnosable by portable ultrasound examination) (Sripa et al., 2009) (Box 1), is confined to the bile ducts and not to the liver at large (Sripa et al., 2009). APF from *O. viverrini* does not result from granulomas around fluke eggs, although fluke eggs are often present in the bile duct and are detected on ultrasonography (Sripa, 2003). Third, APF is likely mediated by a pro-inflammatory cytokine network mediated by IL-6 and TNF-α, and not via Th2 cytokines (Sripa, 2003).

Box 1: Ultrasonography for Advanced Periductal Fibrosis in Opisthorchiasis

During chronic *O. viverrini* infection, the peripheral intrahepatic bile ducts become dilated and thickened from fibrotic deposition, which images in sonograms as echogenic nodules with an echo-free center (Mairiang et al., 1993, 2006; Sripa and Kaewkes, 2000).

- Advanced periductal fibrosis (APF) is defined as 2+ echogenic nodules in more than one segment of the liver.
- Non-persistent APF is defined as 2+ echogenic nodules in more than one segment of the liver at baseline and no echogenic nodules 12 months after treatment with praziquantel (PZQ) for *O. viverrini* infection.
- Persistent APF is defined as 2+ echogenic nodules in 2 or more segments of the liver at baseline and again 12 months after treatment with PZQ (Mairiang et al., 1993, 2006).
- We hypothesize that persistent APF is a risk factor for development of cholangiocarcinoma (Sripa et al., 2009).

Table 2
Top 10 diseases with high mortality in Thai population stratified by sex for the year 2004 (modified from Sripa and Pairojkul, 2008).

Rank	Male			Female		
	Disease	Deaths (thousands)	%	%	Deaths (thousands)	Disease
1	HIV/AIDS	26	11.1	14.7	26	Stroke
2	Stroke	23	10.0	8.0	14	Diabetes
3	Traffic accidents	23	9.9	6.6	12	Ischemic heart disease
4	**Liver and bile duct cancer**	**19**	**8.0**	6.2	11	HIV/AIDS
5	COPD	14	5.9	**4.9**	**9**	**Liver and bile duct cancer**
6	Ischemic heart disease	13	5.6	3.8	7	Lower respiratory tract infections
7	Bronchus and lung cancer	9	3.7	3.4	6	COPD
8	Diabetes	8	3.5	3.2	6	Nephritis and nephrosis
9	Cirrhosis	8	3.4	2.9	5	Traffic accidents
10	Lower respiratory tract infections	7	2.9	2.5	4	Cervix uteri cancer

5.2. Cholangiocarcinoma

Cholangiocarcinoma (CCA) is a primary cancer originating in the biliary epithelium, i.e. the cholangiocytes, of the extrahepatic and intrahepatic biliary ducts. It is extremely invasive, develops rapidly, often metastasizes, and has a very poor prognosis (Blechacz and Gores, 2008). CCA has a worldwide distribution and accounts for about 10–15% of all cases of primary hepatobiliary malignancy (Parkin et al., 1993; Parkin, 2006; Shin et al., 2010). They are slow growing tumors which spread longitudinally along the bile ducts with neural, perineural and subepithelial extensions (Parkin et al., 1993; Malhi and Gores, 2006). While in the Western countries, 5–10% of cholangiocarcinomas arise from the intrahepatic ducts and 90–95% from the extrahepatic ducts, in countries where *O. viverrini* is endemic (Thailand, Lao PDR), intrahepatic CCA is 40% and extrahepatic 60% of all cases; most of the cases identified in Khon Kaen are intrahepatic (Sripa and Pairojkul, 2008). The location at the upper hepato-duodenal ligament, extension into the liver and proximity to major blood vessels make early evaluation challenging. Hence, the prognosis of patients with non-resectable tumors is poor, and the majority of patients die within a year of diagnosis. No medical treatment is available, and surgery and supportive treatment are complicated and often not accessible for victims in developing countries (Malhi and Gores, 2006). The risk factors for CCA in areas where liver flukes are not endemic in Thailand include primary sclerosing cholangitis, hepatolithiasis, and choledochal cysts (Sripa and Pairojkul, 2008). These factors share long-standing inflammation and chronic injury of the biliary epithelium as a common determinant.

5.3. A putative pathogenicity factor – granulin, a mitogen from O. viverrini

The mechanisms by which chronic infection with *O. viverrini* results in cholangiocarcinogenesis are presumed to multi-factorial (Sripa and Pairojkul, 2008), but one such mechanism is the secretion of parasite proteins with mitogenic properties into the bile ducts, driving cell proliferation and creating a tumorigenic environment (Sripa et al., 2007; Smout et al., 2009). Recently, a proteomic approach identified an orthologue of human granulin, a potent growth factor involved in cell proliferation and would healing, in the excretory/secretory (ES) products of the parasite (Smout et al., 2009). *O. viverrini* granulin, termed *Ov*-GRN-1, was expressed in most parasite tissues, particularly the gut and tegument. Furthermore, *Ov*-GRN-1 was detected *in situ* on the surface of biliary epithelial cells of hamsters experimentally infected with *O. viverrini*. Recombinant *Ov*-GRN-1 was expressed in *Escherichia coli* and refolded from inclusion bodies. Refolded protein stimulated proliferation of murine fibroblasts at nanomolar concentrations and induced shape changes in affected cells. Antibodies raised to recombinant *Ov*-GRN-1 inhibited the ability of *O. viverrini* ES products to induce proliferation of murine fibroblasts and a human cholangiocarcinoma cell line *in vitro*, indicating that *Ov*-GRN-1 is the major growth factor present in *O. viverrini* ES products. The report of a secreted growth factor from a parasitic helminth that induces proliferation of host cells supports a role for this fluke protein in establishment of a tumorigenic environment that may ultimately manifest as cholangiocarcinoma.

6. Epidemiology of liver fluke-induced bile duct cancer

O. viverrini is one of only three metazoan pathogens classified as Group 1 carcinogens by the World Health Organization's International Agency for Research on Cancer (the other two being *C. sinensis* and *Schistosoma haematobium*) (Bouvard et al., 2009). Epidemiological studies have determined that the incidence of cholangiocarcinoma (CCA) correlates strongly with the prevalence of *O. viverrini* infection (Parkin et al., 1993; Jongsuksuntigul and Imsomboon, 2003; Parkin, 2006; Shin et al., 2010). CCA is the predominant type of liver cancer in Thailand (Sripa and Pairojkul, 2008). Fig. 3 shows that the incidence of CCA in the four major regions of Thailand varies by at least 12-fold and has a strong positive correlation with the prevalence of *O. viverrini* infection (Sripa et al., 2007). For example, in adult males, there is an 8.5-fold increase in CCA incidence in the upper Northeast Province of Nakhon Phanom (85.5%) over the Southern province of Prachuap Khiri Khan (10.0%) (Sripa et al., 2007). This difference is an example of the marked regional variation of liver cancer deaths, with the highest mortality rate (per 100,000) in Northeast provinces, which harbors 9 out of the top 10 provinces with highest number of liver cancer deaths in Thailand (Sripa and Pairojkul, 2008). Few data on CCA incidence in Lao PDR have been reported (Shin et al., 2010). However, suspected CCA diagnosed by ultrasonography has been observed in ~5% of people in villages endemic for opisthorchiasis in the Lao PDR (S. Phompida, personal communication). A disproportionate number of deaths occur in male heads of households, who often are the family's primary wage earner. Therefore, opisthorchiasis and CCA also exert a heavy economic toll that in Thailand costs at least $120 million annually in both medical care and lost wages (Andrews et al., 2008). Typically *O. viverrini* infection begins in childhood, residents harbor chronic infections and, after three to four decades, liver cancer is diagnosed in a subset of individuals, with males affected more than females. CCA has a poor prognosis; death is swift, usually occurring within 3–6 months of diagnosis (Andrews et al., 2008). In northern Thailand, an estimated 5000 cases of CCA are diagnosed annually (Parkin, 2006), which translates into 5000 deaths superimposed on a chronic burden of liver and bile duct disease (Table 2).

In the human bile ducts, chronic *O. viverrini* infection results in a chronic inflammatory state that leads to severe hepatobiliary abnormalities. The cumulative damage caused by this chronic inflammation can lead to advanced, pathogen-specific disease

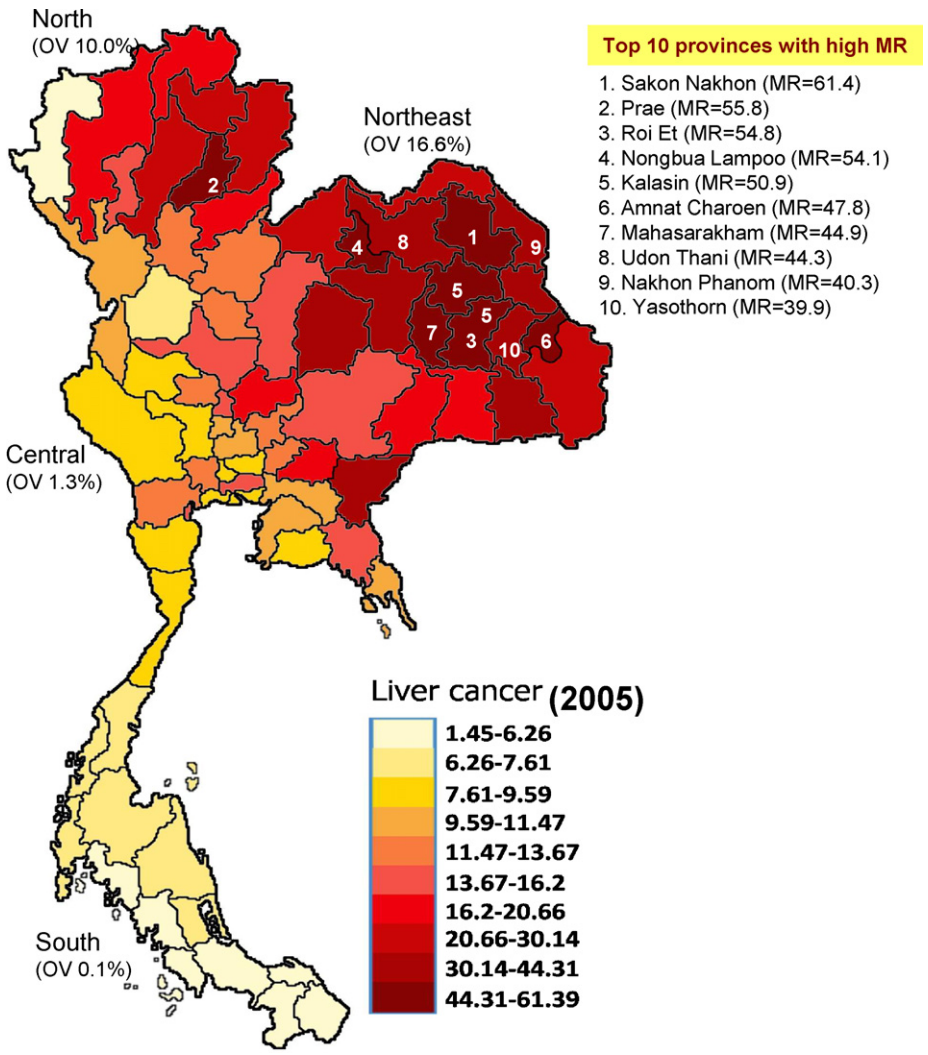

Fig. 3. *O. viverrini* infection in Thailand in relationship to the incidence of cholangiocarcinoma (CCA) by province.

sequelae including obstructive jaundice, hepatomegaly, cholecystitis, and CCA. Moreover, aside from these obvious signs of morbidity in opisthorchiasis, important inflammatory changes to the liver can occur early in the course of the disease (Mairiang et al., 1992, 2006; Sripa et al., 2009), most of which will remain clinically silent unless actively detected by ultrasound or other imaging modalities (Mairiang et al., 2006). Community-based ultrasound studies in *O. viverrini* endemic areas of Northeastern Thailand suggest that hepatobiliary abnormalities such as enlargement of the left hepatic lobe and the gallbladder, loss of gallbladder contractility, presence of sludge, and increased periductal fibrosis are common (Elkins et al., 1990, 1996; Mairiang et al., 1992, 2006; Sripa et al., 2009). Due to the high prevalence of *O. viverrini* infection in East Asia, which can be as high 60–70% in populations resident in endemic areas (Sithithaworn and Haswell-Elkins, 2003; Sripa and Pairojkul, 2008) asymptomatic hepatobiliary abnormalities may represent the greatest part of the disease burden associated with opisthorchiasis.

Overall, approximately one-quarter of people with *O. viverrini* infection develop periductal fibrosis (Sripa et al., 2007), and many will develop CCA (Parkin, 2006; Sripa et al., 2007; Shin et al., 2010). Studies conducted in Khon Kaen Province, the most populated area of northeastern Thailand, reveal a bleak picture of hyperendemicity of *O. viverrini* infection that results in an alarming death rate from CCA (Sripa and Pairojkul, 2008). Here, the prevalence of *O. viverrini* infection focally reaches 70% or higher in villages along the Chi River Basin. The incidence of CCA in this province may be the highest in the world, with an age-standardized incidence rate (ASR) of 78.4 per 100,000 in males and 33.3 in females. As noted, the treatment of choice for opisthorchiasis is oral administration of praziquantel. Anthelmintic treatment with praziquantel will not reliably reverse periductal fibrosis and inflammation (Mairiang et al., 1993) and, therefore, may not prevent the onset of cholangiocarcinoma.

7. Sustainable control of opisthorchiasis: lessons from Thailand

7.1. History of opisthorchiasis control

Opisthorchiasis control in Thailand began more than 50 years ago (Table S1). During 1950–1958, with support from USAID, the Thai Department of Health established Helminthiasis Control Units in five provinces – Nakorn Ratchasima, Udon Thani, Sakon Nakon, Ubon Ratchathani and Songkhla (Jongsuksuntigul and Imsomboon, 2003). Services provided by the units included diagnosis and treatment of intestinal parasites, including liver fluke. At the conclusion of USAID support, the Helminthiasis Control Units were dissolved and control activities integrated into the Rural Health Development Project. From 1967 to 1974, an Opisthorchiasis Control Unit was established in Sakol Nakorn province, Northeast Thailand, as

part of the Rural Health Development Project. The activities of the Opisthorchiasis Control Unit focused on community health education, utilizing a variety of control strategies including demonstrations of how to properly cook fish and the distribution of low cost cooking pots. After the introduction of the anthelmintic praziquantel in Thailand in the early 1980s, a joint field trial organized by Mahidol University and the Helminthiasis Section of the Department of Communicable Disease Control, Ministry of Public Health was conducted in Northeast Thailand. From 1980 to 1983, this field trial demonstrated that the cure rate for liver fluke of a single dose praziquantel, 40 mg/kg was as high as 96% (Jongsuksuntigul and Imsomboon, 2003). During 1984–1987, the Department of Communicable Disease Control organized four opisthorchiasis treatment units in Khon Kaen, Roi-Et, Sakol Nakorn and Ubon Ratchathani provinces in Northeast Thailand. The units undertook stool exams of ~630,000 residents, and treatment for 400,452 cases.

Development of opisthorchiasis control operations on a region-wide scale began in 1987, when an opisthorchiasis control program was included in the Sixth Five-year National Public Health Development Plan (1987–1991) under the auspices of the Department of Communicable Disease Control. During that period, ~5.3 million stool samples were examined, with ~1.8 million positive cases treated. During 1989–1992 the Federal Republic of Germany provided technical and operational support for the Promotion of Community Health through Parasite Control Project in seven Northeastern provinces, covering approximately 3 million people; 1,839,813 individuals received stool exams, with 531,175 positive cases treated. In the Seventh National Health Development Plan (1992–1996), the plan was extended to an additional 17 provinces in Northern Thailand and 6 Central region provinces. Currently, control activities for opisthorchiasis have been integrated into a comprehensive rural health service that targets all provinces. According to the 8th National Health Plan (1997–2001), the opisthorchiasis control program was integrated into the Nationwide Disease Control Aims, with the objective of reducing the prevalence of *O. viverrini* infection to <10%. Many campaigns against the consumption of "raw fish" have been organized by different governmental agencies and non-governmental organizations (NGOs). This extensive National Opisthorchiasis Control Program has resulted in marked reductions in level of *O. viverrini* infection, with the national prevalence falling from 63.6% (1984–1987) to 9.6% in 2001 (Jongsuksuntigul and Imsomboon, 2003).

After 2000, the National Opisthorchiasis Control Program activities subsided due to the Asian economic crisis, which forced a reduction in government funding and diversion of resources to other priorities. Recent data from the National Helminthic Survey showed that the prevalence of *O. viverrini* was 8.7%, with the highest prevalence in the Northeast (16.6%), followed by the North (10.0%), the Central (1.3%) and the Southern (0.1%) regions (T. Wongsaroj, unpublished). The target prevalence for liver fluke infection projected by the Department of Disease Control, Ministry of Public Health, Thailand is 5% by the year 2016. The prevalence of *O. viverrini* infection at national level during 1984 to 2009 is shown in Figure S1.

7.2. Strategic approaches for liver fluke control

Although the prevalence of *O. viverrini* has declined at the national level, it remains high in Northeast Thailand (16.6% in 2009 vs. 15.8% in 2001), with a correspondingly high incidence of CCA (Sripa and Pairojkul, 2008). Therefore, there is a pressing need to maintain an effective control program. However, due to budgetary and health policy constraints (Sripa, 2008), integrated strategic approaches should be targeted in high endemic areas in Northeast and North Thailand. The idea here is similar to what has been successfully done before in Thailand (Jongsuksuntigul and Imsomboon, 2003), but this time with a more systematic and focused approach. Figure S2 presents our suggestions for a systematic and focused approach to opisthorchiasis control in Northeastern Thailand. We have divided "control" into four elements: (1) modifications to current control measures; (2) the subsequent behavioral changes in the population; (3) the public health impact of these behavioral changes, and (4) the epidemiological impact, which would be a decline in the prevalence of *O. viverrini* infection and in the future a decline in the incidence of CCA. We suggest that simple modification to current control policies can result in a marked decrease in the prevalence of *O. viverrini* infection and subsequently the incidence of CCA. For example, the use of mobile stool examination teams, equipped to treat positive cases could increase the frequency (yearly) and the geographic range of fecal exams, resulting in a reduction in the host reservoir (humans) and possibly a decline in transmission to reservoir hosts, e.g. cats. These activities should be carried out several consecutive years with >60% coverage for each round. Whenever the situation of the prevalence in any area falls below 10%, the active services then gradually switch to passive services (Jongsuksuntigul and Imsomboon, 2003). Another modification to current health practices would be strengthening the health education aspects, aiming to reduce consumption of uncooked fish to reduce infection/re-infection intensity. Public health promotion including information, education and communication (IEC) devices should emphasize the need to modify personal hygiene (defecation) behavior, specifically the use of latrines, to reduce environmental contamination with *O. viverrini* eggs. We consider modifications to current practices would result in substantial decreases in the prevalence of *O. viverrini* infection, which would eventually be followed by a decrease the incidence of CCA. To achieve the goal, multisectoral partnerships such as government (Ministry of Public Health, Ministry of Education, Ministry of Sciences and Technology, Ministry of Agriculture), granting agencies, and private sectors, including NGOs must be orchestrated in the plan. This would start with modifications to government health policy, which should prioritize liver fluke as a national health problem as in previous campaigns. The support would include increasing the infrastructure for the diagnosis and surveillance of *O. viverrini* infection, as well as a national "anti-liver fluke" campaign, with concomitant health education.

8. Role for a vaccine?

From a public health perspective, thorough cooking of the cyprinoid fish efficiently blocks infection with these parasites. In this regard, liver fluke-associated CCA is preventable by changes in eating habits. Unfortunately, age-old culinary preferences for uncooked dishes such as *koi-pla* (Fig. 1) do not readily allow for this possibility. Moreover, fish farming of grass carp and other susceptible species in ponds that are routinely contaminated by untreated sewage has resulted in the establishment of infection in fish populations at large, which, along with the involvement of animal reservoir hosts, makes control of liver fluke infection even more challenging (Lun et al., 2005; Sripa et al., 2007). Nonetheless, given this extraordinary linkage between a metazoan parasite and a tumour, characterization of the nature and action of carcinogens of *O. viverrini* or *C. sinensis* may provide fundamental insights into carcinogenesis at large. Moreover, our previous work points to the feasibility of developing an opisthorchiasis vaccine. Based on a gene discovery project for adult stage *O. viverrini*, we have predicted more than 5000 protein encoding genes (Laha et al., 2007; Young et al., 2010). Among these, we characterized a subset with predicted functions at the host–parasite interface, specifically protein likely to be secreted or to be membrane associated in the adult fluke, and thereby likely to be in direct contact with the epithelial cells of the

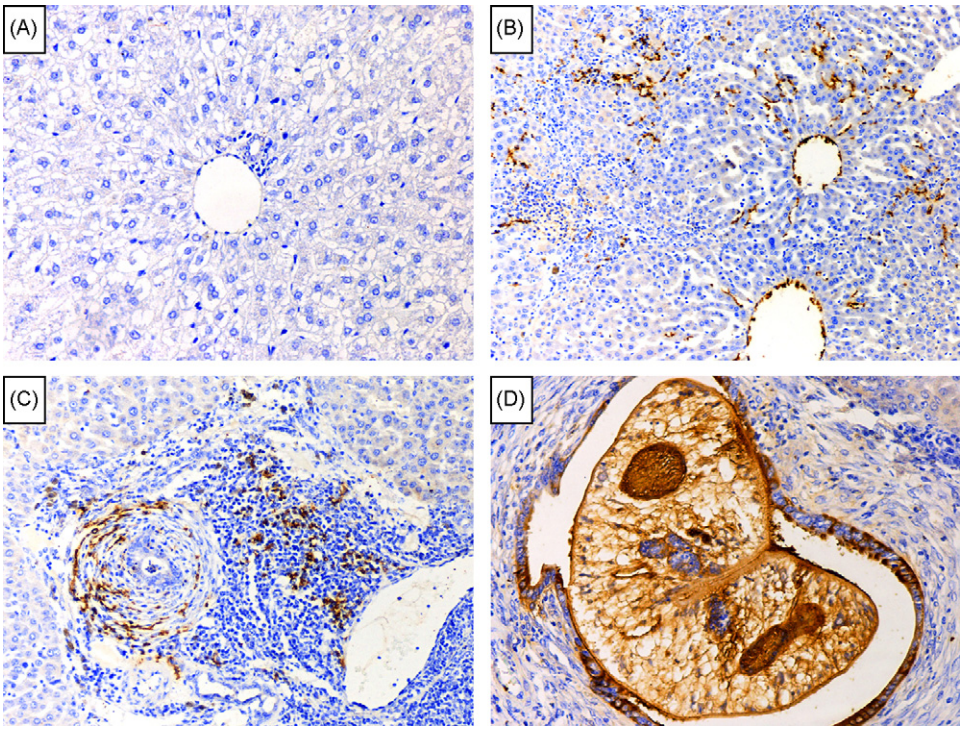

Fig. 4. Immunolocalization of cathepsin F cysteine protease (*Ov*-CF-1) in *O. viverrini* infected hamster liver. Thin sections of paraffin embedded liver tissues were probed with rabbit antiserum (Panel A) Representative section of liver from an uninfected hamster, spanning a portal triad including a secondary bile duct, probed with rabbit anti-*Ov*-CF-1serum (negative control). Infected hamster liver in the vicinity of the secondary bile ducts too small in internal diameter to include an adult fluke, probed with the rabbit anti-*Ov*-CF-1 serum (Panels B and C). Immunoperoxidase stain (brown) indicates the presence of *Ov*-CF-1 in bile ducts epithelial cells (Panel B) and in sinusoidal Kupffer and mononuclear cells (Panel C). Section through bile duct containing an adult *O. viverrini*, showing strong reactivity to organs and tissues of the fluke (including the gut), and to the epithelial cells lining the infected bile duct (Panel D). Immunoperoxidase staining, original magnification, 100× (from Pinlaor et al., 2009).

infected bile ducts, e.g. Pinlaor et al. (2009), Smout et al. (2009). Characterization of the nature and action of secreted proteins of *O. viverrini* such as cathepsin F (Pinlaor et al., 2009) (Fig. 4) may provide insights into liver fluke-induced cholangiocarcinogenesis, and indeed fundamental insights into carcinogenesis at large. In addition, fluke proteins at the host–parasite interface have potential as intervention targets including as vaccine candidates, given recent successes with chemotherapy targeting proteolytic enzymes and tetraspanins in schistosomes and liver flukes (McManus and Dalton, 2006; Tran et al., 2006; Abdulla et al., 2007). Indeed, in view of the acclaimed vaccine against papilloma-virus infection that protects against cervical cancer (Schiller et al., 2008), it is feasible that vaccination to prevent *O. viverrini* infection could provide protection against another infection-related cancer, liver fluke-induced cholangiocarcinoma.

Acknowledgement

We gratefully acknowledge support from award number UO1AI065871 from the National Institute of Allergy and Infectious Disease (the content is solely the responsibility of the authors and does not necessarily represent the official views of the NIAID or the NIH).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.actatropica.2010.07.006.

References

Abdulla, M.H., Lim, K.C., Sajid, M., McKerrow, J.H., Caffrey, C.R., 2007. *Schistosomiasis mansoni*: novel chemotherapy using a cysteine protease inhibitor. PLoS Med. 4 (1), e14.

Ando, K., Sithithaworn, P., Nuchjungreed, C., Tesana, S., Srisawangwong, T., Limviroj, W., Chinzei, Y., 2001. Nucleotide sequence of mitochondrial CO I and ribosomal ITS II genes of *Opisthorchis viverrini* in northeast Thailand. Southeast Asian J. Trop. Med. Publ. Health 32 (Suppl. 2), 17–22.

Andrews, R.H., Sithithaworn, P., Petney, T.N., 2008. *Opisthorchis viverrini*: an underestimated parasite in world health. Trends Parasitol. 24, 497–501.

Armignacco, O., Caterini, L., Marucci, G., Ferri, F., Bernardini, G., Natalini Raponi, G., Ludovisi, A., Bossù, T., Gomez Morales, M.A., Pozio, E., 2008. Human illnesses caused by *Opisthorchis felineus* flukes, Italy. Emerg. Inf. Dis. 14, 1902–1905.

Blechacz, B., Gores, G.J., 2008. Cholangiocarcinoma: advances in pathogenesis, diagnosis, and treatment. Hepatology 48, 308–321.

Bouvard, V., Baan, R., Straif, K., Grosse, Y., Secretan, B., El Ghissassi, F., Benbrahim-Tallaa, L., Guha, N., Freeman, C., Galichet, L., Cogliano, V., WHO International Agency for Research on Cancer Monograph Working Group, 2009. A review of human carcinogens. Part B. Biological agents. Lancet Oncol. 10, 321–322.

Chai, J.Y., Park, J.H., Han, E.T., Guk, S.M., Shin, E.H., Lin, A., Kim, J.L., Sohn, W.M., Yong, T.S., Eom, K.S., Min, D.Y., Hwang, E.H., Phommmasack, B., Insisiengmay, B., Rim, H.J., 2005. Mixed infections with *Opisthorchis viverrini* and intestinal flukes in residents of Vientiane Municipality and Saravane Province in Laos. J. Helminthol. 79, 283–289.

Chai, J.Y., Han, E.T., Guk, S.M., Shin, E.H., Sohn, W.M., Yong, T.S., Eom, K.S., Lee, K.H., Jeong, H.G., Ryang, Y.S., Hoang, E.H., Phommasack, B., Insisiengmay, B., Lee, S.H., Rim, H.J., 2007. High prevalence of liver and intestinal fluke infections among residents of Savannakhet Province in Laos. Korean J. Parasitol. 45, 213–218.

Chaicumpa, W., Ruangkunaporn, Y., Kalambaheti, T., Limavongpranee, S., Kitikoon, V., Khusmith, S., Pungpak, S., Chongsa-nguan, M., Sornmani, S., 1991. Specific monoclonal antibodies to *Opisthorchis viverrini*. Int. J. Parasitol. 21, 969–974.

Duenngai, K., Sithithaworn, P., Rudrappa, U.K., Iddya, K., Laha, T., Stensvold, C.R., Strandgaard, H., Johansen, M.V., 2008. Improvement of PCR for detection of *Opisthorchis viverrini* DNA in human stool samples. J. Clin. Microbiol. 46, 366–368.

Elkins, D.B., Haswell-Elkins, M.R., Mairiang, E., Mairiang, P., Sithithaworn, P., Kaewkes, S., Bhudhisawasdi, V., Uttaravichien, T., 1990. A high frequency of hepatobiliary disease and suspected cholangiocarcinoma associated with heavy *Opisthorchis viverrini* infection in a small community in north-east Thailand. Trans. R. Soc. Trop. Med. Hyg. 84, 715–719.

Elkins, D.B., Sithithaworn, P., Haswell-Elkins, M., Kaewkes, S., Awacharagan, P., Wongratanacheewin, S., 1991. *Opisthorchis viverrini*: relationships between egg counts, worms recovered and antibody levels within an endemic community in northeast Thailand. Parasitology 102, 283–288.

Elkins, D.B., Mairiang, E., Sithithaworn, P., Mairiang, P., Chaiyakum, J., Chamadol, N., Loapaiboon, V., Haswell-Elkins, M.R., 1996. Cross-sectional patterns of hepatobiliary abnormalities and possible precursor conditions of cholangiocarcinoma associated with *Opisthorchis viverrini* infection in humans. Am. J. Trop. Med. Hyg. 55, 295–301.

Glaser, S.S., Gaudio, E., Miller, T., Alvaro, D., Alpini, G., 2009. Cholangiocyte proliferation and liver fibrosis. Expert Rev. Mol. Med. 11, e7 (review).

Harinasuta, C., Harinasuta, T., 1984. *Opisthorchis viverrini*: life cycle, intermediate hosts, transmission to man and geographical distribution in Thailand. Arzneimittelforschung 34, 1164–1167.

Haswell-Elkins, M., Sithithaworn, P., Mairiang, E., Elkins, D., Wongratanacheewin, S., Kaewkes, S., Mairiang, P., 1991. Immune responsiveness and parasite-specific antibody levels in human hepatobiliary disease associated with *Opisthorchis viverrini* infection. Clin. Exp. Immunol. 84, 213–218.

Haswell-Elkins, M.R., Mairiang, E., Mairiang, P., Chaiyakum, J., Chamadol, N., Loapaiboon, V., Sithithaworn, P., Elkins, D.B., 1994. Cross-sectional study of *Opisthorchis viverrini* infection and cholangiocarcinoma in communities within a high-risk area in northeast Thailand. Int. J. Cancer 59, 505–509.

Hong, S.T., Choi, M.H., Kim, C.H., Chung, B.S., Ji, Z., 2003. The Kato-Katz method is reliable for diagnosis of *Clonorchis sinensis* infection. Diag. Microbiol. Inf. Dis. 47, 345–347.

Hotez, P.J., Molyneux, D.H., Fenwick, A., Kumaresan, J., Sachs, S.E., Sachs, J.D., Savioli, L., 2007. Control of neglected tropical diseases. New Engl. J. Med. 357, 1018–1027.

Hotez, P.J., Fenwick, A., Savioli, L., Molyneux, D.H., 2009. Rescuing the bottom billion through control of neglected tropical diseases. Lancet 373, 1570–1575.

Jongsuksuntigul, P., Imsomboon, T., 2003. Opisthorchiasis control in Thailand. Acta Trop. 88, 229–232.

Kaewkes, S., Elkins, D.B., Sithithaworn, P., Haswell-Elkins, M.R., 1991. Comparative studies on the morphology of the eggs of *Opisthorchis viverrini* and lecithodendriid trematodes. Southeast Asian J. Trop. Med. Publ. Health 22, 623–630.

Keiser, J., Utzinger, J., 2005. Emerging foodborne trematodiasis. Emerg. Inf. Dis. 11, 1507–1514.

Laha, T., Pinlaor, P., Mulvenna, J., Sripa, B., Sripa, M., Smout, M.J., Gasser, R.B., Brindley, P.J., Loukas, A., 2007. Gene discovery for the carcinogenic human liver fluke *Opisthorchis viverrini*. BMC Genom. 8, 189.

Laha, T., Sripa, J., Sripa, B., Pearson, M., Tribolet, L., Kaewkes, S., Sithithaworn, P., Brindley, P.J., Loukas, A., 2008. Asparaginyl endopeptidase from the carcinogenic liver fluke *Opisthorchis viverrini*, and its potential for serodiagnosis. Int. J. Inf. Dis. 12, e49–59.

Le, T.H., Van De, N., Blair, D., Sithithaworn, P., McManus, D.P., 2006. *Clonorchis sinensis* and *Opisthorchis viverrini*: development of a mitochondrial-based multiplex PCR for their identification and discrimination. Exp. Parasitol. 112, 109–114.

Lun, Z.R., Gasser, R.B., Lai, D.H., Li, A.X., Zhu, X.Q., Yu, X.B., Fang, Y.Y., 2005. Clonorchiasis: a key foodborne zoonosis in China. Lancet Inf. Dis. 5, 31–41.

Mairiang, E., Elkins, D.B., Mairiang, P., Chaiyakum, J., Chamadol, N., Loapaiboon, V., Posri, S., Sithithaworn, P., Haswell-Elkins, M., 1992. Relationship between intensity of *Opisthorchis viverrini* infection and hepatobiliary disease detected by ultrasonography. J. Gastroenterol. Hepatol. 7, 17–21.

Mairiang, E., Haswell-Elkins, M.R., Mairiang, P., Sithithaworn, P., Elkins, D.B., 1993. Reversal of biliary tract abnormalities associated with *Opisthorchis viverrini* infection following praziquantel treatment. Trans. R. Soc. Trop. Med. Hyg. 87, 194–197.

Mairiang, E., Chaiyakum, J., Chamadol, N., Laopaiboon, V., Srinakarin, J., Kunpitaya, J., Sriamporn, S., Suwanrungruang, K., Vatanasapt, V., 2006. Ultrasound screening for *Opisthorchis viverrini*-associated cholangiocarcinomas: experience in an endemic area. Asian Pacific J. Cancer Prev. 7, 431–433.

Malhi, H., Gores, G.J., 2006. Cholangiocarcinoma: modern advances in understanding a deadly old disease. J. Hepatol. 45, 856–867.

McManus, D.P., Dalton, J.P., 2006. Vaccines against the zoonotic trematodes *Schistosoma japonicum*, *Fasciola hepatica* and *Fasciola gigantica*. Parasitology 133, S43–61.

Ministry of Health, National Institute of Parasitic Diseases, China CDC, 2005. Report on the National Survey of Current Situation of Major Human Parasitic Diseases in China. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi 23 (5 Suppl.), 332–340 (in Chinese).

Parkin, D.M., 2006. The global health burden of infection-associated cancers in the year 2002. Int. J. Cancer 118, 3030–3044.

Parkin, D.M., Ohshima, H., Srivatanakul, P., Vatanasapt, V., 1993. Cholangiocarcinoma: epidemiology, mechanisms of carcinogenesis and prevention. Cancer Epidemiol. Biomarkers Prev. 2, 537–544.

Parvathi, A., Sanath Kumar, H., Kenchanna Prakasha, B., Lu, J., Xu, X., Hu, W., Feng, Z., Karunasagar, I., Karunasagar, I., 2007. *Clonorchis sinensis*: development and evaluation of a nested polymerase chain reaction (PCR) assay. Exp. Parasitol. 115, 291–295.

Parvathi, A., Umesha, K.R., Kumar, S., Sithithaworn, P., Karunasagar, I., Karunasagar, I., 2008. Development and evaluation of a polymerase chain reaction (PCR) assay for the detection of *Opisthorchis viverrini* in fish. Acta Trop. 107, 13–16.

Pauly, A., Schuster, R., Steuber, S., 2003. Molecular characterization and differentiation of opisthorchiid trematodes of the species *Opisthorchis felineus* (Rivolta, 1884) and *Metorchis bilis* (Braun, 1790) using polymerase chain reaction. Parasitol. Res. 90, 409–414.

Pinlaor, P., Kaepitoon, N., Laha, T., Sripa, B., Kaewkes, S., Morales, M.E., Mann, V.H., Parriott, S.K., Suttiprapa, S., Robinson, M.W., To, J., Dalton, J.P., Loukas, A., Brindley, P.J., 2009. Cathepsin F cysteine protease of *Opisthorchis viverrini*. PLoS Negl. Trop. Dis. 3 (3), e398.

Poopyruchpong, N., Viyanant, V., Upatham, E.S., Srivatanakul, P., 1990. Diagnosis of opisthorchiasis by enzyme-linked immunosorbent assay using partially purified antigens. Asian Pacific J. Allergy Immunol. 8, 27–31.

Radomyos, P., Bunnag, D., Harinasuta, T., 1984. Worms recovered in stools following praziquantel treatment. Arzneimittelforschung 34, 1215–1217.

Radomyos, P., Radomyos, B., Tungtrongchitr, A., 1994. Multi-infection with helminths in adults from northeast Thailand as determined by post-treatment fecal examination of adult worms. Trop. Med. Parasitol. 45, 133–135.

Rim, H.J., Chai, J.Y., Min, D.Y., Cho, S.Y., Eom, K.S., Hong, S.J., Sohn, W.M., Yong, T.S., Deodato, G., Standgaard, H., Phommasack, B., Yun, C.H., Hoang, E.H., 2003. Prevalence of intestinal parasite infections on a national scale among primary schoolchildren in Laos. Parasitol. Res. 91, 267–272.

Ruangsittichai, J., Viyanant, V., Vichasri-Grams, S., Sobhon, P., Tesana, S., Upatham, E.S., Hofmann, A., Korge, G., Grams, R., 2006. *Opisthorchis viverrini*: identification of a glycine-tyrosine rich eggshell protein and its potential as a diagnostic tool for human opisthorchiasis. Int. J. Parasitol. 36, 1329–1339.

Sato, M., Thaenkham, U., Dekumyoy, P., Waikagul, J., 2009. Discrimination of *O. viverrini*, *C. sinensis*, *H. pumilio* and *H. taichui* using nuclear DNA-based PCR targeting ribosomal DNA ITS regions. Acta Trop. 109, 81–83.

Sayasone, S., Odermatt, P., Phoumindr, N., Vongsaravane, X., Sensombath, V., Phetsouvanh, R., Choulamany, X., Strobel, M., 2007. Epidemiology of *Opisthorchis viverrini* in a rural district of southern Lao PDR. Trans. R. Soc. Trop. Med. Hyg. 101, 40–47.

Schiller, J.T., Castellsague, X., Villa, L.L., Hildesheim, A., 2008. An update of prophylactic human papillomavirus L1 virus-like particle vaccine clinical trial results. Vaccine 26 (Suppl. 10), K53–K61.

Shekhovtsov, S.V., Katokhin, A.V., Romanov, K.V., Besprozvannykh, V.V., Fedorov, K.P., Yurlova, N.I., Serbina, E.A., Sithithaworn, P., Kolchanov, N.A., Mordvinov, V.A., 2009. A novel nuclear marker, Pm-int9, for phylogenetic studies of *Opisthorchis felineus*, *Opisthorchis viverrini*, and *Clonorchis sinensis* (Opisthorchiidae, Trematoda). Parasitol. Res. 106, 293–297.

Shin, H.-R., Oh, J.-K., Masuyer, E., Curado, M.-P., Bouvard, V., Fang, Y.-Y., Wiangnon, S., Sripa, B., Hong, S.-T., 2010. Epidemiology of cholangiocarcinoma: an update focusing on risk factors. Cancer Sci. 101, 579–585.

Sirisinha, S., Chawengkirttikul, R., Sermswan, R., 1991. Immunodiagnosis of opisthorchiasis. Southeast Asian J. Trop. Med. Publ. Health 22, 179–183.

Sirisinha, S., Chawengkirttikul, R., Haswell-Elkins, M.R., Elkins, D.B., Kaewkes, S., Sithithaworn, P., 1995. Evaluation of a monoclonal antibody-based enzyme linked immunosorbent assay for the diagnosis of *Opisthorchis viverrini* infection in an endemic area. Am. J. Trop. Med. Hyg. 52, 521–524.

Sithithaworn, P., Haswell-Elkins, M., 2003. Epidemiology of *Opisthorchis viverrini*. Acta Trop. 88, 187–194.

Sithithaworn, P., Tesana, S., Pipitgool, V., Kaewkes, S., Pairojkul, C., Sripa, B., Paupairoj, A., Thaiklar, K., 1991. Relationship between faecal egg count and worm burden of *Opisthorchis viverrini* in human autopsy cases. Parasitology 102, 277–281.

Sithithaworn, P., Pipitgool, V., Srisawangwong, T., Elkins, D.B., Haswell-Elkins, M.R., 1997. Seasonal variation of *Opisthorchis viverrini* infection in cyprinoid fish in north-east Thailand: implications for parasite control and food safety. Bull. WHO 75, 125–131.

Smout, M.J., Laha, T., Mulvenna, J., Sripa, B., Suttiprapa, S., Jones, A., Brindley, P.J., Loukas, A., 2009. A granulin-like growth factor secreted by the carcinogenic liver fluke *Opisthorchis viverrini*, promotes proliferation of host cells. PLoS Pathog. 5 (10), e1000611.

Sripa, B., 2003. Pathobiology of opisthorchiasis: an update. Acta Trop. 88, 209–220.

Sripa, B., 2008. Concerted action is needed to tackle liver fluke infections in Asia. PLoS Negl. Trop. Dis. 2 (5), e232.

Sripa, B., Kaewkes, S., 2000. Localisation of parasite antigens and inflammatory responses in experimental opisthorchiasis. Int. J. Parasitol. 30, 735–740.

Sripa, B., Pairojkul, C., 2008. Cholangiocarcinoma: lessons from Thailand. Curr. Opin. Gastroenterol. 24, 349–356.

Sripa, B., Haswell-Elkins, M.R., Sinawat, P., 2003. Histological analysis of gallbladder diseases in relation to opisthorchiasis in endemic areas of Thailand. Acta Trop. 88, 239–246.

Sripa, B., Kaewkes, S., Sithithaworn, P., Mairiang, E., Laha, T., Smout, M., Pairojkul, C., Bhudhisawasdi, V., Tesana, S., Thinkamrop, B., Bethony, J.M., Loukas, A., Brindley, P.J., 2007. Liver fluke induces cholangiocarcinoma. PLoS Med. 4 (7), e201.

Sripa, B., Mairiang, E., Thinkhamrop, B., Laha, T., Kaewkes, Sithithaworn, P., Tessana, S., Loukas, A., Brindley, P., Bethony, J.M., 2009. Advanced periductal fibrosis from infection with the carcinogenic human liver fluke *Opisthorchis viverrini* correlates with elevated levels of IL-6. Hepatology 50, 1273–1281.

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.

Stensvold, C.R., Saijuntha, W., Sithithaworn, P., Wongratanacheewin, S., Strandgaard, H., Ornbjerg, N., Johansen, M.V., 2006. Evaluation of PCR based coprodiagnosis of human opisthorchiasis. Acta Trop. 97, 26–30.

Suttiprapa, S., Loukas, A., Laha, T., Wongkham, S., Kaewkes, S., Gaze, S., Brindley, P.J., Sripa, B., 2008. Characterization of the antioxidant enzyme, thioredoxin peroxidase, from the carcinogenic human liver fluke, *Opisthorchis viverrini*. Mol. Biochem. Parasitol. 160, 116–122.

Thaenkham, U., Visetsuk, K., Dung do, T., Waikagul, J., 2007. Discrimination of *Opisthorchis viverrini* from *Haplorchis taichui* using COI sequence marker. Acta Trop. 103, 26–32.

Thammapalerd, N., Tharavanij, S., Nacapunchai, D., Bunnag, D., Radomyos, P., Prasertsiriroj, V., 1988. Detection of antibodies against *Opisthorchis viverrini* in patients before and after treatment with praziquantel. Southeast Asian J. Trop. Med. Publ. Health 19, 101–108.

Touch, S., Komalamisra, C., Radomyos, P., Waikagul, J., 2009. Discovery of *Opisthorchis viverrini* metacercariae in freshwater fish in southern Cambodia. Acta Trop. 111, 108–113.

Tran, M.H., Pearson, M.S., Bethony, J.M., Smyth, D.J., Jones, M.K., Duke, M., Don, T.A., McManus, D.P., Correa-Oliveira, R., Loukas, A., 2006. Tetraspanins on the surface of *Schistosoma mansoni* are protective antigens against schistosomiasis. Nat. Med. 12, 835–840.

Traub, R.J., Macaranas, J., Mungthin, M., Leelayoova, S., Cribb, T., Murrell, K.D., Thompson, R.C., 2009. A new PCR-based approach indicates the range of *Clonorchis sinensis* now extends to central Thailand. PLoS Negl. Trop. Dis. 3 (1), e367.

Viyanant, V., Brockelman, W.Y., Lee, P., Ardsungnoen, S., Upatham, E.S., 1983. A comparison of a modified quick-Kato technique and the Stoll dilution method for field examination for *Opisthorchis viverrini* eggs. J. Helminthol. 57, 91–195.

Wongratanacheewin, S., Pumidonming, W., Sermswan, R.W., Maleewong, W., 2001. Development of a PCR-based method for the detection of *Opisthorchis viverrini* in experimentally infected hamsters. Parasitology 122, 175–180.

Wongratanacheewin, S., Pumidonming, W., Sermswan, R.W., Pipitgool, V., Maleewong, W., 2002. Detection of *Opisthorchis viverrini* in human stool specimens by PCR. J. Clin. Microbiol. 40, 3879–3880.

Wongratanacheewin, S., Sermswan, R.W., Sirisinha, S., 2003. Immunology and molecular biology of *Opisthorchis viverrini* infection. Acta Trop. 88, 195–207.

Wongsaroj, T., Sakolvaree, Y., Chaicumpa, W., Maleewong, W., Kitikoon, V., Tapchaisri, P., Chongsa-nguan, M., Cross, J.H., 2001. Affinity purified oval antigen for diagnosis of *Opisthorchiasis viverrini*. Asian Pacific J. Allergy Immun. 19, 245–258.

Wynn, T.A., 2007. Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases. J. Clin. Invest. 117, 524–529.

Wynn, T.A., 2008. Cellular and molecular mechanisms of fibrosis. J. Pathol. 214, 199–210.

Young, N.D., Campbell, B.E., Hall, R.S., Jex, A.J., Cantacessi, C., Laha, T., Sohn, W.-M., Sripa, B., Loukas, A., Brindley, P.J., Gasser, R.B., 2010. Unlocking the transcriptomes of two carcinogenic parasites *Clonorchis sinensis* and *Opisthorchis viverrini*. PLoS Negl. Trop. Dis. 4 (6), e719.