Epidemiology and morbidity of food-borne trematodiasis in Lao People's Democratic Republic with particular consideration to opisthorchiasis

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Prof. Dr. Eberhard Parlow

Dekan

To my wife and children

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Summary

Background: Food-borne trematodes, parasitizing the liver, lung and intestinal tract of humans, are an emerging public health problem in countries of tropical regions. Today, an estimated 40 million people are infected worldwide. More than half of those occur in Asia, particularly in Southeast Asian countries. Infection with food-borne trematode is associated with divers and severe morbidity, i.e. a long-lasting infection with *Opisthorchis viverrini* gives rise to liver fibrosis, cholecystitis and cholangitis and may induce a malignant cholangiocarcinoma (CCA).

In Lao People's Democratic Republic (Lao PDR), particularly in the rural settings the habit of consumption of undercooked or insufficiently cooked food is frequent and deeply culturally rooted. In addition access to clean water is low and adequate sanitary facilities are virtually absent, which reflects a socio-economically disadvantaged situation. These areas are at high risk for the transmission of food-borne trematodiasis (FBT) and intestinal helminhiasis. The prevalence of these parasites has an extensive geographical overlap; thus leading in a single person to an infection with several different species of parasites. The concurrent infection of multiple species, in turn, might aggravate the morbidity of the host.

Objectives: Five specific objectives were pursued in this Ph.D. thesis: (i) to investigate the epidemiology of *O. viverrini* and assess the extent of co-infection with other intestinal parasites in a highly endemic setting, (ii) to describe the diversity of FBT with intestinal and hepato-biliar tropism in different eco-epidemiological settings and assess their contribution to the overall morbidity, (iii) to assess the relationship between socio-economic status and food-borne trematode infection, in particular *O. viverrini* in the distinct eco-epidemiological settings, and (iv) to assess the concomitant infections of intestinal parasites in the distinct eco-epidemiological settings and their inter-linking to the environmental, socio-economic and behavioural risk factors.

Population and method: The data presented in this Ph.D. thesis was obtained from a series of epidemiological studies. The data pertaining to the epidemiology of *O. viverrini* and other intestinal parasites were first obtained from a cross-sectional study carried out in February and March 2004 in the Saravane district, province of Saravane. Eligible persons were randomly selected from the district in two steps, i.e. study villages and households, based on the village register and household register available at the district health office (DHO) and at village. Two questionnaires were administrated to collect the data at the individual level (demographic, behavioural and personal hygienic data) and at the household level (food preparing method with fish, characteristics of household head). One single stool sample was obtained from each study participant and analysed by using Kato-Katz technique (KK-technique). Fish species from different local rivers were collected and examined for the infection with metacercariae.

A second cross-sectional study was carried out in March and May 2006 in three distinct eco-epidemiological settings in Champasack province, i.e. islands in Mekong River (Khong district), plain area bordering Mekong River (Mounlapamok district) and highlands (Paksong district). A pre-tested individual questionnaire was used. All enrolled household members were interviewed for demographic data (e.g. age, sex, educational attainment and professional activity) and behavioural risks (e.g. food consumption habits and personal hygiene), whereas a household questionnaire was administered to the heads of household to collect the socio-economic characteristics (e.g. building type and water supply), asset ownership (e.g. farm engine and bicycle) and ownership of animals (e.g. buffalo cow and pig). From each participant three stool samples were collected on consecutive days. Stool analysis was performed with KK-technique on each sample and supplemented with formalin-ethyl-acetate concentration technique (FECT) on one of the samples. The geographical coordinates of each household were registered, using a hand-held global positioning system receiver (Garmin Ltd., Olathe, USA).

The data on morbidity induced by *O. viverrini* infection and multiparasitism, especially morbidity related to a single infection with *O. viverrini* versus species and double infection with *O. viverrini* and *S. mekongi* were obtained from an in-depth hospital- and community-based study carried out in the year of 2005 and 2006 in the two referral hospitals (Mahosot and Savannakhet provincial hospital) and communities of three provinces in central and southern Lao PDR (Savannakhet, Saravane and Champasack). A purgative was added after praziquantel treatment (single oral dose: 40 mg/kg). All diarrhoeal stools produced were collected and repeatedly washed until the supernatant became clear. The sediment was examined for the presence of adult worms as follows. First, adult *Taenia* spp., *Echinostoma* spp., and *O. viverrini* worms were visually searched. Second, the remaining sediment was examined with a stereo-microscope for the presence of minute intestinal flukes (MIF). The number of each species of parasites was recorded. Species identification was confirmed under light microscope after specimens were colored. The detailed clinical data (physical examination, ultrasound examination, liver function test and whole blood count) were obtained and were additionally associated to the infection status.

Results: Our first study on the epidemiology of *O. viverrini* infection carried out in Saravane province showed a high *O. viverrini* prevalence rate of 58.5% among 814 study participants. Infection occurred in all age-groups including pre-school children of less than 6 years (20.0%). The highest prevalence (> 80.0%) and infection intensity (mean 200 epg) was observed among the adult people aged between 45 and 55 years, indicating an accumulation of this food-borne trematode infection over time. Soil-transmitted helminths were found at lower rates, e.g. hookworm at 46.1%, *A. lumbricoides* at 15.7%, and *T. trichiura* at 11.1%. Almost two-third of study participants harboured with two or more parasite species. Examination of *cyprinoid* fish species in the local rivers showed the high rates of infection with metacercariae. From 98 fish samples out of 23 *cyprinoid* species, almost two-third; the metacercariae were found.

Our investigation in Champasack province revealed an overall prevalence of *O. viverrini* infection of 64.3%. There were remarkable differences found between the settings. High prevalence rates were observed in the low-lands of the Mekong plain (e.g. Khong district 92.0% and Mounlapamok district 90.9%). In Paksong district the prevalence was only 5.7%. The occurrence of soil-transmitted helminth infections was distinctly different. All three major soil-transmitted helminths were higher in the mountainous Paksong district compared to the settings in the plain (e.g. hookworm 94.8%, *A. lumbricoides* 85.9% and *T. trichiura* 55.7%). *S. mekongi* was highly prevalent in Kong district (68.0%: 153/225), while only 3.9% (9/232) was observed among study

participants living in Mounlapamok. There was no *S. mekongi* infection in Paksong district. Regarding multiparasitism, 4 out of 5 study participants harboured two or more parasite species. Habit of raw food consumption, unavailability of sanitary facilities and socio-economic disadvantages were identified as being the key underlying risk factors for *O. viverrini* infection and multiparasitism.

The results from our in-depth study on morbidity showed that 83% of the examined person harboured at least 2 parasite species. Six different species of small intestinal trematodes and one of Echinostomatidae were identified in the purging process. The intensity of infection with *O. viverrini* worms varied in our patients. The worm burden was significantly associated with observed hepato-biliary pathologies, i.e. study participants diagnosed with common bile duct dilatation, liver fibrosis and intrahepatic bile duct dilatation had 2.4 times, 3.1 times and 7.7 times higher worm burden than those without such pathologies. Co-infections of *O. viverrini* and *S. mekongi* increased consistently the risk for liver pathologies. Study participants infected with later co-infections were at 2 and 6 times and 30 and 75 times higher risk of having the observed liver fibrosis and hepatomegaly compared to infection with *O. viverrini* alone and non-infected individuals, respectively in the ultrasound examination.

Conclusions: The prevalence rates and intensity of infection with *O. viverrini*, and associated multiparasitism with soil-transmitted helmints, food-borne trematodes and *S. mekongi*, and the hepto-biliar morbidity related to these infections call for concerted actions for control. Long-term and integrated efforts must improved access to preventive and curative treatment courses in health facilities and the communities coupled with health education and improved access to clean water and adequate sanitation.

Zusammenfassung

Hintergrund: Nahrungsmittelübertragene Trematoden (FBT) parasitieren die menschliche Leber, den Intestinaltrakt und die Lunge und wurden in den letzten Jahren als neues aufkommendes Gesundheitsproblem identifiziert. Es wird geschätzt, dass heute weltweit 40 Millionen Menschen infiziert sind. Mehr also die Hälfte leben in Asien, insbesondere in Ländern Südostasiens. Infektionen mit FBT führen zu diversen und schwerer Erkrankung, z.B. eine chronische Infektion mit dem Leberegel *Opisthorchis viverrini* geht mit einer Leberfibrose, Cholecystitis und Cholangitis einher und kann zur Entwicklung eines tödlichen Cholangiokarzinoms (CCA) führen.

In Laos, vor allem in seinem ländlichen Teil, ist die Angewohnheit rohe und nur ungenügend gekochte Nahrungsmittel z.B. Fleisch, Gemüse oder Fisch zu essen sehr verbreitet und kulturell tief verwurzelt. Ein grosser Teil der Bevölkerung hat nur einen geringen Zugang zu sauberem Wasser und ausreichenden Sanitäreinrichtungen, was mit den geringen sozio-ökonomischen Status der Bevölkerung zusammenhängt. Die Lebensbedingungen sind entscheidende Risikofaktoren für die Übertragung von FBT und intestinalen Wurminfektionen. Die Verbreitung dieser Parasitenarten überschneidet sich stark so dass ein grosser Teil der Bevölkerung mit mehreren Parasitenarten infiziert ist. Gleichzeitige Infektionen mit verschiedenen Parasitenarten führt zu einer grösseren Krankheitslast.

Ziele: Die vorliegende Dissertation verfolgte die folgenden Ziele: (i) Die Epidemiologie des Leberegels *O. viverrini* zu untersuchen und das Ausmass von Ko-Infektionen mit anderen intestinalen Parasiten zu bestimmen. (ii) Die Diversität der nahrungsmittelübertragenen Trematoden im Intestinaltrakt und in der Leber zu beschreiben und deren Beitrag zur Gesamtpathologie zu bestimmen. (iii) Die Beziehungen zwischen dem sozio-ökonomischen Status der Bevölkerung und der Infektion mit FBT, insbesondere dem Leberegel, in verschiedenen öko-epidemiologischen Gebieten, zu quantifizieren. (iv) Die Wichtigkeit von gleichzeitigen Infektionen mit intestinalen Parasiten in verschiedenen öko-epidemiologischen Gebieten zu bestimmen und den Zusammenhang mit Faktoren der Umwelt, des sozio-ökonomischen Status und des Verhaltens der Bevölkerung zu untersuchen.

Methoden: die Daten dieser Dissertation wurden durch eine Serie von epidemiologischen Studien erhoben.

Die ersten Daten zur Epidemiologie des Leberegels (*O. viverrini*) und anderen intestinalen Parasiten wurde in einer Querschnittstudie im Saravane Distrikt (Saravane Provinz) zwischen Februar und März 2004 erhoben. Studienteilnehmer wurden in einem 2-Schritt Verfahren identifiziert. Zuerst wurden die Dörfer und danach in jedem Dorf die Haushalte zufällig ausgewählt. Mittels Fragebogen wurden individuelle Daten (Daten zur Demographie, Verhalten und persönlicher Hygiene) und Daten zum Haushalt (Daten zu Nahrungsmittelvorbereitung, Wasserversorgung und Sanitäreinrichtungen) erhoben. Von jedem Studienteilnehmer wurde eine Stuhlprobe mit der Kato-Katz Diagnostikmethode untersucht. Fische von verschiedenen lokalen Flüssen wurden gesammelt und auf die Präsenz von Metazerkarien (infektiöse Stadien von *O. viverrini*) untersucht.

Eine zweite Querschnittsstudie wurde zwischen März und May 2006 in drei unterschiedlichen öko-epidemiologischen Gebieten der Champasack Provinz durchgeführt: In je drei Dörfer von (i) Inseln im Mekong (Khong Distrikt), (ii) der Mekongebene (Mounlapamok Distrikt) und (iii) der Hügellandschaften (Paksong Distrikt). Alle Mitglieder der zufällig ausgewählten Haushalte wurden in die Untersuchung eingeschlossen. Mit getesteten Fragebogen wurden Daten zur Demographie (u.a. Alter, Geschlecht, Ausbildung, berufliche Aktivitäten) und zum Verhalten von den individuellen Studienteilnehmern erhoben (u.a. Ernährung und persönliche Hygiene). Mit einem Haushaltfragebogen wurden Daten zu den sozio-ökonomischen Lebensbedingungen des Haushaltes (u.a. Material und Typ von Wasserversorgung), zu den Besitzverhältnissen (u.a. Farmmachinen und Fahrräder) und zu Tieren (u.a. Büffel, Kühe und Schweine) erhoben. Von jedem Studienteilnehmer wurden Stuhlproben von drei aufeinander folgenden Tagen erhalten, die mit der Kato-Katz Diagnostikmethode und der Formalin-Ether Konzentrationsmethode untersucht wurden. Mit einem Hand GPS (Garmin Ltd., Olathe, USA) wurden die geographischen Koordinaten der Haushalte registriert.

In den Jahren 2005 und 2006 wurde eine vertiefte Studie in zwei Referenzspitälern (Mahosot und Savannakhet Provinzspital) und in Dörfern von drei

Provinzen im zentralen und südlichen Laos (Savannakhet, Saravane, Champasack) durchgeführt. Erwachsene Personen wurden in die Studie aufgenommen. Nach einer Stuhluntersuchung mit der Kato-Katz Diagnostikmethode wurden Patienten mit einer vermuteten Leberegelinfektion mit Praziquantel (einfache orale Dosis von 40mg/kg) behandelt und ihnen anschliessend ein Abführmittel verabreicht. Alle drauffolgenden Durchfallstühle wurden vollständig auf die Anwesenheit von Parasiten untersucht und die Anzahl Parasiten gezählt. Zusätzlich wurden detaillierte klinische Informationen (klinische Untersuchung, Ultraschalluntersuchung der Leber, Leberfunktionstests) der Studienteilnehmer erhoben.

Resultate: Die erste Studie zur Epidemiologie des Leberegels, die in Saravane durchgeführt wurde, zeigte eine hohe Infektionsrate von 58.5% unter den 814 Studienteilnehmern. Eine Infektion wurden in allen Altersgruppen nachgewiesen, einschliesslich in Kindern im Vorschulalter unter 6 Jahren. Die höchste Prävalenz und Intensität der Infektion wurde in Erwachsenen im Alter zwischen 30 und 45 Jahren diagnostiziert, was auf eine Akkumulation des Leberegels hinweist. Die boden-übertragenen Würmer wurden auch diagnostiziert, u.a. wurden Hakenwürmer (46.1%), Spulwürmer (15.7%) und Peitschenwürmer (11.1%) nachgewiesen. Zwei Drittel der Studienteilnehmer litten an zwei oder mehr Parasitenarten. Die Untersuchungen in *cyprinoiden* Fischarten ergaben hohe Infektionsraten. Von 98 Fischproben von 23 verschiedenen Arten, waren zwei Drittel mit Metazerkarien infiziert.

In unserer Studie in der Champasack Provinz fanden wir eine globale Infektionsrate mit dem Leberegel von 64.3%. Es gab beträchtliche Unterschiede in den verschiedenen Untersuchungsgebieten. Die höchsten Prävalenzen wurden in der Mekongebene gefunden (Khong District 92.0% und Mounlapamock District 90.9%). Im Paksong Distrikt betrug die Prävalenz 5.7%. Das Auftreten von boden-übertragenen Würmern war ebenfalls gebietsspezifisch unterschiedlich. Alle drei boden-übertragenen Wurmarten waren häufiger im gebirgigen Paksong Distrikt als in der Mekongebene (z.B. Hakenwurm 94.8%, Spulwurm 85.9% and Peitschenwurm 55.7%). Die Mekong Bilharziose (*S. mekongi*) war im Kong Distrikt (68.0%) am häufigsten während dem nur 3.9% der Studienteilnehmer im Mounlapamok Distrikt positive waren. Diese Infektion konnte im Paksong Distrikt nicht nachgewiesen werden. In Bezug auf den

Multiparasitismus, 4 von 5 Studienteilnehmer litten an zwei oder mehr Parasitenarten. Die Gewohnheit rohe Esswaren zu sich zu nehmen, die Abwesenheit von Sanitäreinrichtungen und die sozio-ökonomisch benachteiligten Lebensbedingungen wurden als Schlüsselrisikofaktoren für die hohen Leberegelinfektionsraten und den Multiparasitismus identifiziert.

Die Resultate der Vertiefungsstudie zeigte, dass 83% der untersuchten Personen zwei oder mehr Parasitenarten trugen. Sechs verschiedene intestinale Trematodenarten wurden nachgewiesen, darunter auch *Echinochasmus japonicus*, der zum ersten Mal in Laos diagnostiziert wurde. Die Intensität der Infektionen mit dem Leberegel variierte beträchtlich. Leber und Gallen Morbidität und Leberfibrosen und generelle Vergrösserungen der Gallengänge waren assoziiert mit den beobachteten Infektionsintensitäten. Die Ko-Infektionen vom Leberegel mit der Mekongbilharziose resultierte in ein erhöhtes Risiko für eine Leberfibrose und Lebervergrösserung.

Schlussfolgerung: Die Prävalenzen und Intensitäten von *O. viverrini* Infektionen und assoziierten boden-übertragenen Parasiten, nahrungsmittelübertragene Trematoden und *S. mekongi* und die hepatobiliäre Morbidität, die mit diesen Infektionen einhergeht, machen eine Bekämpfung erforderlich. Langzeitliche und integrative Bemühungen müssen den Zugang zu präventiven und kurativen Behandlungen in den Gesundheitszentren und der Bevölkerung gewähren. Diese Bemühungen müssen mit Gesundheitserziehung und einem verbesserten Zugang zu sauberem Wasser und angepassten Sanitäreinrichtungen gekoppelt werden.

1. Introduction

The purpose of this Ph.D. thesis was to study the epidemiology of and morbidity due to FBT in Lao PDR. Particular emphasis was placed on opisthorchiasis, which is the most common and from a public health point of view the most important human trematodiasis in the country. Therefore, this introduction provides an overview of opisthorchiasis and other FBT, their geographical distribution, and their burden, and describes the complexity of their life cycles. Other intestinal helminthiasis, particularly the soil-transmitted helminthiasis (STH) and schistosomiasis mekongi investigated during the field work are also reviewed.

1.1 Biology and life cycle of FBT, STH and schistosomiasis

An infection with food-borne trematodes occurs through consumption of raw or insufficiently cooked aquatic products such as fish, craps and water plants. Food-borne trematodes parasitize human liver, lung, and intestinal tract. Their public health impact of FBT is tremendous, particularly in places where raw food consumption is widespread and deeply culturally rooted. FBT is an emerging public-health problem, but remain largely neglected (Keiser & Utzinger 2005; Lun *et al.* 2005a; Marcos *et al.* 2008; Sripa 2008). The species of medical importance belong to the liver flukes (*Opisthorchis* spp., *Faciola* spp.), the intestinal flukes (*Haplochis* spp., *Fasciolopsis buski* and *Echinostoma* spp.) and the lung flukes (*Paragonimus* spp.) (Keiser & Utzinger 2005).

FBT exhibit a complex life cycle in which the adult worm parasitizes humans or other suitable mammalian hosts. Both the adult worm and the eggs can cause morbidity. Eggs of the parasite are leaving humans mainly with faeces (or sputum in the case of *Paragonimus* spp.) and infect intermediate hosts. Trematodes possess two intermediate hosts, namely (i) freshwater snail, and (ii) fishes, crustacean or water plants (Radomyos *et al.* 2004; Garcia 2007).

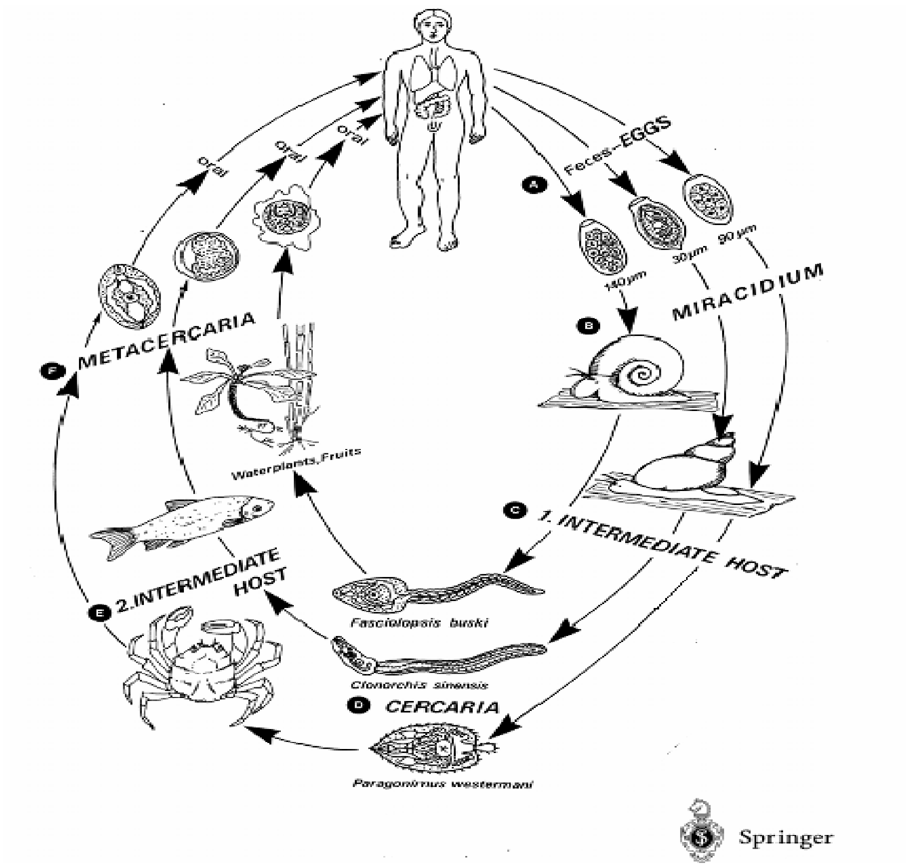

Figure 1.1.1: Life cycle of liver, intestinal and lung flukes (sources: http://parasitology.informatik.uni-wuerzburg.de/login/frame.php)

1.1.1 Liver flukes

The main species of trematode in group of the liver are *Clonorchis sinensis, Fasciola gigantica, Fasciola hepatica, Opisthorchis felinouse* and *Opisthorchis viverrini* (Keiser & Utzinger 2005; Marcos *et al.* 2008).

Human opisthorchiasis results from an infection of the bile duct by small trematode (*O. viverrini* and *O. felineus*), which cause hepato-biliary pathologies (e.g. bile duct obstructive, cholecystitis, cholangitis as well as development of CCA). *Opisthorchiasis* is of particular public health and economic importance in many parts of the developing world, particularly in Southeast Asian countries (Sripa *et al.* 2007; Andrews *et al.* 2008; Kaewpitoon *et al.* 2008; Pinlaor *et al.* 2009). Transmission in human is through the consumption of raw fish (Sripa *et al.* 2003b; Kaewpitoon *et al.* 2008; Cook & Zumla 2009). After the metacercariae contaminated in the fish are digested, the metacercariae excyst in the duodenum and migrate to the liver through the ampulla of Vater and common bile duct (Sripa & Kaewkes 2000) and travel to the distal bile capillaries, where they develop into adult worms (Radomyos *et al.* 2004; Garcia 2007) and start to produce eggs. Eggs may be trapped in the epithelium tissue of the bile duct walls and cause inflammations. Other eggs are transported into the intestine and are excreted into the environment with the faeces. Miracidia are released from the eggs in freshwater where they are taken up by *Bithynia* spp. water snails. In the snails, the parasite develops over several stages (rediae, and sporocytes) to cercariae. Once released into the water they infect the second intermediate host, namely *cyprinoid* fish species (Garcia 2007).

Human fascioliasis is a worldwide zoonotic disease that results from an infection by the trematodes *F. gigantica* and *F. hepatica*. The parasite induces a hepato-bilar disease, i.e. jaundice, hepatomegaly and bile duct ulcer (Hassan *et al.* 2004 ; Moghaddam *et al.* 2004; Aksoy *et al.* 2005; Ashrafi *et al.* 2006; Valero *et al.* 2008; Yesildag *et al.* 2009 ; Valero *et al.* 2009). Human acquire fascioliasis by ingestion of metacercariae attached to certain aquatic vegetations.

The infection is also possible through drinking of contaminated water or consumption of food items washed in such water. After ingestion, the larvae excyst in the small intestine (duodenum), then they penetrate the intestinal wall and migrate into the peritoneal cavity (Garcia 2007). The larvae invade the liver capsule and migrate though the liver parenchyma, which provokes the parenchyma disease that is called “liver rot”. Finally the larvae reach the bile ducts where they develop into adults, mate and produce eggs. The eggs are unembryonated, ovoid, operculate and brownish yellow. They have a length and a width of 130-150 µm and 63-90 µm, respectively. Eggs are released to the gastrointestinal tract with the bile fluid and excreted to the outside environment with the faeces (Aksoy *et al.* 2005; Yesildag *et al.* 2009). In the water, the miracidium develops inside the eggs within a period of 1 to 2 weeks. The miracidium is released and infects freshwater snails of the genus *Lymnaea*. In the snails, the miracidium develops into sporocyst, redial generations and cercariae. The cercariae are then released from the snails and encysted on water plants to metacercariae (Radomyos *et al.* 2004; Garcia 2007).

1.1.2 Intestinal flukes

Intestinal flukes are a group of various trematodes that the adult stage inhabits in the intestinal tract. The main species of this group are *Fasciolosis buski,* and species of the family *Echinostomatidae* and *Heterophyidae* (Chai & Lee 2002; Chai *et al.* 2005a; Trung *et al.* 2007). The adult intestinal trematodes vary in size from the barely visible sized 0.3-0.7 mm wide and 1.0-2.5 mm long (heterophyid flukes) to a large size of 20-75 mm in length and 8-20 mm in width (*F. buski*) (Graczyk *et al.* 2001; Radomyos *et al.* 2004; Garcia 2007). Although the adult worms share a common source of inhabitant but their modes of transmission differ in each species as follows:

F. buski is the biggest human intestinal fluke. Pigs and rabbit are important reservoir hosts (Le *et al.* 2004; Roy *et al.* 2009). The adult worms live in the small intestine attached to the mucosa. Females lay numerous unembryonated eggs. The eggs leave the body via faeces. In the water, eggs require 3 to 7 weeks to develop into mature miracidia. Once released, they infect the intermediate snail hosts of the genus *Segmentina* and *Hippeutis*. In the snails the miracidia develop into a generation of sporocysts and two generations of rediae before forming cercariae. The cercariae leave the snails and encysted on water vegetations (metacercariae) which are infectious for humans. On ingestion, the metacercariae hatch from the cyst in the small intestine and find their final location in the intestinal mucosa (Graczyk *et al.* 2001; Mas-Coma *et al.* 2005; Garcia 2007).

Echinotomatid flukes are orally acquired through ingestion the metacercariae encysted in the different second intermediate hosts depending on the specific species, e.g. freshwater snails, molluscs, tadpoles, frogs, clams and freshwater fish (Chai & Lee 2002; Le *et al.* 2004; Radomyos *et al.* 2004; Garcia 2007). There are 14 species parasitizing human worldwide, belonging to the genera *Echinostoma, Echinochasmus, Echinoparyphium, Euparyphium* and *Himasthla* (Seo *et al.* 1985; Chai & Lee 2002). On ingestion, the metacercariae encapsulate in the small intestine. The metacercariae then migrated to their final locations by attachment to the mucosal wall of the small intestine and develop into the mature worms. The adult worms lay eggs, which are excreted to the outside environment with the faeces (Radomyos *et al.* 2004; Garcia 2007).

The eggs are immature and take 1 to 2 weeks to develop the miracidia. The miracidia hatch from the eggs and infect the snail intermediate hosts. In the snails, miracidia develop into several steps such as sporocyst, rediae and cercariae. Finally, the cercariae are released from the snails and encysted in suitable second intermediate hosts such as freshwater snails, molluscs, tadpoles, frogs, clams and freshwater fish. Humans acquire an infection through consumption of the second intermediate hosts (Chai & Lee 2002; Le *et al.* 2004; Radomyos *et al.* 2004; Garcia 2007).

Heterophyid flukes are small intestinal trematodes that are acquired by ingestion of uncooked infected fish (Murrell *et al.* 2001; Kumchoo *et al.* 2005; Thu *et al.* 2007). After ingestion the metacercariae excysted in the small intestine attached to the mucosal wall and develop into the mature worms. The numerous eggs laid by the adult worms are excreted with the faeces. The eggs are ingested by snail intermediate hosts. In the snails, the parasites develop in three or more generations before they reach cercarial stage that are released into the water. Cercariae penetrate in freshwater fish of the genus *Cyprinoid* and encysted under the scales or in the flesh.

The transmission is through the consumption of infected fish. The natural reservoirs of heterophyids are fish-eating birds and mammals (Chai & Lee 2002; Chai *et al.* 2005a; Skov *et al.* 2009). The main species that infect humans are *Haplorchis* spp., *Metagonimus* spp., *Gastrodiscoides hominis, Centrocestus formosanus, Diorchitrema* spp., *Stellanthasmus falcatus* and *Pygidiopsis summa* (Chai & Lee 2002; Keiser & Utzinger 2005).

1.1.3 Lung flukes

Paragonimus spp. are lung parasitizing parasites of human and carnivores. Today, more than 10 species of lung flukes are known to infected human and mammals: *P. westermani, P. heterotremus, P. maxicanus, P. africanus, P. kellicotti, P. miyazakii, P. philippinensis, P. skrjabini, P. hueitunginensis* and *P. uterobilateralis*

(Garcia 2007). The adult worms of *Paragonimus* spp. are plump, ovoid, reddish brown and encapsulate in the lung of humanoid and carnivores.

The ovoid, operculated and unembryonated eggs sized 80-120 µm with prominent shoulder are laid out by the worms into the void of worms. They eggs are then passed into the bronchioles and are coughed up with sputum, swallowed and excreted with faeces (Radomyos *et al.* 2004; Garcia 2007). After, 2-3 weeks in the water, the miracidium hatches from the egg and infects the first (snails) and second (crabs, prawns) intermediate hosts. Human are infected by ingestion of raw or insufficient cooked crabs and prawn. After encapsulation in the duodenum, the larvae penetrate the intestinal wall into the abdominal cavity and migrate first to the skin and then to the diaphragm and into the pleural cavity (Nakamura-Uchiyama *et al.* 2002).

The larvae finally reach the lung and encapsulate at the vicinity of the bronchioles and develop into the mature forms. Human acquired an infection by ingestion of raw or insufficient cooked crabs or prawns containing metacercariae. After encapsulation in the duodenum, the larvae penetrate the intestinal wall into the abdominal cavity and migrate around or through the diaphragm into the pleural cavity. The larvae finally reach the lung and encapsulate at the vicinity of the bronchioles and develop into the mature worms (Radomyos *et al.* 2004; Garcia 2007).

1.1.4 Soil-transmitted helminthiasis

Soil-transmitted helminths are another group of frequent intestinal parasites with a huge public health and economic impact, particularly in the developing countries of the tropical region (de Silva *et al.* 2003; Bethony *et al.* 2006; Hotez *et al.* 2008b). Four species such as *Ascaris lumbricoides, Trichuris trichiura* and hookworm (*Ankylostoma duodenale* and *Necator americanus*) are widespread species (WHO 2002; Utzinger & Keiser 2004; Ellis *et al.* 2007; Keiser & Utzinger 2008). Although these soil-transmitted helminths share the common source of infection (soil) and inhabitant (intestinal tract), but their modes of transmission differ somewhat (Figure 1.1.2).

A. lumbricoides and *T. trichiura* are orally acquired by ingestion of embryonated eggs. Unembryonated eggs are shed with the faeces in the environment where the embryo matures within a period of 1-2 weeks on the soil (de Silva *et al.* 2003; Brooker *et al.* 2004; Bethony *et al.* 2006). On ingestion, the eggs of *A. lumbricoides* hatch into the larvae in the stomach and duodenum. First, the larvae actively penetrate the intestinal wall. Then, they reach the right heart via the hepatic portal circulation. Next, they are carried out into the pulmonary circulation and reach the pulmonary capillaries. After a 1-week maturation period in the lung, the larvae break into the alveoli and migrate into trachea and pharynx via bronchi. The larvae are then swallowed and finally develop into the mature worm in the intestine. Whilst the eggs of *T. trichiura* hatch in the small intestine, then the larvae eventually attached to the colon and develop into the mature worm there (Radomyos *et al.* 2004; Bethony *et al.* 2006; Garcia 2007).

An infection with hookworms is acquired by skin penetration of filariform larvae (L3) which live freely on the soil. After penetration the larvae reaches the right heart via venules and then lung via pulmonary circulation. The larvae are filtered in the pulmonary capillaries and they break into the alveoli, migrate to the trachea and pharynx via bronchi. The larvae are then swallowed into the small intestine where they develop into the mature worms (Hotez *et al.* 2004; Brooker, Bethony, & Hotez 2004; Bethony *et al.* 2006; Garcia 2007).

Strongyloides stercoralis has one of the more complex life cycles. Human infection is aquired by skin penetration of the infective larvae (filariform larvae). The larvae develop into the adult worm in the small intestine after they immigrate through blood, lung, trachea and pharynx. Female worms produce eggs in the intestinal lumen. The eggs usually hatch in the intestinal tract and the rhabditiform larvae (non-infective larvae) are excreted via faeces. However, the larvae can develop to the filariform in the intestinal tract and cause autoinfection (Garcia 2007).

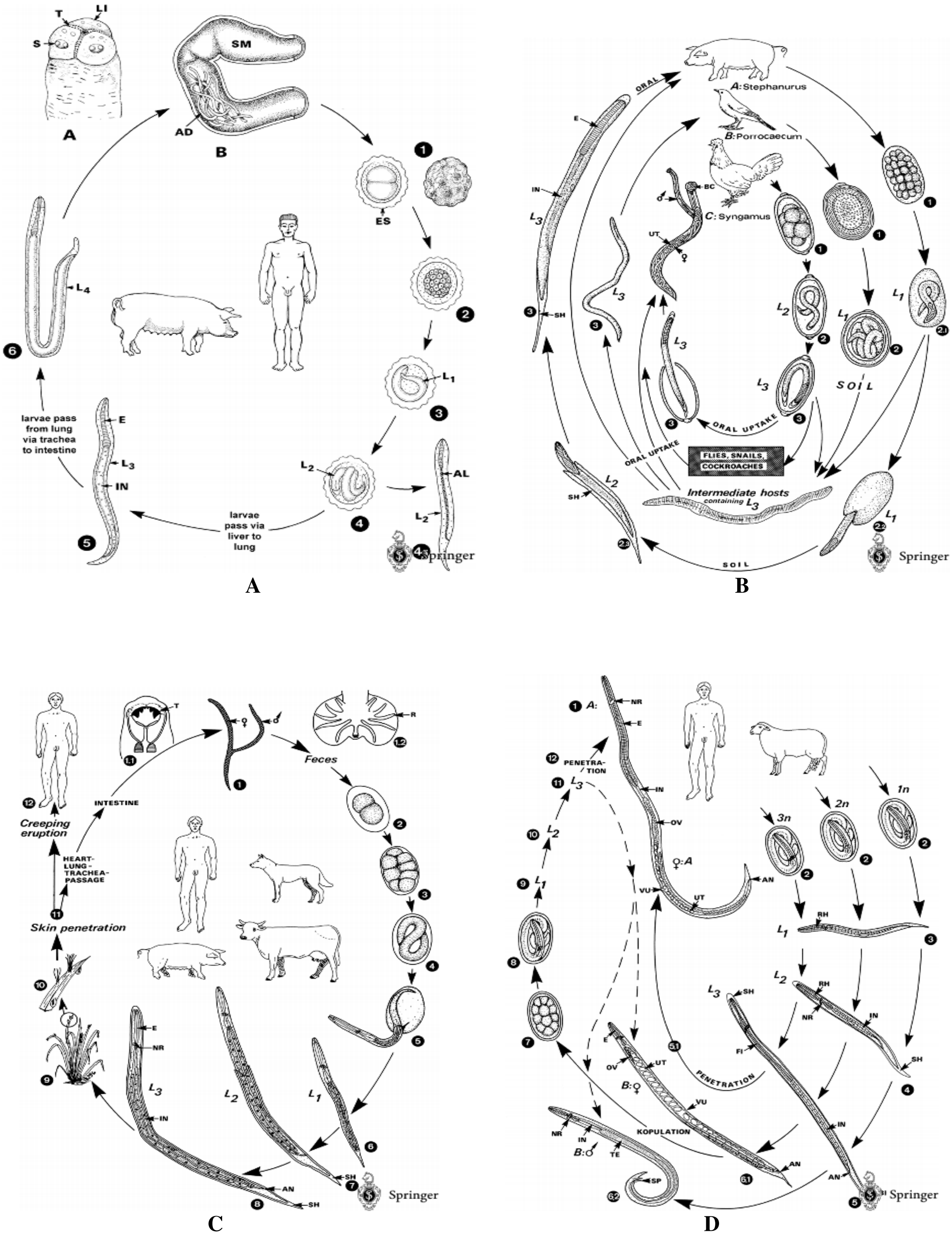

Figure 1.1.2: Life cycles of *A. lumbricoides* (A), *T. trichiura* (B), hookworm (C) and *Strongyloides* spp. (sources: http://parasitology.informatik.uni-wuerzburg.de/login/frame.php)

1.1.5 Schistosomiasis

Trematodes of the genus *Schistosoma* (e.g. *Schistosoma haematobium, Schistosoma mansoni, Schistosoma japonicum, Schistosoma mekongi* and *Schistosoma intercalatum*) are the blood flukes parasitizing human and mammalian animals (Ross *et al.* 1997; Steinmann *et al.* 2006; Wang *et al.* 2009). Three species of these are considered as having an important public health impact, e.g. *S. haematobium, S. mansoni* and *S. japonicum* (WHO 2002; Utzinger & Keiser 2004; Gryseels *et al.* 2006). Schistosomes differ from those of other human trematodes since the worms: (i) have two distinct sexes, (ii) live in the blood vessels, (iii) have non-operculated eggs, and (iv) have no encysted metacerial stage in the life cycle (Gryseels *et al.* 2006; Garcia 2007). The life cycle of schistosomiasis involve the human and, in the case of *S. japonicum* also animals (water buffalo) and the different species of aquatic snails: (i) *Bulinus* snails act as intermediate hosts for *S. haematobium* and *S. intercalatum*, (ii) *Biomphalaria* snails are intermediate hosts for *S. mansoni,* (iii) *Neotricula aperta* snail is intermediate host for *S. mekongi,* and (iv) amphibious *Oncomelania* snails are intermediate hosts for *S. japonicum* (Gryseels *et al.* 2006; Garcia 2007).

Transmission occurs while human contacts infected water. The cercariae in the water penetrate the human skin, then they develop into schistosomula and migrate to the final locations where they mature and produce eggs, e.g. mesenteric venules of intestine for *S. mansoni, S. intercalatum, S. japonicum* and *S. mekongi*) and vesical plexuses for *S. haematobium.* Eggs circulated into the different host organs by blood stream and is trapped the tissues causing inflammation and chronic disease. A part of the eggs are discharged to environment via excretion system (faeces or urine), then the eggs hatch on reaching water and liberating meracidia, which must penetrate a suitable snail intermediate host and produce the infective cercarial stage (Gryseels *et al.* 2006; Garcia 2007; El & Tallima 2009).

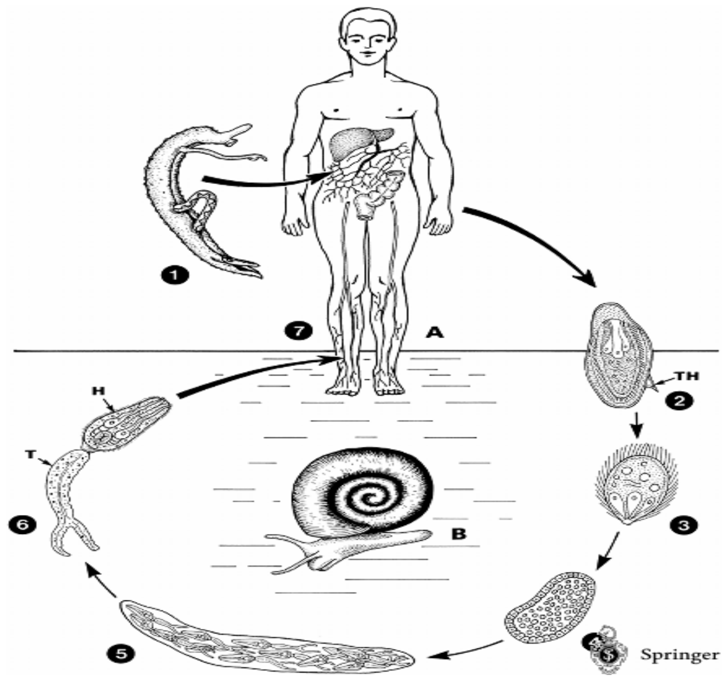

Figure 1.1.3: Life cycle for schistosomiasis (sources: http://parasitology.informatik.uni-wuerzburg.de/login/frame.php)

1.2 Epidemiology and burden of FBT, STH and schistosomiasis

1.2.1 Food-borne trematodiasis

Until today, more than 70 food-borne trematode species have been reported worldwide (Chai & Lee 2002; Keiser & Utzinger 2005; Keiser & Utzinger 2007). It is estimated that at least 750 million people are at risk of infection and 40 million people are currently infected (Chai & Lee 2002; Keiser & Utzinger 2005; Keiser & Utzinger 2007). It is believed that most of the global burden is currently concentrated in Asia (Table 1.2.1).

O. viverrini is endemic in Southeast Asia, particularly in Thailand, and Lao PDR, but can also be found in Vietnam and Cambodia. In highly endemic areas of Thailand and Lao PDR, the prevalence rates may be over 60% of the general population (Giboda *et al.* 1991b; Kobayashi *et al.* 2000; Rim *et al.* 2003; Chai *et al.* 2007; Sripa 2008). An estimated 10 millions of people in this region is infected (Sripa, Sithithaworn, & Sirisinha 2003b; Garcia 2007).

C. sinensis is endemic in China, Japan, Korea, Malaysia, Singapore, Taiwan and Vietnam. An estimated 7 millions of people living in these countries are infected. Of those, 4.7 millions are China and 1 million in Korea (Keiser & Utzinger 2004; Traub *et al.* 2009; Kim *et al.* 2009).

O. felineus infections in human have been recorded from Poland, Germany, Russia and Kazakhstan. Over 1.5 millions of people living in these countries are infected with this parasite (Sithithaworn & Haswell-Elkins 2003; Keiser & Utzinger 2004; Garcia 2007).

Intestinal flukes are mainly endemic in Asia (Graczyk *et al.* 2001; Murrell *et al.* 2001; Wiwanitkit *et al.* 2002; Kumchoo *et al.* 2005; Thu *et al.* 2007; Skov *et al.* 2009). *F. buski* has been reported from reservoir hosts (dogs, pigs and rabbits) in Bangladesh, Cambodia, China, Indonesia, Lao PDR, Malaysia, Pakistan, Taiwan, Thailand and Vietnam (Giboda *et al.* 1991a; Graczyk, Gilman, & Fried 2001; ; Wiwanitkit *et al.* 2002; Le *et al.* 2004; Rohela *et al.* 2005). Several species of *Echinostome* have the focal distribution in Thailand, Taiwan, Indonesia, Malaysia, Philippines, Korea and China (Seo *et al.* 1985; Chai & Lee 2002; Radomyos *et al.* 2004). Small intestinal trematodes of family *Heterophydae* are endemic in China, Egypt, India, Indonesia, Iran, Philippines, Sudan, Tunisia, Korea, Japan, Lao PDR, Vietnam and Turkey. Currently, the number of people at risk and infected individuals is unknown (Giboda *et al.* 1991b; Chai *et al.* 2005b; Kumchoo *et al.* 2005; Chai *et al.* 2007; Rim *et al.* 2008).

Fascioliasis is endemic in several regions across the world. Today, *F. hepatica* is endemic in Europe, the Americas, Oceania, Africa and Asia. *F. gigantica* has a focal distribution in Africa and Asia (Moghaddam *et al.* 2004; Ashrafi *et al.* 2006; Le *et al.* 2008; Valero *et al.* 2009). An estimated 180 million people is at risk and 2.4 million are infected (Watkins 2003; Keiser & Utzinger 2004).

Paragonimiasis occurs in Asia, Africa and the Americas (Garcia 2007; Odermatt *et al.* 2007; Liu *et al.* 2008; Nkouawa *et al.* 2009). *P. westermani* is responsible for a large portion of human infections. It is generally endemic in Asia. *P. heterotremus* has a patchy distribution in Lao PDR, China and Thailand. An estimated 200 million people worldwide are at risk of acquisition the paragonimiasis and 22 million people are infected. Most of human cases occur in Asia (Keiser & Utzinger 2004; Liu *et al.* 2008; Nkouawa *et al.* 2009).

1.2.2 Soil-transmitted helminthiasis

STH mainly affected the poorest region of the developing world with an estimated 2.95-39.0 million disability adjusted life years lost (DALYs) (WHO 2002; Hotez *et al.* 2007). It is currently estimated that 1,200 million people are infected with *A. lumbricoides,* 795 and 740 million people are infected with *T. trichiura* and hookworm worldwide (de Silva *et al.* 2003; Hotez *et al.* 2004; Bethony *et al.* 2006; Hotez *et al.* 2008b). Table 1.2.1 summarizes the global burden due to helminth infections. *A. lumbricoides* and *T. trichiura* infections are very frequent in Asia. The highest prevalence of *A. lumbricoides* is accounted for China (39%); while *T. trichiura* is highly prevalent in the East Asia and pacific islands (28%). Hookworm infections are of highest prevalence in the sub-Saharan Africa (de Silva *et al.* 2003). Transmission is closely associated with low socio-economic, lacking of sanitation and clean water (de Silva *et al.* 2003; Steinmann *et al.* 2007).

The geographical distribution of *S. stercoralis* is in both tropical and temperate climatic zones. Today, there is no data on the global burden available. An estimate 100 million people are infected worldwide (Keiser & Nutman 2004; Bethony *et al.* 2006; Vadlamudi *et al.* 2006).

1.2.3 Schistosomiasis

Human schistosomiasis is highly endemic in Africa and Asia (WHO 2002; Utzinger & Keiser 2004; Steinmann *et al.* 2006). Infection with *S. mansoni* and *S. haematobium* occur mainly in the African continent and the Middle East. *S. japonicum* is endemic in China, the Philippines, Indonesia and India (Utzinger & Keiser 2004; Steinmann *et al.* 2006; El & Tallima 2009). *S. mekongi* is endemic in the Lower Mekong River basin, particularly in Lao PDR and Cambodia (Attwood *et al.* 1997; Attwood 2001; Urbani *et al.* 2002; Keang *et al.* 2007). Global burden due to schistosomiasis is shown in table 1.2.1. An estimated 779 million people is living at risk of infection and 207 million are infected with global DALYs lost of 4.5 million, mainly in Africa (Keiser *et al.* 2002; Steinmann *et al.* 2006; Hotez *et al.* 2007; Hotez *et al.* 2008a; WHO 2002).

Table 1.2.1: Summary global burden due to FBT, STH and schistosomiasis

Disease	Global prevalence (million)	Population at Risk	DALYs (million)	Source
Food-borne trematodiases	> 40	750	n.d	Keiser &Utzinger 2004, Hotez *et al.* 2008
Ascariasis	807-1221	4.2 billion	1.2-10.5	Chan 1997; Bethony *et al.* 2006; Hotez *et al.* 2008
Trichuriasis	604--795	3.2 billion	1.6-6.4	Chan 1997; Bethony *et al.* 2006; Hotez *et al.* 2008
Hookworm infection	576-740	3.2 billion	1.5-21.1	Chan 1997; Bethony *et al.* 2006; Hotez *et al.* 2008
Strongyloidiasis	30-100	n.d	n.d	Hotez *et al.* 2008
Schistosomiasis	200-207	779	1.3-4.5	Chan 1997; Bethony *et al.* 2006; Hotez *et al.* 2008

1.3 Situation of parasitic infections and multiparasitism in Lao PDR

Lao PDR is a landlocked country situated in the Great Mekong sub-region. The Mekong River passes though the country from North to South and its widely interconnected ecosystems are areas of highest risk for FBT and other intestinal parasitic infections as these environmental conditions favour the development of the intermediate hosts of these parasites. In addition, human behaviour and customs favour the transmission. Of particular note, the consumption of raw or insufficiently cooked fish through a preferred traditional dish ‘koy-pa and lap-pa’ is a potential underlying key for acquiring the fish borne trematode infections.

The most frequent food-borne trematode is *O. viverrini*. Prevalence studies carried out over the last decade have shown that this parasite is prevalent in all 18 provinces of the country (Figure 1.3.1) but that it is particularly prevalent in the southern part of the country (Chai & Hongvanthong 1998; Kobayashi *et al.* 2000; Rim *et al.* 2003). There is a lack of community-based interventions, which might explain the persistence of this parasitic infection throughout the country. However, those studies were only counted on the analysis of single stool sample using the KK-method. This shortcoming method challenges the diagnosis of *O. viverrini* in two different ways: (i) underestimation the real prevalence of infection in the community since this diagnostic tool has recognized as being low sensitivity if only one single sample is examined (de Vlas & Gryseels 1992; Kongs *et al.* 2001; Utzinger *et al.* 2001; Booth *et al.* 2003), (ii) confounding the *O. viverrini* dignosis with MIF, since several MIF are present in the country (Giboda *et al.* 1991a; Chai *et al.* 2005b; Chai *et al.* 2007). Those parasites, their eggs are similar in shape and size, using KK-technique the differentiation is impossible.

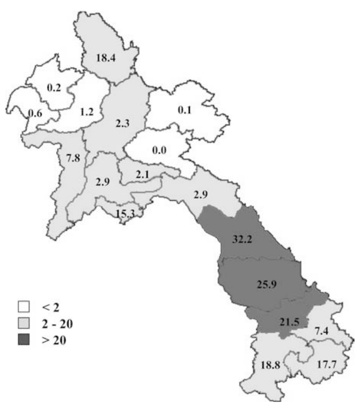

Figure 1.3.1: Distribution of *O. viverrini* among schoolchildren in Lao PDR (Rim et *al.* 2003)

FBT with intestinal tropism have also been reported in Lao PDR. For example, 3.8% of *F. buski* infected human was recorded during the parasitological survey conducted in 1991 among people living in the Nan-gneum wetland of (Giboda *et al.* 1991a). Most probably species of the genus *Echinostoma* are present too, as they have been reported from a neighbouring country (Waikagul 1991; Radomyos *et al.* 2004).

Haplorchis spp. reach prevalences as high as 37.1% among the people infected with small trematode eggs (Chai *et al.* 2005b). The presence of *Haplorchis* spp. is particularly confusing. Eggs of *Haplorchis* have a similar morphology to those of *O. viverrini* and are, therefore, frequently confused with this parasite of hepato-biliar importance. Previous studies have shown that more patients were actually suffering from haplorchiasis than opisthorchiasis (Giboda *et al.* 1991b; Chai *et al.* 2005b; Rim *et* *al.* 2008). The risk of FBT is closely related to the socio-economic and socio-cultural conditions of the communities concerned. Factors that govern FBT are poverty, malnutrition, an explosively growing free-food market, a lack of sufficient food inspection and sanitation, and co-infections with other parasites, particularly helminths (Hotez *et al.* 2008b).

F. gigantica has been reported from cattle in Lao PDR, but no cases in humans have been confirmed to date. In neighbouring Vietnam, since 1997, a dramatic increase in the number of patients with *F. hepatica* has been reported (Tran *et al.* 2001). Underlying reasons for this increase are currently under investigation. Due to unprecedented economic development in Vietnam commencing in the mid-1990s, there was an increase in the cattle market and rearing, mainly by free grazing. These observations combined with the fact that the population has the habit to eat raw vegetables might be responsible for the observed epidemic increase. Thus, there is considerable and concern that *F. hepatica* might gain in importance in Lao PDR.

Soil-transmitted helminths are also highly endemic in the country. A nationwide study among the schoolchildren has shown that the prevalence of *A. lumbricoides, T. trichiura* and hookworms were as high as 35%, 26% and 19%, respectively. In some province the prevalence was higher than 80%, particularly for *A. lumbricoides*. *A. lumbricoides* and *T. trichiura* are highly prevalent in the high-lands, particularly in the northern part of Lao PDR, while hookworms have a more patchy distribution throughout the country (Rim *et al.* 2003).

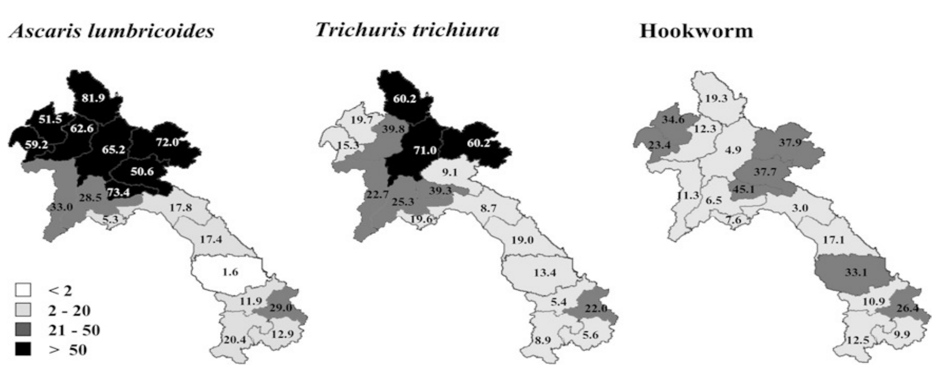

Figure 1.3.2: prevalence of soil-transmitted helminths in Lao PDR (Rim et *al.* 2003)

S. mekongi is the only water-borne trematode endemic in the lower Mekong River basin. Foci of high transmission persist in northern Cambodia and southern Lao PDR. Socio-ecological conditions are very similar in these parts of the Mekong stretch. Rock formations combined with small rapids prevail, which form the bases for the micro-habitats for *Neotricula aperta*, the freshwater snails transmitting the infection (Attwood *et al.* 1997; Attwood 2001). Low socio-economic status of the rural population forces the direct and daily utilization of the Mekong River for household hygiene. Recent investigations showed that also tributaries of the Mekong River can harbour infectious intermediate hosts (Attwood *et al.* 1997; Attwood 2001; Attwood *et al.* 2008).

However, their role for the transmission dynamics is not fully understood. The control programme for this parasite had commenced in the late 1980s and implemented for 10 years with the goal to eliminate this disease in the endemic areas of Champasack province (Urbani *et al.* 2002). However, in 2003 high infection rates with *S. mekongi* of up to 47% were found in communities in the Khong district, Champassak province (MoH/WHO 2003).

Multiparasitism is the norm rather than the exception in the developing world (Raso *et al.* 2004; Steinmann *et al.* 2008). This is also the case for Lao PDR, where many parasite species have been reported. Although some parasites are locally distributed e.g. paragonimiasis in high-lands and schistosomiasis in Khong island of southern part, but many parasites such as *O. viverrini, A. lumbricoides, T. trichiura* and hookworm share their high prevalence in the same eco-epidemiological setting. Hence polyparasitism may occur in the country. However, there is currently a paucity of the epidemiological study focusing on this multiparasitism. Previous studies conducted in central part of country suggested that co-infection between *O. viverrini* and small intestinal trematodes is highly prevalent (Chai *et al.* 2005b; Chai *et al.* 2007). This is underestimated the situation of multiparasitism in the country. The real situation could be many folds higher.

1.4 Morbidity of infection with FBTs and multi-parasitism

Food-borne trematode parasites can be associated with serious morbidity. Of particular severity is the resulting hepato-biliar and intestinal disease (Sripa *et al.* 2007; Kaewpitoon *et al.* 2008; Sripa & Pairojkul 2008; Andrews *et al.* 2008). Liver diseases due to trematode infections include fibrosis and calcifications of liver parenchyma and portal veins or migration of adult parasite stages (fascioliasis) (Aksoy *et al.* 2005). The resulting retention of blood can lead to hypertension of the portal vein, splenomegaly and oesophageal varices. Death due to rupture of oesophageal varices may occur (Haswell-Elkins 2003). Liver fluke infections (opisthorchiasis, fascioliasis) can lead to disease of the bile ducts and gall bladder (Sripa *et al.* 2003a), ranging from mild gall bladder disease to obstructive jaundice, cholecystitis and cholelithiasis. Furthermore, it had been shown that opisthorchiasis is associated with CCA (Sripa & Pairojkul 2008; Andrews *et al.* 2008). Although the mechanism of the pathology is not clearly understood to date, epidemiological and hospital-based studies carried out in different settings consistently found this relationship.

Infections with intestinal flukes such as *F. buski, Haplochis sp*. and *Echinostoma sp*., are generally associated with light various intestinal diseases. However, they aggravate, particularly among pre-school children, and patients with malnutrition and anaemia (Graczyk & Fried 1998; Graczyk *et al.* 2001; Fried *et al.* 2004; Aksoy *et al.* 2005). In Lao PDR the malnutrition rate in pre-school children reaches up to 40% (FAO 2003; Miyoshi *et al.* 2005). These trematode infections, together with other intestinal parasites, might contribute significantly to these ill health conditions. It has been shown that multiple intestinal parasitisms are associated with hampered child growth and a low nutritional status (McGarvey *et al.* 1993; Ezeamama *et al.* 2005). Based on the virulence of trematodes and the high prevalence in endemic areas their public health impact is substantial. Furthermore, these infections drain the social and economic development of effected regions through multiple channels, including loss of productivity, health care costs incurred and impact on the development of children. A recent study carried out in China with an emphasis on *Clonorchis sinenesis* has elucidated the social and economic burden of this infection (Lun *et al.* 2005b; Shen *et al.* 2009).

A substantial amount of research has been conducted on opisthorchiasis and clonorchiasis over the past two decades. Therefore a considerable body of literature is available mainly from Thailand on the pathology (Sripa 2003), disease (Mairiang & Mairiang 2003), epidemiology (Sithithaworn & Haswell-Elkins 2003), as well as taxonomy and biology (Kaewkes 2003; Sithithaworn *et al.* 2006) and immunology (Wongratanacheewin *et al.* 2003). However, the bulk of this work has been conducted with the single parasite-single disease perspective. Difficulties arise if multi-parasitic infections are present. In Southeast Asia the population is exposed to risk factors of numerous trematodes and a host of other intestinal parasitic infections. Thus, multiple species infections are the norm rather then the exception across the developing world (Utzinger & deSavigny 2006). In turn, multiparasitism may lead to combined morbidity and increased disease burden in a manner yet to be examined (Ezeamama *et al.* 2005).

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2. Goal and study objectives

2.1 Goal

The goal of this PhD thesis is to enhance our understanding of infection status, geographical distribution of *O. viverrini* and other food borne trematodes, their environmental and socio-economic risk factors. Furthermore, a comprehensive assessment of multiparasitism, relationship of the parasitic infections and their individual and collective association with observed morbidity in Lao PDR.

2.2 Objectives

2.2.1 To investigate the epidemiology of *O. viverrini* and assess the extent of co-infection with other intestinal parasites in a highly endemic setting.

2.2.2 To describe the diversity of FBT with intestinal and hepato-biliar tropism in different eco-epidemiological settings in southern Lao PDR and assess their contribution to the overall morbidity.

2.2.3 To assess the relationship between socio-economic status and food-borne trematode infection, in particular *O. viverrini* in the distinct eco-epidemiological settings of Champasack province, southern Lao PDR.

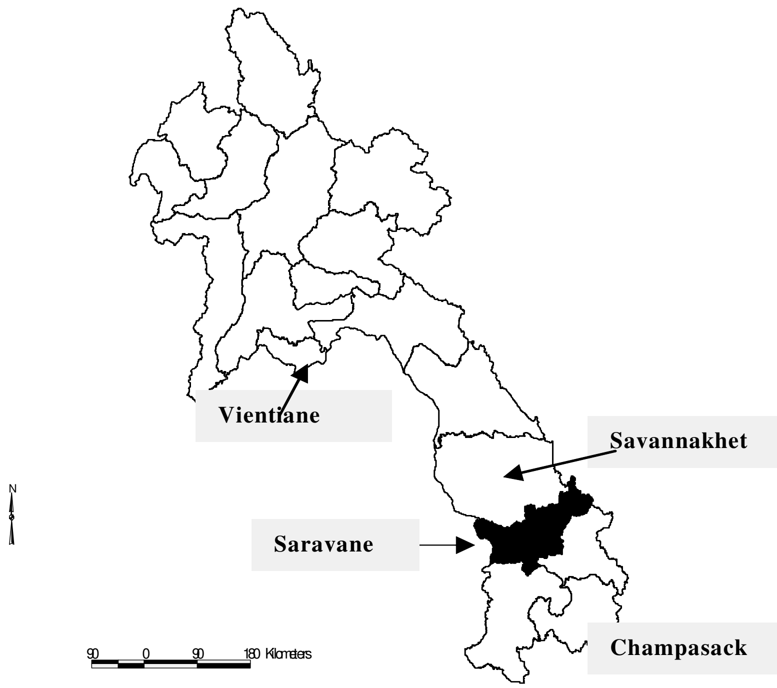
2.2.4 To assess the concomitant infections of intestinal parasites in the distinct eco-epidemiological settings and their inter-linking to the environmental, socio-economic and behavioural risk factors.

3. Study sites

The data presented in this PhD thesis were derived from the different community- and hospital-based studies carried out in the different sittings in the central and southern parts of Lao PDR.

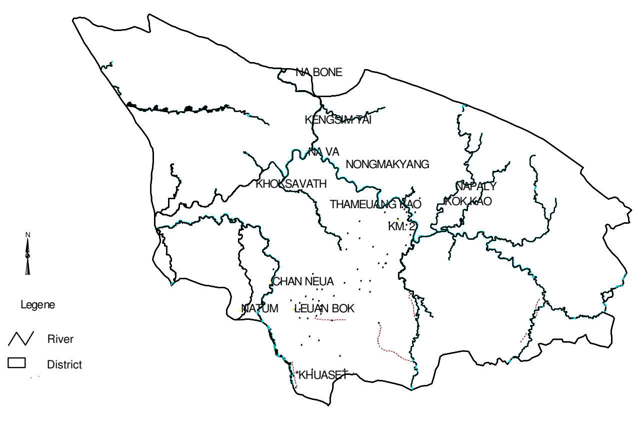
A first cross-sectional study on epidemiology of opisthorchiasis was conducted in February and March 2004 in a southern province of the country Saravane province (population: 350,000; approximately~700 km from Vientiane capital). This study was conducted in the Sarane district (central district of province). A total 157 households in 13 villages were randomly selected. The second cross-sectional study was carried out in March and May 2006 in Champasack province (population 500,000), southern part of country (approximately 600 km from Vientiane capital). Three districts (Khong, Mounlapamok and Paksong) with distinct landscapes characterize the country's profile were selected. A total of 176 households in 9 villages (3 villages per each district) were randomly enrolled in the study.

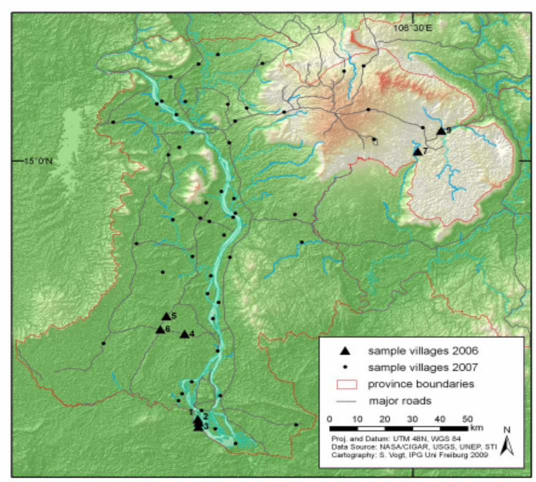
An in-depth study (hospital- and community-base) on the diversity of helminthiasis and its related morbidity was carried out in September and October 2005 in two referral hospitals in central part of Lao PDR (Mahosot hospital in Vientiane capital and Savannakhet provincial hospital in Savannakhet province) and in November 2005 and June 2006 in two communities (Khamsida, Savannakhet province and Thammouangkhao, Saravane province)



Lao People's Democratic Republic

Saravane District





Saravane district

Champasack province
Khong district
Mounlapamok district
Paksong district

4. Epidemiology of *Opisthorchis viverrini* in a rural district of southern Lao PDR

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4.1 Abstract

In Lao PDR, detailed investigations on *Opisthorchis viverrini* are scarce. The objective was to document epidemiological features of *O. viverrini* infections in a highly endemic district. A random sample was selected (13 villages, 15 households / village, all household members > 6 months). Clinical examinations, short interviews, and stool examinations (Kato-Katz technique) were performed. Fish samples were dissected for the presence of metacercaria. A total of 814 persons were enrolled (median 16 years, 51.5% women). The prevalence was 58.5%. Infection rates increased with age (from 20.0% to 85.5%; $p<0.001$). Intensity of infection and the habit of consuming insufficiently cooked fish also increased with age. 75.2% of the study participants reported cooking fish insufficiently. Of the 23 different species of cyprinoid fish consumed in the study villages, 20 species were infected. At the village level the prevalence of raw fish consumption was strongly associated with the infection status of *O. viverrini* ($r=0.76$, $p=0.003$). At individual level, age, the consumption of insufficiently cooked fish, and the absence of sanitation were strongly associated. The study describes the high endemic level of *O. viverrini* infection and the key risk factors in southern Lao PDR. The disease associated with an *O. viverrini* infection needs to be assessed in order to develop and conduct adequate interventions.

Keywords: *Opisthorchis viverrini*, epidemiology, food-borne trematode, Lao PDR

4.2 Introduction

Food-borne trematode infection is an emerging public health issue (Keiser et al., 2005). The disease is endemic in many parts of the Far-East, Southeast Asia and Eastern Europe and can be spread over large distances via infected fish (Yossepowitch et al., 2004). The main public health problem in Southeast Asia arises from infection with trematodes belonging to the family Opisthorchiidae such as *Opisthorchis viverrini*, *O. felineus* and *Clonorchis sinensis*. An estimated 9 million people are infected with *O. viverrini*, 1.6 million with *O. felineus* and 35 million with *C. sinensis* (Lun et al., 2005; Sithithaworn et al., 2003; WHO, 1995). *C. sinensis* is endemic in China, Korea, Taiwan, Vietnam and Japan and *O. felineus* is widespread in Russian Federation and Eastern Europe. *O. viverrini* is of public health importance in South East Asia, particularly in Lao People's Democratic Republic (Lao PDR) and Thailand (Sithithaworn et al., 2003).

In Lao PDR, it is estimated that over 2 million people are infected with *O. viverrini* (WHO, 1995). The parasite was first diagnosed in 1929 by Bedier and Chesneau in the cities of Thakek (Khammouane province) and Vientiane, where infection rates of 23% and 15% were found, respectively (Upatham et al., 2003). Today, the prevalence is still very high. Rim and colleagues (2003) recently completed a nationwide survey among schoolchildren and found infection rates often exceeding 50%, in the southern provinces. Recently, Thakek still had a prevalence rate of approximately 60% (Kobayashi et al., 2000).

Essential knowledge about *O. viverrini* infections has been gained over the last decade in Thailand. There, prevalence rates of *O. viverrini* vary between very low in the south and central provinces, to high towards the border with Lao PDR in the North and North East (19.3% and 15.7%) (Sithithaworn et al., 2003; Upatham et al., 2003). In the north, the population mostly belongs to the Lao-Thai ethnic group where raw food consumption is common (Upatham et al., 2003).

The adult *O. viverrini* parasite causes hepatobiliar disease. Most infections are free of symptoms (Mairiang et al., 2003). Clinical manifestations may vary from non-severe, such as right hypochondrial pain or intestinal irritation, to severe manifestation, such as cholangitis, cholecystitis and choletithiasis. Moreover, *Opisthorchis* infection is a main risk factor for cholangiocarcinoma (Pinlaor et al., 2004; Sriamporn et al., 2004). North East Thailand has the highest prevalence of cholangiocarcinoma worldwide (Pinlaor et al. 2004). Over 60% of cancers are attributed to *O. viverrini* (Honjo et al., 2005).

The life-cycle of *O. viverrini* involves two intermediate hosts: a freshwater snail of the genus Bithynia (Sadun, 1955) and then a cyprinoid fish species host (Haswell-Elkins et al., 1992). The adult parasite lives in the biliary ducts. Eggs are shed with the faeces. Transmission of the parasite occurs when sanitation is inadequate, outdoor defecation occurs and raw fish is consumed.

The aim of the present study was to describe epidemiological features of *O. viverrini* in a highly endemic district of Lao PDR. Hence, a cross-sectional household survey was carried out in order to elucidate determinants of disease and transmission relevant for subsequent intervention.

4.3 Materials and methods

4.3.1 Study setting

The survey was conducted in the Saravane district, province of Saravane, southern Lao PDR (Figure 4.1) where recently a high prevalence of *O. viverrini* was found among schoolchildren (Rim et al., 2003). The province has a population of approximately 318,100. 20% are children under five-years of age. The birth rate and mortality rate in this province are 39.8 and 8.3 per 1,000 people per year, respectively. Only 61.9% have access to safe drinking water and as few as 15.1% have sanitary facilities in the households (NSC, 2003; PHO, 2002). Saravane district is located in the low plain and has an estimated population of 83'000 peoples inhabiting 168 villages. 65.0% of the population belong to the Lao-Theung ethnic group (PHO, 2002). The district has a main river (Xedone) with five important tributaries (Xeseth, Xekhone, Huay-pao, Hauy-pa-ae and Hauy-sleng). All are extensively used for fishing. On the Xeseth River, the second largest dam of Lao PDR has been constructed. The Xeset Hydropower Plant is a run-of-river scheme utilizing about 1.5 km of the river with a drop of 157 m. The plant provides electricity for the southern provinces of Lao PDR and Thailand and feeds local irrigation systems.

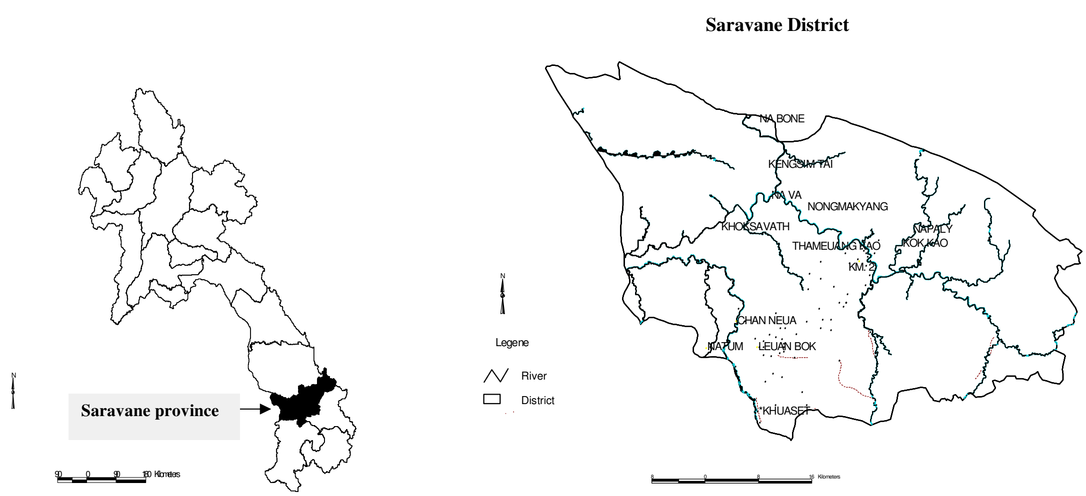

Figure 4.1: Map of Lao PDR and Saravane district

4.3.2 Study design and population surveyed

A cross-sectional study was carried out in February and March 2004. Households were selected by two-stage random sampling. First, 13 villages were selected from the list of villages at the District Health Office. Second, in each village, 15 households were selected from the list of households provided by the head of the village. All members of the selected households older than 6 months and present on the survey day were enrolled in the study.

Ethical approval was obtained from the Ministry of Health, Lao PDR. Informed consent was obtained from all authorities involved and from each individual prior to enrolment.

4.3.3 Field and laboratory investigation

Each study participant underwent a short clinical examination by a general physician and was interviewed on socio-demographic factors and other potential risk factors for *O. viverrini* infection. Age, level of education, profession, use of sanitary household facilities and food (habit of eating raw or insufficiently cooked fish) and personal hygiene were taken for each person. Availability of toilets or latrines and information on food preparing habits were obtained by interviewing the head of the household. Parents or caretakers were interviewed for children under 10 years of age.

A stool sample was obtained from each individual. A single (42mg) Kato-Katz thick smear was prepared on microscope slides and examined with a light microscope for the presence of intestinal parasites (Katz et al., 1972). *O. viverrini* egg counts were obtained for each sample. The presence/absence of *Ascaris lumbricoides*, *Trichuris trichiura*, *Taenia* spp., hookworms and other parasites was also recorded.

In each village, a fisherman was interviewed on the availability of fish and fish species in the village using a pre-tested questionnaire. A picture manual of local fish species of Lao PDR edited by the *Live Aquatic Resources and Research Centre* (Vientiane, Lao PDR) was used to identify fish species.

In each village, a sample of available fish species was obtained and dissected into small scraps, pressed under a cover slide and examined for the presence of trematode meta-cercaria using a light microscope (Rhee et al., 1983).

4.3.4 Data management and statistical analysis

All data was entered in EpiData, v. 3.01 (www.epidata.dk). Analysis was performed using STATA, version 8 (Stata Cooperation, College Station, TX, USA). The study participants were subdivided into seven age groups: (1) < 6 years, (2) 6-15 years, (3) 16-25 years, (4) 26-35 years, (5) 36-45 years, (6) 46-55 years and (7) > 55 years. Prevalence rates were used to assess helminth infections and risk factors. Standard statistical procedures were used to compare proportions and means. Geometric mean of egg counts with *O. viverrini* was calculated for infected persons. *O. viverrini*-positive individuals were grouped into three categories: light infections (1-999 eggs per gram of faeces [epg]), moderate infections (1,000-9,999 epg) and heavy infections (≥10,000 epg) according to a classification proposed by Maleevong and colleagues (1992). Associations between *O. viverrini* infection and risk factors were performed on grouped data at village level and on individual level. Analyses on grouped data were correlations between prevalence of infection and prevalence of risk factors. Multivariate logistic regression was applied to relate *O. viverrini* infection and risk factors at an individual level. The following predictors were included in the regression model: age, sex, habit of eating raw or insufficiently cooked fish, availability of a latrine at home and educational level of head of households. 95% confidence intervals (CI) are provided where appropriate.

4.4 Results

4.4.1 Study population

A total of 814 persons from 157 households and 13 villages were investigated. 65.2% (513) and 34.8 % (283) were ethnic Laoloum and Laotheung, respectively. 51.5% (419) were females (sex ratio M/F: 0.94) and 18.9% (159) were children below 6 years of age. Age ranged from 6 months to 98 years with a median age of 16 years. The average number of people per household enrolled was 5.2.

Among the 157 heads of households, 69.4% were male (Table 4.1). The median age was 43.0 years (45.0 years for males and 39.0 years for females, p = 0.01). Almost all heads of household were married (86.6%) and their illiteracy rate was 31.2%. The illiteracy rate of women was two-fold higher than that of males (47.9% vs. 23.9%, p = 0.001). Subsistence farming was the main occupation of most heads of households (91.7%) while very few were government employees (1.9%) or traders (1.9%).

Table 4.1: Characteristics of studied head of households (n=157)

	Total (n = 157)	**Male (n = 109)**	Female (n = 48)
Age in years			
Median	43.0	45.0	39.0
Age range	18.0-81.0	25.0-81.0	18.0-81.0
Ethnic group % (95% CI)			
Laoloum	61.1 (53.1-68.8)	66.1 (56.4-74.9)	50.0 (35.2-64.8)
Laotheung	38.9 (31.2-46.9)	33.9 (25.1-43.6)	50.0 (35.2-64.8)
Education % (95% CI)			
Illiterate	31.2 (24.1-39.1)	23.9 (16.2-33.0)	47.9 (33.3-62.8)
Primary school	56.1 (47.9-64.0)	61.5 (51.7-70.6)	43.8 (29.5-58.8)
Secondary school	11.5 (6.9-17.5)	13.8 (7.9-21.7)	6.3 (1.3-17.2)
University	1.3 (0.2-4.5)	0.9 (< 0.1-5.0)	2.1 (< 0.1-11.1)
Profession % (95% CI)			
No work	4.5 (1.8-9.0)	3.7 (1.0-9.1)	6.3 (1.3-17.2)
Farmer	91.7 (86.3-95.5)	90.8 (83.8-95.5)	93.8 (82.8-98.7)
Trader	1.9 (0.4-5.5)	2.8 (0.6-7.8)	0
Government employee	1.9 (0.4-5.5)	2.8 (0.6-7.8)	0

4.4.2 Sanitation facilities

In only one village, closest to the provincial capital, were sanitation facilities available (Table 2). 95.0% (773 of 814) of all study participants reported to defecate regularly outdoor. Of these, 95.2% defecated in the bush surrounding their village, 4.3% used a dugout hole and 0.5% into a river.

4.4.3 Clinical examination

A physical abnormality was diagnosed in 192 (23.6%) study participants. 91 (47.4%) and 31 (16.1%) reported a diarrhoeal episode (more than 3 bowel movements within 24 hours) in the past week or weight-loss, respectively. 30 persons (15.6%) had a skin-eruptions and 22 patients (11.5%) had chronic itching (chronic urticaria). 16 persons (8.3%) had clinical anaemia (sub-conjunctiva pallor) and 2 patients (1.0%) had sub-icterus and hepatomegaly.

4.4.4 Stool sample analysis

The prevalence rates of intestinal parasitic infections are given in Table 4.2. The most prevalent parasite infection was *O. viverrini,* found in more than half of the study participants (58.5%). This infection was prevalent in all villages ranging from 14.3% to 79.9%. The two villages closest to the district capital, Banxeseth (14.3%) and Km2 (26.7%) had the lowest prevalence rates. Infection rates increased with age and reached a plateau in the 16-25 years age group (Figure 4.2) and did not differ between gender (male 59.5% versus female 57.5%, $p=0.570$).

Of all *O. viverrini* infected subjects (476 individuals), 92.5% were classified as having a light infection, 7.3% a moderate one and 0.2 % as heavily infected. The geometric mean infection intensity was 154.3 (range 24-15,552 epg). There was no statistical difference between males (172.3 epg) and females (138.6 epg; $p = 0.357$). The intensity of infection increased with age in a way comparable to prevalence of infection (Figure 2). The pre-school children (aged < 6 years) had the lowest intensity of infection (90.7 epg) while the highest was found in adults aged 45-55 years (206.6 epg).

We found hookworm infections in 46.1% of subjects. The highest prevalence at village level reached 79.6% (Table 4.2). Infections with *A. lumbricoides* and *T. trichiura* were detected in 16.0% and 11.0% of the participants, respectively. Eggs of the *Taenia* spp. were recorded in 5.0% of the stool examinations. In three villages, the prevalence of this parasite attained 10% of the subjects.

In 83.5% (680/814) of the stool specimens analysed, at least one intestinal parasite species was found. 40.9% (333/814) of the subjects were infected with one, 33.1% (269/814) with two, 8.9% (73/814) with three and 0.6% (5/814) with four different parasite species. The infection rates did not vary with gender (83.5% vs. 83.5%, p = 0.996).

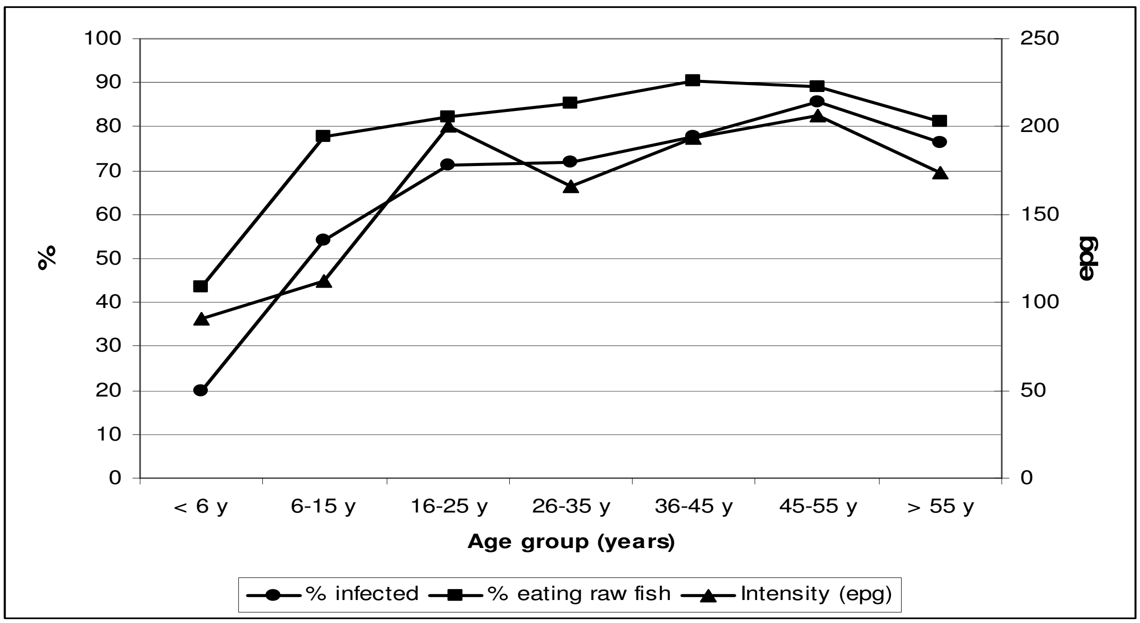

Figure 4.2: **Prevalence (%) and intensity (eggs per gram stool [epg]) of infection with *Opisthorchis viverrini* and prevalence (%) of eating raw or insufficiently cooked fish by age.**

Table 4.2: Prevalence of intestinal parasites and selected risk factors by village (n=814)

Villages	*O. viverrini*	Hookworm	*A. lumbricoides*	*T. trichiura*	*Taenia* spp.	Habit of eating raw or insufficiently cooked fish	Never heard about *O. viverrini*	Availability of toilet at home
Kengsim	79.7	29.0	1.5	4.4	5.8	79.7	88.4	0.0
NongMakyYang	78.3	43.5	1.5	5.8	2.9	87.0	88.4	0.0
Napari	70.7	40.0	52.0	1.3	1.3	69.3	98.7	0.0
Nabone	68.8	29.9	2.6	9.1	10.4	87.0	83.1	0.0
Channeua	66.7	54.0	0.0	14.3	4.8	74.2	96.8	0.0
Thamuongkao	62.3	39.1	0.0	2.9	10.1	73.9	66.7	0.0
Khoksavath	59.7	47.4	1.8	1.8	0.0	91.2	71.9	0.0
Nava	59.6	32.7	9.6	1.9	3.9	86.5	62.1	0.0
Natum	59.5	52.7	8.1	4.1	1.4	86.5	51.9	0.0
Leunbok	44.9	79.6	59.2	32.7	8.2	73.5	100	0.0
Khokkao	42.4	55.9	0.0	1.7	10.2	50.9	88.1	0.0
Km2	26.7	40.0	20.0	11.1	4.4	62.2	48.8	70.0
Benxeseth	14.3	67.9	62.5	66.1	1.8	44.6	91.1	0.0
Total	58.5	46.1	15.7	11.1	5.0	75.2	80.5	4.5

4.4.5 *Fish consumption habits*

75.2% (611/814) of persons reported frequently consuming raw or insufficiently cooked fish (Table 2). The habit was observed in all villages with rates between 44.6% and 91.2%. Men did it significantly more frequently (79.2% vs. 71.3%, p = 0.009). The reporting rate showed a marked increase with age paralleling infection rates (Figure 4.3).

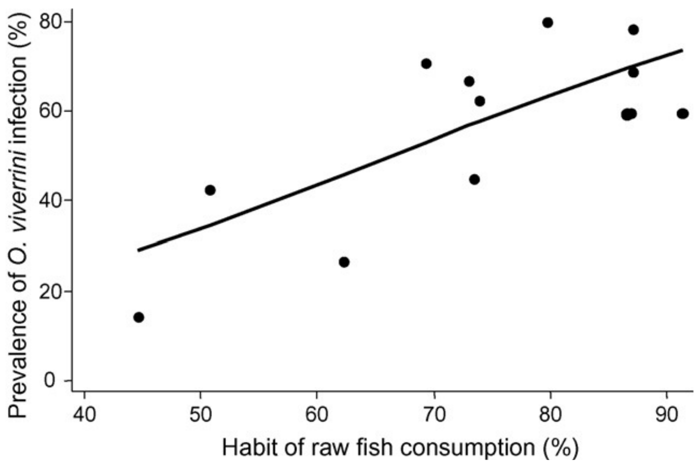

Figure 4.3: Association between the prevalence of *Opisthorchis viverrini* infection and the consumption of raw or insufficiently cooked fish at village level (*n* = 13). Two villages have very similar associations and the dots overlap. The black line represents the line of best fit.

4.4.6 *Fish examination*

43 different fish species were found in the study villages. Of these, 23 species (53.4%) belonged to the family of cyprinoidiae. In a sample of 98 fishes of cyprinoidiae fish, originating from 6 different rivers, metacercaria were found in 58 samples (59.2%).

High metacercaria infection rates were found in a group of seven species. The following fish species belonged to this group (Lao name in bracket, # infected of # examined): *Hampala macrolepidota* (Pa-sout, 7 of 7), *Lobocheilus melanotaen* (Pa-langnam, 9 of 9), *Poropuntius cf laoensis* (Pa-chat, 9 of 10), *Puntius brevis* (Pa-khaomon, 6 of 7, *Cyclocheilichthys enoplos* (Pa-chok, 4 of 5), *Oreichthys parvus smit* (Pa-siew-na, 2 of 5) and *Rasbora Ourotacniatiran* (Pa-siew-our, 2 of 6).

Low metacercaria infection rates were found in a second group of 13 fish species (Lao name in brackets): *Amblynchichthystrunca* (Pa-tapo), *Amblynchihthys truncatus* (Pa-mang), *Cyclocheiliehthysarmatus* (Pa-dokgnue), *Discherodontusahme* (Pa-hangdeng), *Garra fuligirosa* fowler (Pa-khome), *Hypsibarbus pierrei* (Pa-park), *Notopterus* (Pa-tong), *Osteochilus schlegeli* (Pa-namoum), *Osteochilus hasselti* (Pa-thai), *Parachela maculicauda* (Pa-tebhuabird), *Puntioplites falcifer* (Pa-sakang), *Raiamas guyttatua* (Pa-mitsanak-noy), *Systomus orphoides* (Pa-pok). Only three species of fish were not found infected (Lao name in bracket): *Cyprinus carpiolinnaeus* (Pa-nai), *Oreochromis niloticus* (Pa-nin) and *Barbichthys nitibus* (Pa-soy).

4.4.7 Associations of risk factors with O. viverrini infection

Analysis on grouped data at village level showed a strong positive correlation between the prevalence of *O. viverrini* infection and the habit of consuming raw fish or insufficiently cooked fish (r=0.76, p=0.003, Figure 3). No correlation was found between *O. viverrini* infection and the proportion of persons who reported not having heard about opisthorchiasis (r=0.18, p=0.564).

Moderate negative correlations were detected between the *O. viverrini* infection and the prevalence of hookworms (r=-0.57, p=0.041), *T. trichiura* (r=-0.69, p=0.009) and *A. lumbricoides* (the latter was not significant r=-057, p=0.060). No correlation was found with *Taenia* spp. infection rates (r=0.03, p=0.918).

Only two cases of clinical icterus and hepatomegaly findings were clinically diagnosed. Both were infected with *O. viverrini*, one person lightly while the others with moderate infection intensity.

In all age groups the consumption of raw or insufficiently cooked fish was reported at prevalence rates above 75%, except in children under 6 years of age (Figure 2).

Logistic regression was used to investigate the association of risk factors and *O. viverrini* infections at the individual level (Table 4.3). The analysis revealed that age, fish consumption habits, and availability of latrines were independently associated with the risk of *O. viverrini* infection. Schoolchildren aged 6 to 15 years had a 2.52 fold higher times risk for an infection with *O. viverrini* over preschool children (p=0.001). Likewise adults aged 46-55 years had a 12.57 fold increased risk than preschool children (p<0.001). Study participants who reported to consume raw or uncooked fish had a 2.31 fold higher risk of *O. viverrini* infection (p<0.001). Having a sanitation facility in the household was associated with a 74% risk decrease (OR: 0.26, p=0.001).

Gender did not contribute to the overall risk of infection (OR: 1.18, p=0.318). Study participants with primary school educational level had a 79% increased risk for an *O. viverrini* infection (OR: 1.79, p=0.004) compared with individuals with no formal schooling. Persons with a secondary school or higher education did not have an increased risk for an *O. viverrini* infection compared with persons with no schooling (OR: 1.02, p=0.955).

Table 4.3: Results of multivariate analysis for risk factors of *O. viverrini* infection

Risk factor	**Odds ratio**	**p**	**95% CI**
Age group:			
< 6 years	1.00		
6-15 years	2.52	0.001	1.45-4.40
16-25 years	5.60	<0.001	2.88-1092
26-35 years	5.84	<0.001	3.00-11.48
36-45 years	8.07	<0.001	3.96-16.46
46-55 years	12.57	<0.001	5.18-30.50
> 55 years	9.40	<0.001	4.52-19.56
Gender			
Female	1.00		
Male	1.18	0.318	0.85-1.64
Habit of eating raw fish			
No	1.00		
Yes	2.31	<0.001	1.57-3.40
Presence of any sanitation facility at home:			
Sanitation facility absent	1.00		
Sanitation facility present	0.26	0.001	0.11-0.57
Educational level:			
No formal schooling	1.00		
Primary school	1.79	0.004	1.20-2.66
Secondary school & higher	1.02	0.955	0.54-1.91

4.5. Discussion

4.5.1 Prevalence and impact of O. viverrini

Stool examinations show a very high endemic level of *O. viverrini*. This finding confirms the previous reports by Rim *et al.* in the same province (2003) and by Kobayashi *et al.* who showed high infection rates in the neighboring Khammouane province (2000). In addition, our study is most likely to underestimate infection rates. Due to time and financial constraints only one stool sample per person could be examined. Repeated stool analysis with Kato-Katz technique has shown to increase substantially the sensitivity (de Vlas et al., 1992; Marti et al., 1993), hence we can assume that the "true" prevalence may be higher than assessed in the present study.

However, misdiagnosis of *O. viverrini* by light microscopy can occur. Small intestinal trematodes such as species from the *Haplorchis* genus have morphologically very similar eggs. Using the Kato-Katz technique, the difference cannot be made easily and confusion with other flukes, in particular small intestinal flukes, cannot be excluded. Haplorchis taichui and H. yokogawai are both endemic in Lao PDR. However, they were found in much lower prevalence than O. viverrini (Ditrich et al., 1990; Giboda et al., 1991a; Giboda et al., 1991b). The clear extent, however, of their importance is not known and needs further assessment.

O. viverrini infection is known to be a major risk factor for cholangiocarcinoma (Honjo et al., 2005). The prevalence rate of infection in Saravane district is considerably higher than recent estimates from districts of North-Eastern Thailand (Sriamporn et al., 2004) where the incidence of cholangiocarcinoma reached 302 cases per 100,000 people (Sriamporn et al., 2004). In addition, recent findings suggest that 60% of the cholangiocarcinoma can be attributed to *Opisthorchis* infections (Honjo et al., 2005). Based on these data and taking into account a total population of 80,000 people for the district of Saravane, then up to 152 cases of cholangiocarcinoma per year due to *Opisthorchis* infections may be expected for this district alone. This, however, is based on the assumption that no confusion was made between *O. viverrini* eggs and intestinal trematodes.

Although *O. viverrini* was observed at high prevalence rates, the intensity of infections was low; an observation which was consistently made in Thailand (Sithithaworn and Haswell-Elkins 2003). This does not necessarily mean that the public health impact is low. The evaluation of the risk for cholangiocarcinoma performed by Honjo and colleagues (2005) was based on the presence of serological antibodies and therefore even light infections and transient infections may lead to liver cancer. In our study, only 2 persons were detected with symptoms associated with other hepatobiliar disease such as jaundice or hepatomegaly. This agrees with earlier findings from Thailand (Pungpak et al., 1989). However, recent community-based ultrasonographic studies showed that hepatobiliar abnormalities are associated with an *O. viverrini* infection and are possible precursor conditions for cholangiocarcinoma (Elkins et al., 1996).

Our study draws attention to the sharp increase with age in infection rates, intensity of infection, and raw fish consumption reaching a plateau in young adults. This finding suggests an accumulation of infection over time due to continuous exposure. Furthermore, no notable gender differences were seen. Comparable observations were recently made in Thailand (Sithithaworn et al., 2003).

Furthermore, our study also demonstrates a high rate of multi-helminth infections of the intestine. These infections in turn contribute to additional functional and developmental morbidity (Raso et al., 2005).

Human infection occurs through ingestion of infectious metacercaria within fish. Our investigation draws attention to the large number of different cyrinoid fish species that are consumed regularly by the Lao rural population, and also to the high rate of fish infection. This information is in agreement with an expert committee report put forth by WHO (1995) in which more than 80 cyprinoid fish species are listed as potential second intermediate fish species for *O. viverrini*. Cyprinoid fish of genera *Puntius*, *Cyclocheilichtys* and *Hampala* were reported to be highly infected with *O. viverrini* metacercaria (Wykoff et al., 1965), which was confirmed in our study. Unfortunately, we were not able to use the pepsin digestion technique for the metacercarial diagnosis

(Tesana et al., 1985), and hence were not able to determine the exact parasite species. Some of the fish infections may therefore be also due to other trematode infections.

Cats, dogs and rats are also definitive hosts for *O. viverrini* (Sadun, 1955). The relative contribution of these hosts to the transmission of the parasite to the intermediate snail hosts are not known but are considered minor compared with the higher egg excretion and hygiene behaviour of humans (Sadun, 1955). Estimations of infection rates in these animals have not been studied and remain unknown for Lao PDR.

4.5.2 Risk factors for O. viverrini infection

The preference for raw and insufficiently cooked fish dishes and the low availability of sanitation facilities are the main factors contributing to a high prevalence level of O. viverrini infection.

The overall illiteracy rate of the heads of household in our study was very high. This might be an underlying risk factor for parasitic infections. Many surveys confirm that a poor educational level is significantly associated with intestinal parasitic infection. For instance a study conducted in Iran showed that with an increased educational level of parents, the infection rates of intestinal parasites, consisting of protozoan (mainly Giardia lamblia and Blastocystis hominis) and helminths (mainly Enterobius vermicularis), declined 50% in children (Nematian et al., 2004). In our study we could observe a trend of higher prevalence for an O. viverrini infection in households with a lower educational level of heads of household. However, this finding was not statistically significant.

In general, lack of sanitation is a key determinant for helminth infections. Its correlation is well documented (Esrey et al., 1991; Fewtrell et al., 2005). Our current survey shows that only in one of 13 villages any sanitation facilities were present and that virtually all study participants reported defecating outdoors.

The present study describes the high endemic level of O. viverrini in the rural and remote district of Saravane in southern Lao PDR. It documents the associated factors for O. viverrini infection such as the consumption of raw fish and the absence of sanitation facilities. As a next step, the disease burden associated with this infection needs to be assessed at the individual and community levels to allow designing adequate interventions. In Thailand, interventions focussing on mass-treatment with Praziquantel and sanitation and health education have shown to be effective (Jongsuksuntigul et al., 2003). They might be of equal importance in areas similar to Saravane district.

Conflict of interest: The authors have no conflicts of interest concerning the work reported in this paper.

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5. Diversity of human intestinal helminthiasis in Lao People's Democratic Republic

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5.1 Abstract

Food-borne trematodiasis is an emerging public-health problem, also in Lao PDR. We investigated the diversity of trematodes and polyparasitism in patients with hepato-biliary or intestinal symptoms in hospital and community-based surveys. In 232 individuals aged ≥ 15 years, stool samples were examined by Kato-Katz (3 samples) and a formalin-ethyl-acetate concentration technique (FECT, 1 sample). *Opisthorchis viverrini* and minute intestinal flukes (MIF) were common; respective prevalences were 86.0% and 63.4%. Hookworm was the predominant soil-transmitted helminth (65.7%).

The prevalences of *Taenia* spp., *Strongyloides stercoralis* and *Trichuris trichiura* were 22.8%, 10.3% and 8.6%, respectively. Additionally, 97 individuals were purged; *O. viverrini* and *Haplorchis taichui* were found in 95 and 76 participants, respectively. Other trematodes included *Phaneropsolus bonnei* (22.7%), *Prosthodendrium molenkampi* (14.4%), *Haplorchis pumilio* (5.2%), *Haplorchis yokogawai* (3.8%), and *Echinochasmus japonicus* (3.1%). Co-infection with *O. viverrini* and MIF was rampant (81.4%). Poly-trematode infection is highly prevalent in Lao PDR; hence requires urgent attention.

Key words Food-borne trematodiasis, *Opisthorchis viverrini*, *Haplorchis taichui*, polyparasitism, purging, Lao PDR

5.2 Introduction

Food-borne trematodes parasitizing the liver, lung, and intestinal tract of humans are an emerging public-health problem.[1-3] It is assumed that over half of the worldwide infections occur in Southeast Asia.[2] Two species of liver flukes, i.e. *Clonorchis sinensis* and *Opisthorchis viverrini*, and more than 50 species of intestinal flukes (species belonging to the genera *Echinostoma*, *Fasciolopsis*, and *Haplochis*) have been described in the literature.[4] An estimated 750 million individuals are at risk, approximately 40 million are infected, and yet food-borne trematodiasis is often neglected.[5-7]

Lao PDR is situated in central Southeast Asia, with the Mekong River running through the country from north to south. The Mekong River basin with its widely interconnected ecosystems has been identified as a high-risk area for food-borne trematodiasis. Previous studies have shown that *O. viverrini* is endemic in all provinces of Lao PDR.[8] The highest prevalence rates were found in central and southern parts, reaching levels of up to 60%.[9] In these studies, however, only '*Opisthorchis*-like' eggs were reported. Minute intestinal flukes (MIF) have eggs that are similar to *O. viverrini* in terms of morphology and size, and hence species-specific diagnosis is difficult.

Adult *O. viverrini* live in the bile duct and can cause chronic inflammation. As a result, cellular injury and partial obstruction of bile flow may occur[10]. However, the majority of individuals with an *O. viverrini* infection are asymptomatic. In ~5-10% of infected individuals, usually among those with heavy infection intensities, non-specific and non-severe clinical manifestations occur, e.g., right hypochondrial pain and intestinal irritation. The most severe manifestations include cholangitis, cholecystitis, and cholelithiasis.[9-11] Moreover, *O. viverrini* is an established risk factor for cholangiocarcinoma; indeed the highest incidence of cholangiocarcinoma in the world has been found in northeast Thailand, with > 60% being attributed to *O. viverrini.*[10, 12,13]

In addition to *O. viverrini*, other food-borne trematodes with intestinal tropism occur in Lao PDR, the most prominent of which is *Haplorchis* spp.[14,15] Stool analysis of Lao patients treated for 'opisthorchiasis' in Czechoslovakia, and a recent survey conducted in the central and southern parts of Lao PDR revealed that a significant number of patients suffered from opisthorchiasis and haplorchiasis concurrently.[14,15] The co-occurrence of *O. viverrini* and *Haplorchis* spp. is not only a challenge for diagnosis, but might also aggravate morbidity.

In the present study we assessed the diversity of trematode infections and intestinal polyparasitism during hospital- and community-based cross-sectional surveys carried out in Lao PDR. Additionally, a sub-sample of patients was purged for worm collection and species identification. Hence, our study allowed validation of the quantitative formalin-ethyl-acetate concentration technique (FECT) for diagnosing *O. viverrini* and MIFs.

5.3 Materials and methods

5.3.1 Study area and population

A hospital-based study was carried out at the infectious disease wards of the Mahosot Hospital (Vientiane, Lao PDR) and the Savannakhet provincial hospital (Savannakhet, Lao PDR) in September and October 2005. Mahosot is the national university leading hospital, with 450 beds. Savannakhet provincial hospital with 80 beds is the largest provincial hospital of Lao PDR, and serves as referral hospital for the southern and central provinces. All patients aged ≥ 15 years who were hospitalized with hepato-biliary or intestinal symptoms such as icterus, stomach-ache, abdominal pain (right hypochondrial quadrant), nausea, vomiting, and abdominal irritation were invited to participate.

The community surveys were carried out in Khamsida (Champhone district, Savannakhet province), and Thamouangkao (Saravane district, Saravane province) in November 2005 and June 2006, respectively. In both areas *O. viverrini* is highly endemic.[8,9] Khamsida is located ~15 km from the central district (population 584 people), and Thamoungkao ~10 km from Saravane (estimated population 1,000 people). Residents in both communities are primarily engaged in farming. Main dishes are prepared with raw or insufficiently cooked fish and other aquatic products[9]. In these community-based surveys, the same inclusion criteria were applied as with in the hospital-based ones.

5.3.2 Field and laboratory procedures

Demographic data (e.g., age, sex, educational attainment, and professional activity), and behavioral data (e.g., food consumption habits) were obtained with a pre-tested questionnaire. All study participants underwent a physical examination by a general physician.

Three stool specimens, collected over consecutive days, were obtained from each individual. One Kato-Katz thick smear (41.7 mg), was prepared from each specimen.[16] Slides were allowed to clear for 30 min prior to examination under a light microscope. The number of eggs was counted and recorded for each parasite species separately.

Exactly 300 mg of stool was placed in a small tube containing 10 ml of sodium acetate acetic-acid formalin (SAF).[17] Samples were subjected to a quantitative FECT at the parasitological department of the Faculty of Medicine, University of Health Sciences (Vientiane, Lao PDR), assisted by laboratory staff from the Swiss Tropical Institute (Basel, Switzerland). Helminth eggs were counted and recorded for each species separately. *O. viverrini* eggs were differentiated from those of MIF by demonstrating the distinct shoulders at the operculum, and eggshell and knob morphology under a light microscope at high magnification.[18]

5.3.3 Purgation of patients

A sub-sample of 97 individuals with heavy '*Opisthorchis*-like' infections was enrolled in a purging procedure.

Each individual received a single 40 mg/kg oral dose of praziquantel after dinner. Albendazole 400 mg was added if patients were co-infected with soil-transmitted helminths. All stool produced after treatment was collected. The following morning, 45 ml of monosodium sulphate solution was administered to patients (Swiff©, Berlin Pharmaceutical Industry Co, Ltd.; Berlin, Germany). All successive diarrheal stools within 24 hours (usually 6-8 bowel movements) were collected and examined. Bottled drinking water was provided and patients were encouraged to drink as much as possible.

5.3.4 Worm collection

Diarrheal stool was poured into a 2 L bottle, filled up with tap water, and stirred until the stool was homogenously mixed. After sedimentation for 10 min the supernatant was discharged, water was added and stirred again. This washing procedure was repeated until the supernatant became clear. The sediment was examined for the presence of adult worms as follows. First, adult *Taenia* spp., *Echinostoma* spp., and *O. viverrini* worms were visually searched. Second, the remaining sediment was examined with a stereo-microscope for the presence of MIF. The number of species-specific parasites was recorded for each individual. Species identification was confirmed under light microscope after specimens were colored with carmine and mounted in permount.

All individuals infected with *O. viverrini*, and soil-transmitted helminth infections were treated according to national guidelines.[19] An anti-spasmodic treatment and oral dehydration was provided in case of side effects due to drug administration.

5.3.5 Data management and statistical analysis

Data were double-entered and validated in EpiData version 3.1 (Epidata Association; Odense, Denmark). Statistical analyses were performed with STATA version 9 (Stata Corporation; College Station, TX). Those individuals with complete data records were included in the final analyses.

Age was subdivided into five groups: (i) 15-25 years, (ii) 26-35 years, (iii) 36-45 years, (iv) 46-55 years, and (v) > 55 years. Infections with hookworm, *Ascaris lumbricoides*, *Trichuris trichiura*, and *O. viverrini* were grouped into light, moderate, and heavy infections (hookworm: 1-1,999; 2,000-3,999; and ≥ 4,000 eggs per gram [epg], *A. lumbricoides*: 1-4,999; 5,000-49,999; and ≥ 50,000 epg, *T. trichiura*: 1-999; 1,000-9,999; and ≥ 10,000 epg, '*O. viverrini*-like': 1-999; 1,000-9,999; and ≥ 10,000 epg, respectively) according to Maleevong and colleagues [20] and WHO guideline.[21]

Fisher's exact test and χ^2 test were employed to investigate associations between categorical variables. Analysis of variance was used to associate the parasite egg counts with either age groups or gender. Arithmetic mean of worm counts was calculated for infected individuals in purged patients. Linear regression and Spearman's correlation were used to investigate the relationship between number of adult worms of *O. viverrini* and MIF flukes and their egg counts in microscopic examination of stool samples. For all analysis the significance level was $p = 0.05$.

5.4 Results

5.4.1 Stool analysis

Complete parasitological data were obtained from 232 individuals, owing to an overall compliance of 97.1%. The majority of subjects participated in the community survey (n = 213; 91.8%).

Table 5.1 summarizes the results from the microscopic stool examination, using either the Kato-Katz technique, or the FECT method. Also shown are the pooled results, with all subsequent analyses performed on these pooled data. '*Opisthorchis*-like' eggs were diagnosed in 216 individuals (93.1%). Examination of multiple Kato-Katz thick smears resulted in significantly higher helminth infection prevalence when compared to a single stool specimen examined by the FECT; hookworm (62.7% vs. 37.5%, $P < 0.001$), *T. trichiura* (7.7% vs. 1.7%, $P = 0.002$), and *Taenia* spp. (21.0% vs. 10.0%, $P = 0.001$). With regard to diagnosing '*Opisthorchis*-like' eggs, the two methods showed borderline statistical significance (93.1% vs. 87.5%, $P = 0.057$).

Table 5.1: Number (%) of individuals diagnosed with intestinal parasites using the Kato-Katz technique (3 samples), and a FECT method (1 sample), and pooled results using both diagnostic approaches (n = 232)

	Pooled results	Kato-Katz	FECT	χ^2	*P*-value
Trematodes					
Small trematode eggs	216 (93.1)	216 (93.1)	204 (87.5)	3.61	< 0.057
Opisthorchis viverrini	200 (86.2)	n.d.	200 (86.0)	n.a.	n.a.
Minute intestinal flukes	146 (63.4)	n.d.	146 (63.4)	n.a.	n.a.
Echinostomatidae	51 (21.9)	40 (17.2)	38 (16.5)	0.06	< 0.804
Nematodes					
Hookworm	153 (65.7)	146 (62.7)	87 (37.5)	30.00	< 0.001
Strongyloides stercoralis	24 (10.3)	n.d.	24 (10.4)	n.a.	n.a.
Trichuris trichiura	20 (8.6)	18 (7.7)	4 (1.7)	9.35	0.002
Enterobius vermicularis	2 (0.9)	0	2 (0.9)	2.01	0.156
Ascaris lumbricoides	1 (0.4)	1 (0.4)	1 (0.4)	0.001	1.000
Capillaria philippinensis	1 (0.4)	1 (0.4)	1 (0.4)	0.001	1.000
Cestodes					
Taenia spp.	53 (22.7)	49 (21.0)	23 (10.0)	11.11	0.001
Hymenolepis diminuta	1 (0.4)	1 (0.4)	0	1.00	0.317

n.a., not applicable; n.d., not determined

There was a tendency that male study participants had higher infection prevalences for individual parasites. Statistically significant sex differences were found for *O. viverrini* (males: 91.6%, females: 81.7%, $P = 0.030$), *Echinostomatidae* (males: 29.9%, females: 15.1%, $P = 0.006$), and *S. stercoralis* (males: 18.7%, females: 3.2%; $P < 0.001$).

Figure 5.1 depicts age-specific prevalence curves for the different parasites investigated. None of the helminths prevalence was significantly associated with age. A distinct age peak of intensity of infection was found for *O. viverrini* and *Taenia* spp. in the 36-45 years and 26-35 years age-group, respectively (Figure 5.2). None of the other helminths showed a statistically significant association between intensity of infection and age or gender.

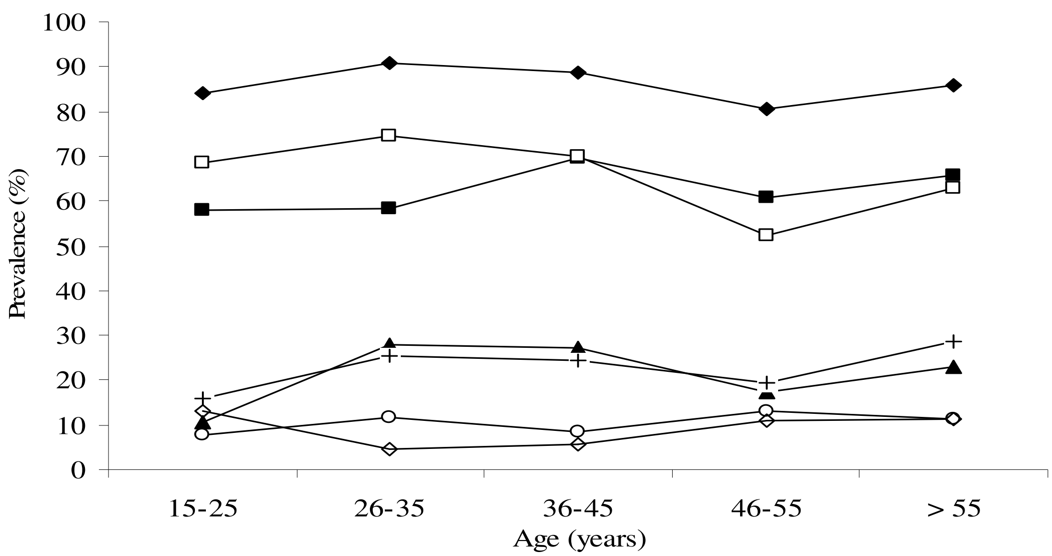

Figure 5.1: Age-prevalence curves for *Opisthorchis viverrini* (♦), minute intestinal flukes (■), hookworm (□), Echinostomatidae (▲), *Taenia* spp. (+), *Strongyloides stercoralis* (○), *Trichuris trichiura* (◊), diagnosed by Kato-Katz and FECT in 232 individuals.

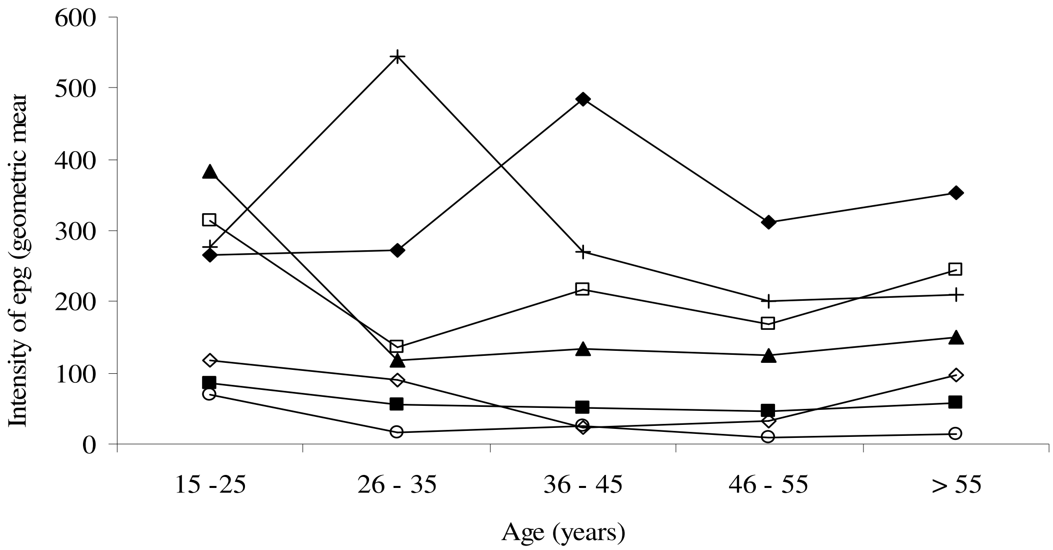

Figure 5.2: Age-intensity (geometric mean of epg) *Opisthorchis viverrini* (♦), minute intestinal flukes (■), hookworm (□), Echinostomatidae (▲), *Taenia* spp. (+), *Strongyloides stercoralis* (○), *Trichuris trichiura* (◊), diagnosed by Kato-Katz method and FECT in 232 individuals. (*Opisthorchis viverrini,* Minute intestinal flukes and *Strongyloides stercoralis* were only counted in the FECT).

5.4.2 Polyparasitism and infection intensity

Among the 232 individuals with complete data sets, only 15 (6.5%) were free of intestinal parasites. Single species parasitic infections were found in 22 individuals (9.5%). Over two-third of the participants (67.2%) harbored 2-4 different parasite species concurrently. Eight individuals (3.5%) were infected with 5 different parasites, and 2 individuals (males, aged 15 and 28 years) harbored 7 parasite species.

Table 5.2 summarizes infection intensities, as expressed in epg, of the different intestinal parasites either detected by the Kato-Katz, or the FECT method, or after combining the results from both diagnostic approaches. The pooled results showed that 117 (50.4%) of the examined patients showed an infection with small trematode eggs of moderate intensity, whereas 20 individuals (8.6%) had a heavy infection.

Table 5.2: Number (%) of individuals with no, light, moderate, or heavy infection intensities with *O. viverrini*, hookworm and *T. trichiura*, according to two different diagnostic approaches, and pooled results (n=232)

Parasite	No infection	Light	Moderate	Heavy
Kato-Katz				
Small trematode eggs	16 (6.9)	66 (28.5)	112 (48.3)	38 (16.3)
Hookworm	86 (37.1)	133 (57.3)	11 (4.7)	2 (0.9)
Trichuris trichiura	214 (92.2)	18 (7.7)	0	0
FECT				
Opisthorchis viverrini	32 (13.8)	66 (28.5)	85 (36.6)	49 (21.1)
Hookworm	145 (62.5)	76 (32.8)	8 (3.5)	3 (1.3)
Trichuris trichiura	228 (98.3)	4 (1.7)	0.0	0.0
Pooled results				
Small trematode eggs	15 (6.5)	80 (34.5)	117 (50.4)	20 (8.6)
Hookworm	79 (34.0)	145 (62.2)	7 (3.0)	1 (0.4)
Trichuris trichiura	212 (91.0)	20 (8.6)	0	0

5.4.3 Characteristics of purged individuals

From the 107 individuals invited for the purging study, 10 were excluded upon clinical examination, as they were severely sick (6 with severe kidney stone disease, and 1 each with liver tumor, ascites, or liver cirrhosis), or pregnant (n = 1). Hence, 97 individuals were purged, 82 (84.5%) in the community-based survey, and 15 (15.5%) in the hospitals. Laoloum was the main ethnic group and patients were aged between 15 and 75 years. The illiteracy rate of females in the community survey was significantly higher than among males (45.0% vs. 34.2%, $\chi^2 = 4.09$, $P = 0.043$), while only one of the participants was illiterate in the hospital study. Subsidence farming was the main occupation of study participants, both in the community and the hospital-based surveys (89.0% and 67.7%, respectively).

5.44 Number and species of worms collected after purgation

Table 5.3 shows the species-specific prevalence of intestinal parasites recovered from study participants after purgation. Adult *O. viverrini* flukes were diagnosed in 95 individuals (97.9%). Additionally, adult worms of 6 different species of MIF were identified, with *H. taichui* being the most common one (78.4%). The prevalences of *P. bonnie* and *P. molenkampi* were 22.7% and 14.4%, respectively. Significantly higher prevalences were observed in the community-based study when compared to hospitalized patients for *O. viverrini* (100% vs. 86.7%, P = 0.001), *H. taichui* (84.2% vs. 46.7%, P = 0.001), and *T. saginata* (21.9% vs. 0%, P = 0.043).

Table 5.3: Number (%) of individuals with adult helminths recovered after purgation among 97 study participants in a community and hospital-based survey in Lao PDR

Parasites	Overall (n = 97)	Community (n = 82)	Hospital (n = 15)	χ^2	*P*-value
Liver flukes					
Opisthorchis viverrini	95 (97.9)	82 (100.0)	13 (86.7)	11.03	0.001
Minute intestinal flukes					
Haplorchis taichui	76 (78.4)	69 (84.2)	7 (46.7)	10.29	0.001
Phaneropsolus bonnei	22 (22.7)	20 (24.4)	2 (13.3)	0.92	0.336
Prosthodendrium molenkampi	14 (14.4)	13 (15.9)	1 (6.7)	0.89	0.344
Haplorchis pumilio	5 (5.2)	5 (6.2)	0	n.a.	n.a.
Echinochasmus japonicus	3 (3.1)	3 (3.7)	0	n.a.	n.a.
Haplorchis yokogawai	2 (3.8)	2 (2.5)	0	n.a.	n.a.
Cestodes					
Taenia saginata	18 (18.6)	18 (21.9)	0	4.10	0.043
Other Taenia spp.	5 (5.3)	4 (4.9)	6.7 (1)	0.08	0.782

n.a., not applicable

Seventy-nine individuals (81.4%) harbored *O. viverrini* and at least one species of MIF concurrently. Conversely, single infections with both *O. viverrini* and MIF were rare; the respective prevalence was 16.5%, and 2.1%. Two of the parasites were significantly associated with age groups, i.e., *P. molenkampi* (χ^2 = 10.63, P = 0.031), and *P. bonnei* (χ^2 = 9.18, P = 0.050) with a peak prevalence in the 36-45 years age-group. Figure 3 shows species-specific age-prevalence curves of adult flukes following purgation.

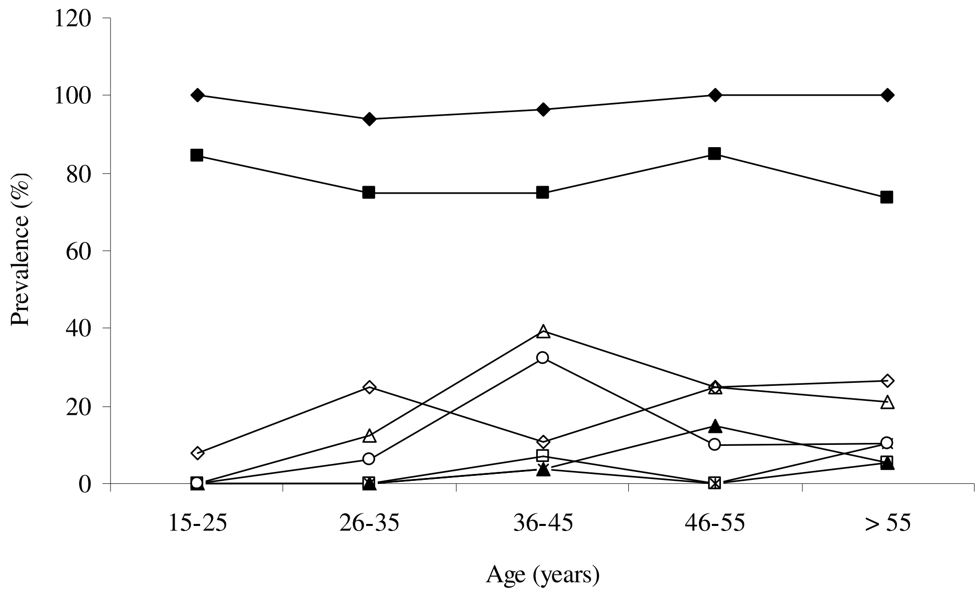

Figure 5.3.: Age-prevalence curve for *O. viverrini* (♦), *Haplorchis taichui* (■), *Echinochamus japonicus* (□), *Taenia saginata* (◊), *Phaneropsolus bonnei* (∆), *Prosthordendrium molenkanpi* (○), *Haplorchis pumilio* (▲), *Haplorchis yokogawai* (*) detected in 97 purged individuals in Lao PDR.

Table 5.4 summarizes the total number of flukes collected from the 97 purged individuals, including median counts and ranges. Very high total counts were recorded for *O. viverrini* (17,755; community: 14,802, hospital: 2,953), and *H. taichui* (15,555; community: 14,530, hospital: 1,025). The arithmetic mean fluke count for *O. viverrini* was 186 (community: 182, hospital: 206), and an arithmetic mean count of 207 was found for *H. taichui* (community: 214, hospital: 146).

Figure 5.4 depicts the association between the number of adult *O. viverrini* flukes recovered after purgation and the infection intensity as expressed by epg upon microscopic stool examination using FECT method. Linear regression analysis shows a significant positive association with a regression equation of $y = 0.98 + 0.92x$ ($r = 0.54$, $P < 0.001$). No association between adult worm recovered and eggs counts was found for MIF ($r = 0.22$, $P = 0.093$); Figure 5 depicts this association between adult worm counts of *O. viverrini* and egg counts per worm. A significant negative correlation was found ($r= -0.40$, $P = 0.0001$) with linear regression equation $y = 2.18 – 0.36x$ ($P < 0.001$).

Table 5.4: Number (including arithmetic mean and 95%, CI) of trematodes discovered after purgation of 97 individuals in Lao PDR

Parasites	Overall	Community	Mean (95%, CI)	Hospital	Mean (95%, CI)
Liver flukes					
Opisthorchis viverrini	17,755	14,802	182 (117 -246)	2,953	206 (53 – 359)
Intestinal flukes					
Haplorchis taichui	15,555	14,530	214 (101 – 326)	1025	146 (54 – 239)
Phaneropsolus bonnei	910	735	37 (6 – 67)	175	88 (13 – 206)
Prosthodendrium molenkampi	519	482	37 (4 – 70)	80	S.N
Haplorchis yokogawai	154	145	S.N	0	0
Haplorchis pumilio	108	108	22 (2 – 41)	0	0
Echinochasmus japonicus	0	10	3 (1 – 6)	0	0

S.N: small number

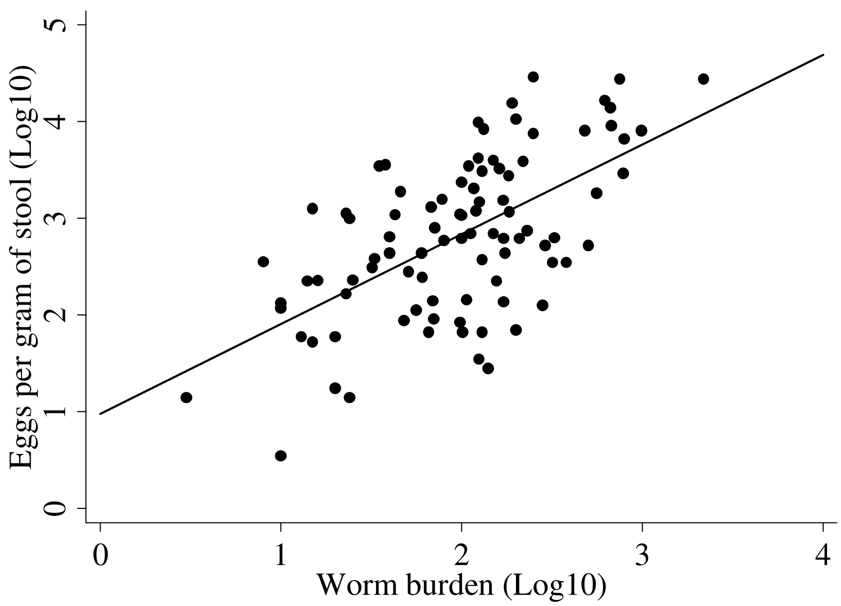

Figure 5.4: Logarithmic transformation of the association between the number of *O. viverrini* flukes recovered after purgation (n = 97) and egg counts in the FECT

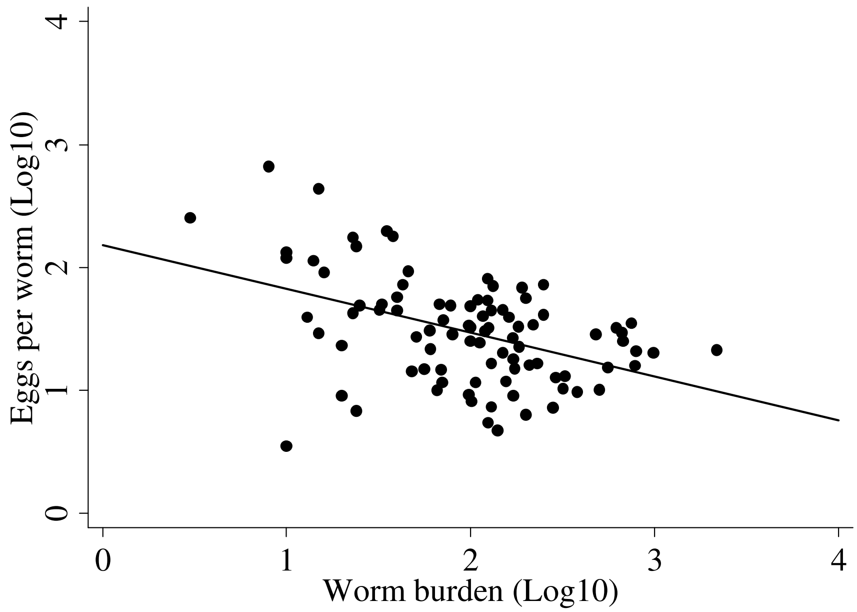

Figure 5.5: Logarithmic transformation of the association between the number of *O. viverrini* flukes recovered after purgation (n = 97) and eggs per worms in the FECT

5.4.5 Diagnostic performance of FECT

The validity of the FECT for diagnosis of *O. viverrini* and MIF was assessed, considering the purgation as the diagnostic ‘gold’ standard. The sensitivity of the FECT for the discovery of *O. viverrini* and MIF eggs was 96.8% (92/95) and 85.0% (68/80), respectively. The specificity and the positive predictive value for diagnosing *O. viverrini* eggs was 100.0% (92/92), regardless of the method. Specificity and positive predictive value of the FECT for MIF diagnosis was 70.6% (12/17) and 93.1% (68/73), respectively. For both parasites the negative predictive value of the FECT was low; 60.0% (3/5) in the case of *O. viverrini*, and 50.0% (12/24) for MIF.

5.5 Discussion

Our data obtained from 232 Lao individuals, aged ≥ 15 years, who complained of hepato-biliary or intestinal symptoms, confirm that multiparasitism is the norm rather than the exception. In fact, more than three-quarters of the participants harbored at least 2 helminth species concurrently, with *O. viverrini*, and MIF being the most common trematodes encountered. We employed a rigorous diagnostic approach with 3 Kato-Katz thick smears performed on consecutive stool specimens, supplemented with a FECT on one of the specimens. Previous studies have shown that multiple Kato-Katz thick smears plus other diagnostic methods are mandatory to achieve a high diagnostic sensitivity.[22,23] In the present study, only 16 individuals (6.9%) were diagnosed free of any intestinal parasite. It is conceivable that the actual number of parasite-free individuals is even lower, as some light infections might have been missed despite our rigorous diagnostic approach. Besides the liver fluke *O. viverrini* and various kinds of intestinal flukes, hookworm infections were also highly prevalent. Interestingly, *S. stercoralis*, arguably the most neglected of the soil-transmitted helminthes [24] was more prevalent than *T. trichiura*, and *A. lumbricoides*. Purgation of a sub-sample of individuals allowed identification of parasites at species level, with MIF and *H. taichui* found at the highest frequency.

Our data underscore that multiparasitism is very common in Lao PDR, and these findings support observations from neighboring countries such as e.g. Vietnam [25-27] and from other parts of the developing world. [6,22,23,28-30] Hence, our results and those from other groups who worked in Lao PDR [8,9] call for concerted action to remedy the issue of multiparasitism. Of particular public-health relevance are our results in relation to the diversity of trematodes identified. In the purgation of 97 patients as many as 7 different trematode species were identified. *O. viverrini* was the most abundant fluke, followed by *H. taichui*. Another 5 intestinal trematodes were recorded, *P. bonnei*, *H. yokogawai*, *P. molenkampi*, *H. pumilio*, and *E. japonicus*, although at significantly lower prevalences, which is consistent with previous reports from other parts of Lao PDR.[14,31] A number of the identified human trematodes have eggs of similar size and shape.[18] Species differentiation is therefore difficult with simple coprologic diagnostic tools such as direct fecal smears or the widely used Kato-Katz thick smear examination. However, the differentiation of MIF from *O. viverrini* is of considerable public-health importance as the latter fluke provokes a range of hepato-biliar diseases, and is a major risk factor for cholangiocarcinoma.[10,32] Hence, monitoring of interventions that are targeted against *O. viverrini* require diagnostic techniques that are capable of differentiating between *O. viverrini* and MIF.

Our analysis showed that *O. viverrini* worm burden is highly significantly associated with the egg counts in the stool samples. Very similar linear regression lines were described in Thailand.[33,34] Hence, we can confirm that egg counts are a valid proxy-measure for intensity of infection with *O. viverrini*. In addition, as Elkins and colleagues [33] we also found a decreasing fecundity of *O. viverrini* with increasing worm burden. A phenomenon which may reflect a density-dependent constraints on fecundity.[34,35]

Our validation of a commonly employed fecal-concentration technique with results of the purging examination, the latter serving as a diagnostic 'gold' standard, showed high sensitivity and specificity for diagnosis of both *O. viverrini* and MIF. Our findings therefore support the use of the FECT in future studies emphasizing parasite species-specific diagnosis. Repeated stool examinations have shown to significantly improve the diagnostic accuracy for parasitic infections.[23,24,36-38] It follows that the FECT performed on multiple stool samples, holds promise for accurate and species-specific diagnosis. However, such an approach is time-consuming and is likely to compromise compliance, as study participants are reluctant to provide multiple stool specimens. Alternatively, species-specific polymerase chain reaction (PCR)-based techniques have been developed. [39-41] However, PCR methods are less suitable for large-scale community-based investigations, as they are costly and still of limited direct applicability under field conditions.

It is important to note that dishes based on raw or insufficiently cooked fish, other aquatic products, and meat are frequently consumed in Lao PDR, and other Southeast Asian countries. The common habit of raw or undercooked fish consumption is a key factor in the transmission of trematode infection, which in turn explains the high prevalence and infection intensity of *O. viverrini*; indeed over half of the subjects examined here harbored a moderate or heavy infection with *O. viverrini*.

Interestingly, we also found 2 MIF species which are transmitted by consumption of raw naiads, i.e., *P. bonnie*, and *P. molenkampi*. Both species were actually diagnosed quite frequently (22.7% and 14.4%, respectively). In the 1970s, the first cases of human infections with these trematodes were described in the Udonthani area of north-eastern Thailand, where communities have similar alimentary costumes than in Lao PDR.[42] Furthermore, in 3 patients living in Kamsida village, a rare echinostomatidae fluke was diagnosed, namely *E. japonicus*. To our knowledge this is the first report of *E. japonicus* from Lao PDR, and details will be presented elsewhere.

Of clinical and public-health importance is the fact that virtually all patients included in the study were infected with trematodes, with more than three-quarters harboring *O. viverrini* and MIFs concurrently. Conversely, only few patients had a single-species infection (*O. viverrini*: 16.5%, MIF: 2.1%). Although it is acknowledged that polyparasitism may negatively impact on health and wellbeing, new research is needed to deepen our understanding of the underlying mechanisms, and how to measure improvements following control measures at the individual and population level. Further investigations, coupled with rigorous monitoring of control interventions, are urgently needed to further our knowledge, and provide a rationale for evidence-based interventions.

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Conflict of interest: The authors have no conflict of interest in relation to this work.

Ethical approval: The study was approved by the institutional review board of the Swiss Tropical Institute and the Ethics Committee of the University and the State of Basel (EKBB; reference no. 255/06). Ethical clearance was obtained from the National Ethics Committee, Ministry of Health in Vientiane (reference no. 027/NECHR). Written informed consent was obtained from each participant prior to enrolment.

Author's contribution: SS and PP designed study; SS, YV, MV, PO collected field data; SS and OR collected hospital data; SS and ST identified parasites in laboratory; SS analyzed data and drafted manuscript; PO, JU and KA contributed to data analysis and revised manuscript; KA held overall responsibility of data collection; all authors read and approved the final manuscript. PO, KA and JU obtained funding; SS and PO are guarantors of the paper.

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6. Rare human infection with the trematode *Echinochasmus japonicus* in Lao PDR

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6.1 Abstract

Food-borne trematodiases are often neglected although millions of people are affected and these zoonotic diseases are emerging in some parts of the world. Humans acquire an infection through consumption of the second intermediate hosts (e.g. freshwater fish) that harbour the metacercariae of the parasite. Here, we report the first three cases of *Echinochasmus japonicus* infection in Lao PDR. Adult *E. japonicus* flukes were recovered from a woman (age: 37 years) and two men (age: 42 and 75 years) following treatment with praziquantel (single oral dose of 40 mg/kg) and purgation (45 ml monosodium sulphate). All three individuals were infected with two other species of trematodes concurrently, namely *Opisthorchis viverrini* and *Haplorchis taichui*. The two male patients moreover harboured *Prosthodendrium molenkampi* and *Phaneropsolus bonnie* trematodes. All three cases suffered from diarrhoeal episodes and other gastro-intestinal discomforts in the month preceding praziquantel administration and purgation. The frequent consumption of raw or insufficiently cooked freshwater fish and other aquatic products is the route cause of acquiring rare zoonotic infections such as *E. japonicus*.

Keywords: Food-borne trematodiasis, *Echinochasmus japonicus*, purgation, case study, Lao PDR

6.2 Introduction

Food-borne parasitic infections, particularly those caused by trematodes, are an emerging public health threat around the world [1,2]. In Lao PDR, the liver fluke *Opisthorchis viverrini* is the most important food-borne trematode species parasitizing humans [3–6]. Indeed, infections with *O. viverrini* negatively impact on the public health, since this parasite has been identified as a main risk factor for cholangiocarcinoma, a fatal liver cancer [7]. Moreover, several small intestinal flukes have been documented in humans. Among those, *Haplorchis taichui* is the most prevalent one [8-10]. Other intestinal flukes that have been documented in the literature include *Haplorchis pumilio*, *Haplorchis yokogawai*, *Phaneropsolus bonnie* and *Prosthodendrium molenkampi*. The adult worms develop restrictedly in the human intestine and cause intestinal parasitic burden.

Several trematodes of the family Echinostomatidiae are important infections in birds and mammals, and a number of these trematodes also parasitize the human intestinal tract. In Lao PDR, previous studies have listed the prevalence of infection with *Echinostoma* spp. To date, however, not a single species of Echinostomatidiae has been identified, neither in humans nor in livestock [9].

Here, we report – to our knowledge for the first time – three cases of *Echinochasmus japonicus* infections in Lao PDR. Adult worms were recovered in patients suffering from intestinal disorders during a cross-sectional hospital and community-based survey carried out in Savannakhet province, in the central part of Lao PDR, after administration of praziquantel followed by purgation.

6.3 Materials and methods

6.3.1 Study area and population

The study area and population surveyed have been described elsewhere [10]. In brief, the three cases of *E. japonicus* were diagnosed in November 2005 within the frame of a larger study pertaining to the epidemiology and control of liver fluke infections and intestinal multiparasitism carried out in Khamsida village, Champhone district, Savannakhet province in central Lao PDR. In this area villagers are mainly engaged in subsistence farming. Common traditional food dishes include raw or insufficiently cooked freshwater fish, locally known as “lap-pa”, “koy-pa” and “som-pa”.

6.3.2 Questionnaire, parasitological and clinical investigations

Individuals aged over 15 years were first interviewed with a pre-tested questionnaire about common food habits and self-reported morbidity indicators (e.g. abdominal pain), using a recall period of one month.

After the questionnaire survey, three stool samples were collected from each individual over consecutive days. On the spot, stool samples were subjected to the Kato-Katz technique [11]. The number of eggs was counted and recorded for each helminth species separately, and eggs per gram of stool (EPG) were calculated. In addition, a portion of fresh stool (300 mg, weighed) was examined using a modified formalin-ether concentration technique [12] for diagnosis and quantification of helminth infection intensity and appraisal of intestinal protozoa.

Finally, each individual was examined by ultrasonography at Champhone inter-district hospital, Champhone district, Savannakhet province by an experienced radiologist. Ten ml of venous blood was drawn and the following parameters were measured: cell blood count (CBC), haemoglobin (Hb) level, mean corpuscular volume (MCV), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and HBs-Ag.

For worm recovery, a single oral dose of praziquantel (40 mg/kg) was administrated to each participant before bedtime. The next morning, people were administrated 45 ml of monosodium sulphate for purgation. Six to eight successive diarrhoeal stools were collected from each individual within the next 10 h. The diarrhoeal stools were poured into a 2 l bottle and stirred until the stool was homogenously mixed. After a sedimentation time of at least 10 min, the supernatant was carefully poured out. The process was repeated until the supernatant became clear. First, adult *Taenia* spp., *Echinostoma* spp. and *O. viverrini* worms were visually searched for in the entire sediment. Then, the solution was examined under a stereo-microscope for minute flukes (e.g. *H. pumilio*, *H. taichui*, *H. yokogawai*, *Prosthodendrium molenkampi* and *P. bonnei*).

Echinostomatidae-like adult worms were brought to the laboratory and stained with standard techniques. Characteristics of *E. japonicus* were observed, i.e. a row of 24 collar spines, large ventral sucker, vitelline follicles and eggs in the uterus as previously described [13–15].

6.3.2 Ethical considerations

The study was approved by the institutional research commission of the Swiss Tropical Institute. Ethical clearance was granted by the Ethics Committee of the University and the State of Basel (EKBB; reference no. 255/06) and the National Ethics Committee, Ministry of Health (MoH) in Vientiane (reference no. 027/NECHR). Written informed consent was obtained from each participant prior to enrolment. At the end of the study, all participants still exhibiting parasitic infections were treated according to national guidelines [16].

6.4 Results

6.4.1 Description of E. japonicus flukes

A total of 10 adult *E. japonicus* flukes were collected from three individuals (2, 4 and 4 adult specimens for cases 1, 2 and 3, respectively). Unfortunately, the tegument and/or parenchymal organs were damaged in six of these flukes upon recovery. However, one fresh specimen and three stained specimens could be observed in detail. In each of these four specimens we observed the characteristically elongated body with a tapering round posterior end. They were 0.9 mm long and 0.4 mm wide. The mouth terminal was surrounded with one alternating row of 24 collar spines, 12 in each side and interrupted at the dorsal side of the oral sucker. There was a large ventral sucker. The vitelline follicles occurred on both lateral sides and extend from the level of the ventral sucker to the posterior end of the body. Two large testes were located in the posterior end of the fluke. The uterus was short and contained only a few eggs (1–3 eggs per specimen). The ovary was round and conducted to seminal receptacle.

6.4.2 Case descriptions

Key characteristics of the three patients infected with *E. japonicus*, including demographics, dietary patterns, recent medical histories, and results of blood, stool and ultrasonography examination are summarised in Table 1. The three patients were identified in a cross-sectional survey among 97 individuals (3.1%) who all suffered from hepato-biliary or intestinal symptoms, and hence underwent detailed examination for parasitic worms [10].

Case 1 was a female, aged 37 years. This patient had a severe '*Opisthorchis*-like' infection (10,660 EPG) and, concurrently, a light infection with hookworm (456 EPG) and *Echinostoma* spp. (72 EPG). The patient reported several diarrhoeal and stomach ache episodes in the previous month and to have consumed regularly raw or insufficiently cooked freshwater fish. Blood testing revealed no particular abnormalities. In the abdominal ultrasound examination, gall bladder stones were identified and the common bile duct was dilated. At purgation, two adult *E. japonicus*, 291 adult *O. viverrini* and 13 adult *H. taichui* worms were found. No intestinal protozoa were identified.

Cases 2 and 3 were males, aged 42 and 75 years, respectively. Both cases had a moderate infection with '*Opisthorchis*-like' eggs upon parasitological examination (6,984 and 1,968 EPG, respectively) and were co-infected with hookworm (light infection intensity; case 2: 114 EPG, case 3: 72 EPG) and *Echinostoma* spp. (case 2: 96 EPG, case 3: 516 EPG). Moreover, case 3 was super-infected with *Taenia* spp. (2,928 EPG). Both patients reported the consumption of raw and uncooked food items one month prior to the interview, in particular dishes prepared from freshwater fish. Both cases reported several diarrhoeal episodes during the preceding month. In addition, case 2 reported severe stomach ache and abdominal pain, whereas case 3 reported hyper bowel movement. Blood tests revealed hypochromic microcytic anaemia in case 2 and leukocytosis and mild liver dysfunction in case 3. The abdominal ultrasound examination was normal in both cases. Upon purgation, four *E. japonicus* worms were recovered from each these male patients, together with multiple other trematodes, i.e. *O. viverrini*, *H. taichui*, *P. bonnei* and *P. molenkampi* (Table 6.1). Case 3, moreover, had 1 adult *Taenia saginata* upon purgation.

Table 6.1: Summary of demography, food habit, medical histories, blood, ultrasound and stool examination of three individuals infected with *E. japonicus*

Characteristics	Case 1	Case 2	Case 3
Demography			
Age (years)	37	42	75
Sex	Female	Male	Male
Ethnicity	Laotheung	Laotheung	Laotheung
Profession	Farmer	Farmer	Farmer
Food habits (last 24 h)			
Consumption of raw vegetable	yes	yes	No
Consumption of raw meat	no	no	No
Consumption of raw fish	yes	yes	yes
Reported morbidity (last month)			
Diarrhoea	yes	yes	yes
Stomach ache	yes	yes	No
Abdominal pain (umbilical area)	no	yes	No
Hyper bowel movement	no	no	yes
Blood test results			
Total white blood cell (cells/mm3)	6,100	7,700	16,200
Haemoglobin (g/dl)	12.5	10.3	15.7
Mean corpuscular volume: MCV (µm3)	77	71	90
Mean corpuscular haemoglobin: MCH (pg/cell)	23	20	28
Asparate aminotransferase (U/L)	31.5	42.6	46.2
Alanine aminotranferase (U/L)	25.3	35.9	41.5
HBs-Ag	neg	neg	neg
Ultrasound findings			
Common bile duct dilated	yes	no	No
Gall bladder stone	yes	no	No
Adult worms recovered			
Echinochasmus japonicus	2	4	4
Opisthorchis viverrini	291	124	71
Haplorchis taichui	13	2	48
Phaneropsolus bonnei	0	10	12
Prosthodendrium molenkampi	0	5	20
Taenia saginata	0	0	1

Normal level : *AST and ALT ≤ 35 U/L; Hemoglobin = male : 13.0-17.0 g/dl; female : 12.0-16.0 g/dl; - MCV = 78-100 μm^3 MCH = 26.0–34.0 pg/cell*

6.5 Discussion

To our knowledge we report the first three cases of *E. japonicus* in Lao PDR. Our study was carried out in the central part of country in the province of Savannakhet, which is known to be highly endemic for food-borne trematodiases [5]. The three cases were identified among 97 individuals suffering from intestinal discomfort who agreed for being purged [10]. A total of 10 adult *E. japonicus* flukes were recovered upon purgation approximately 10 hours after oral administration of a single dose of praziquantel (40 mg/kg). These specimens were difficult to identify. The exposure to praziquantel and the long time interval between treatment and purgation (~10 hours) resulted in damages in six out of the 10 adult worms recovered. However, characteristics of the head part, ventral sucker, vitelline follicles and uterus of *E. japonicus* were clearly visible and allowed for an accurate diagnosis.

E. japonicus was first described some 80 years ago [17]. Adult flukes were recovered from experimentally-infected animals (dogs, cats, mice and birds) by using metacercariae from freshwater fish [1,18]. Infections in humans have been documented by Ujiie in 1936 [15]. However, over the past 70 years, only a few cases have been reported in the peer-reviewed literature. For example, in the Republic of Korea, four cases have been presented in the 1980s [15]. Based on these observations and taking into account the prevailing risk factors of raw/undercooked freshwater fish consumption, Chai and Lee (2002) predicted that as many as 5,000 individuals could be infected with *E. japonicus* in the Republic of Korea alone [1]. In view of the common habit of eating raw or insufficiently cooked food dishes in Lao PDR and Thailand, it is conceivable that the true number of human infections with *E. japonicus* is seriously underestimated in these Asian countries. This claim is underscored by our observations: three cases among 97 individuals suffering from intestinal discomfort, owing to an overall prevalence of 3.1%.

The life cycle of *E. japonicus* has been studied in the Republic of Korea and Japan [19]. It involves two intermediate hosts, a snail (first intermediate host) and a freshwater fish (second intermediate host). The first intermediate host snail identified in Japan and the Republic of Korea was *Parafossarulus* (*Bulimus*) *striatulus japonicus* and

Parafossarulus manchouricus, respectively. Twenty-four different species of freshwater and brackish-water fish (e.g. *Pseudorasbora parvas*, *Plecoglossus altivelis*, *Zacco platypus*, *Carassius carassius* and *Acanthogobius flavimanus*) have been reported to serve as second intermediate hosts. The definitive hosts are predominantly birds and mammals. Human infection most probably occurs through consumption of raw or uncooked freshwater fish [15,19].

The clinical symptoms due to an infection with *E. japonicus* are restricted to the gastro-intestinal system. Some abnormalities such as anaemia, leukocytosis and mild liver dysfunction observed in the present study may not be directly related to an *E. japonicus* infection. The co-infection with hookworm, *O. viverrini* and other trematodes might be responsible for these abnormalities. Previous studies have shown that an infection with *O. viverrini* can lead to severe liver pathologies, including cholecystitis, cholangitis and cholangiocarcinoma [7], whereas infection with hookworm was significantly related to severe anaemia among the study population [20,21]. The three *E. japonicus* cases reported here suffered from repetitive diarrhoeal episodes, stomach ache, abdominal pain and hyper bowel movement in the one month prior to praziquantel treatment and purgation. However, other fluke infections might have caused the same or similar symptoms, particularly in patients with a high infection intensity and/or a multiple species parasitic worm infection. An infection with *E. japonicus* does not lead to severe disease as in the case of *Capillaria philippinensis*, which is another rare intestinal helminthiasis that is endemic in Lao PDR [22] and may be fatal if adequate treatment is not provided in time.

At present, little information is available on echinostomiasis in Lao PDR and elsewhere. A recent study found 18 individuals among 1,869 screened (1.0%) with eggs of echinostomatid flukes in their stool, but the investigators failed to identify the flukes at species level [8]. In Thailand, six fluke species of the echinostomatid family have been recovered from humans [14,23,24], including *E. japonicus*.

The three cases of *E. japonicus* reported here confirm that transmission occurs in rural parts of Lao PDR. The habit of raw food consumption, particularly freshwater fish and other aquatic products, places a large proportion of the population at risk for food-borne trematode infection, including *E. japonicus*. Rapid population growth, migration and expansion of aquaculture, in a place of unchanged socio-economic status and food habits [2] bare the risk of elevated transmission of food-borne trematodiases.

6.6 Acknowledgements

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7. Hepato-biliar and intestinal parasitic infections, multiparasitism and risk factors in Champasack province, southern Lao PDR

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Running title: Intestinal parasitic infections in southern Lao PDR

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7.1 Abstract

Background: Quantitative assessments of multiparasitism are scare in the Mekong basin. We assessed hepato-biliar and intestinal parasitic infections, and multiparasitism in random population samples from three different eco-epidemiological settings in Champasack province, southern Lao PDR, and determined associated risk factors.

Methodology / Principal Findings: Two stool samples were collected from 669 individuals aged ≥6 months over consecutive days and examined by the Kato-Katz (K-K) method. Additionally, one of these samples was subjected to a formalin-ethyl acetate concentration technique (FECT). Questionnaires were administered to obtain individual and household-level data pertaining to behaviour, demography and socio-economic status. Risk factors for hepato-biliar and intestinal parasitic infections and multiparasitism were determined using multiple logistic regressions analyses. Multiple species intestinal parasite infections were highly prevalent: 86.6% of the study participants harboured at least two and up to seven parasite species concurrently. Hookworm was the most common helminth infection (76.8%). The prevalence of '*Opisthorchis viverrini'*-like, *Ascaris lumbricoides*, *Trichuris trichiura* and *Schistosoma mekongi* infections was 64.3%, 31.7%, 25.0% and 24.2%, respectively. Infections with intestinal protozoa were rare.

Significance: There is a pressing need to design, implement and sustain helminth control interventions in the southern part of Lao PDR. Given the very high prevalence rates with hepato-biliar and intestinal parasites and extent of multiparasitism, mass anthelminthic drug administration is warranted. This intervention should be coupled with health education and improved access to clean water and adequate sanitation to consolidate morbidity control and enhance sustainability.

keywords opisthorchiasis, *Schistosoma mekongi*, soil-transmitted helminthiasis, intestinal protozoa multiparasitism, risk factor, Kato-Katz, formalin-ethyl acetate concentration, socio-economic status, Lao PDR

7.2 Author summary

Multiparsitism is a general concern in tropical countries but of particular importance in the Mekong basin. Here we report on results of a thorough examination of hepato-biliar and intestinal multiparasitism and associated risk factors conducted in three settings of the most southern province of Lao PDR. Multiple species intestinal parasite infections were highly prevalent: 86.6% of the study participants harbored at least two and up to seven parasite species concurrently. Of particular concerns are the prevalence rates of *Opisthorchis viverrini* (64.1%) and *Schistosoma mekongi* (24.2%) as they are responsible for severe hepato-biliar morbidity (including cholangiocarcinoma). In our study villages of the Khong district we detected an extremely high prevalence of *S. mekongi* of 68.0%. Hookworm was the most common soil-transmitted helminth (76.8%). We conclude that given the very high prevalence rates of parasite infections and extent of multiparasitism, mass anthelminthic drug administration is warranted. We argue that this intervention should be coupled with health education and improved assess to clean water and adequate sanitation to consolidate morbidity control and enhance sustainability.

7.3 Introduction

Lao People's Democratic Republic (Lao PDR) is a landlocked country situated in the Great Mekong sub-region, where socio-economic and eco-epidemiological characteristics vary greatly according to location. In the northern part similar ecosystems are found as in southern China with mountains and highlands dominating the landscapes. These topological features are natural barriers for the social and economic development, since transportation of goods and commodities, communication and other exchanges are hampered. These issues exacerbate people's accessibility to health care facilities, clean water and adequate sanitation. Indeed, according to the results of the national population and housing census carried out in 2005, less than 20% and only about half of the population living in these areas had access to clean water and sanitation, respectively [1]. Water supply and sanitation are intimately linked with parasitic infections and poverty. Schistosomiasis, opisthorchiasis and infections with the common soil-transmitted helminths (i.e. *Ascaris lumbricoides*, hookworm and *Trichuris trichiura*) are of particular relevance [2]. Improving socio-economic status, including enhanced access to quality health care, safe water and adequate sanitation have the potential to significantly reducing the prevalence and intensity of parasitic infections, and hence reduce disease-related morbidity [3,4,5].

The central and southern parts of Lao PDR are the plain land along the Mekong River basin. In these regions, the socio-economic conditions and means of communication and transport are more advanced. In recent years, through the formation of the ASEAN community, the economy of the Great Mekong sub-regions countries has been bolstered. Along with these changes and ecological transformations (e.g. deforestation and water resources developments), particularly in the lowlands of the Mekong River basin, patterns of parasitic infections are changing [6]. A matter of considerable public health concern is the transmission of *Schistosoma mekongi* which, although several rounds of mass drug administration (MDA) were conducted, is still transmitted in the Mekong River in the most southern province of the country [7]. Of further particular note are high prevalences of *Opisthorchis viverrini* [7,8], a liver fluke that is the main risk factor for the fatal cholangiocarcinoma liver cancer [9,10]. An infection with *O. viverrini* is acquired through the consumption of traditional dishes (e.g.

"Lap-pa" and "Koy-pa") prepared with raw or insufficient cooked fish [11,12]. The habit of eating raw or undercooked freshwater fish or other aquatic products is also a risk for acquiring small intestinal trematode infections, such as heterophyid and lecithodendriid flukes, which are endemic in southern Lao PDR [8,13,14,15]. The habit of raw fish consumption is also a precondition of capillariasis transmission, which was recently documented in Lao PDR [16]. A national survey conducted among schoolchildren found that common soil-transmitted helminth infections are particularly prevalent in the northern provinces, whereas '*O. viverrini'*-like infections are rampant in the central and southern provinces with significant overlaps of different parasite species in all provinces [17]. It follows that multiparasitism must occur, which has been confirmed in recent surveys [8,12,13]. However, data pertaining to multiparasitism have mostly been obtained from small studies (e.g. in a single community in a single village), often looking at a narrow age range (e.g. school-aged children).

The present study was carried out in different eco-epidemiological settings of Champasack province, southern Lao PDR. Using a cross-sectional design, the purpose was to assess the prevalence and intensity of hepato-biliar and intestinal parasitic infections and intestinal multiparasitism, and to determine underlying risk factors.

7.4 Materials and methods

7.4.1 Study area

The study was carried out in three distinct eco-epidemiological settings of Champasack province (Figure 7.1), located in the southern part of Lao PDR, namely (i) Khong, (ii) Mounlapamok and (iii) Paksong districts. Of note, the districts represent different settings in terms of socio-economy and eco-epidemiology and are therefore characteristic for other parts of Lao PDR.

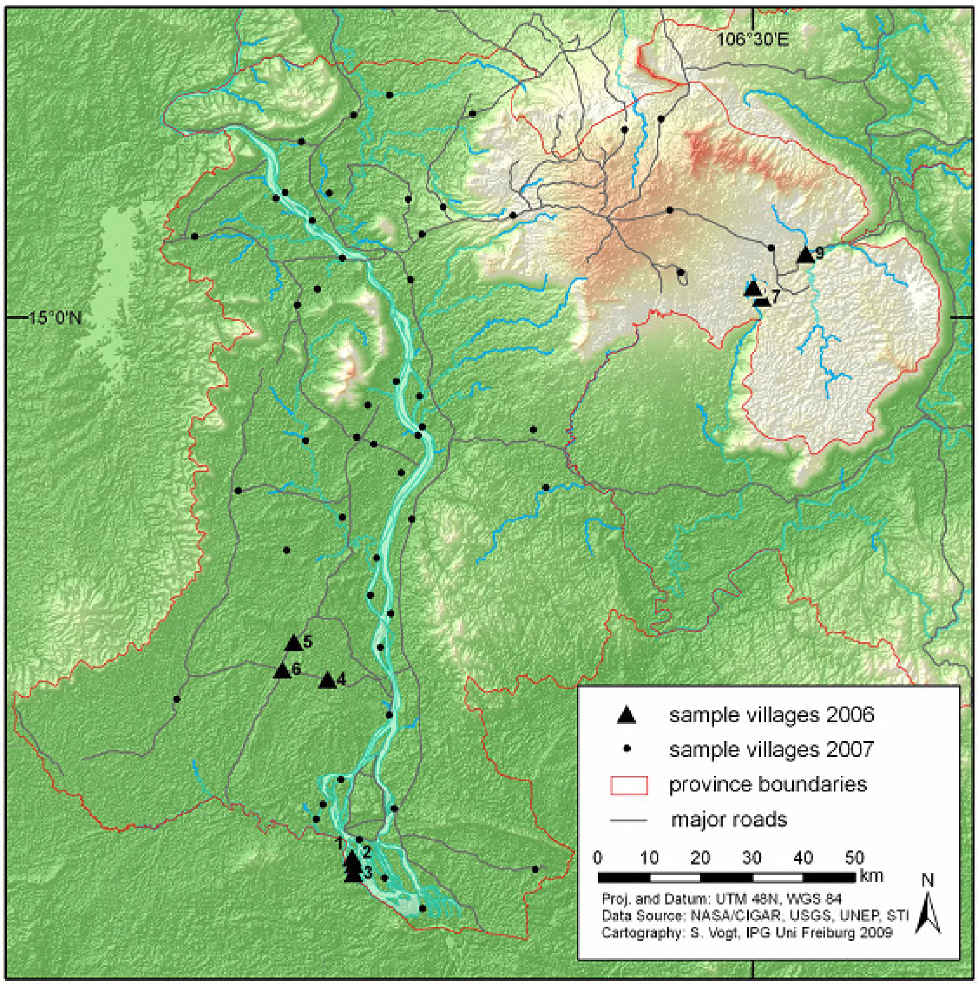

Figure 7.1: Map of Champasack province with location of study villages 2006 (triangles).

Khong district (estimated population: 80,000) (NSC 2003) is an island district which is located in the southern part of the province (~120 km from Pakse city), which borders Cambodia (geographical coordinates: 13.57°-14.14°N latitude and 105.44°-

106.08°E longitude). Khong district comprises dozens of islands in the Mekong River basin and is therefore also known as "district of four thousand islands". The water-fall 'Khon-Phapheng' has put a barrier in the Mekong River and has created a natural reservoir. The ecology of the area is suitable for aquatic snails, the intermediate hosts for *S. mekongi* and food-borne trematodes, such as *O. viverrini* and minute intestinal flukes (MIF).

The Mounlapamok district is also located in the southern part of the province (~80 km from Pakse city) with an estimated population of 40,000 (NSC 2003). It is a lowland district situated along the Mekong River (geographical coordinates: 14.15°-14.25°N latitude and 105.49°-106.11°E longitude). In this area, opisthorchiasis is highly endemic [19].

The Paksong district is located on the Bolovan plateau (geographical coordinates: 14.58°-15.23°N latitude and 105.55°-106.48°E longitude) at an elevation of ~1,000 m above sea level in the north-east of the province (~50 km from Pakse city). It is a mountainous area with an estimated population of 65,000 (NSC 2003). Soil-transmitted helminth infections are highly prevalence in Paksong district [17].

7.4.2 Ethical consideration and treatment

The study was approved by the Ethics Committee of the University and the State of Basel (EKBB; reference no. 255/06). Ethical clearance was obtained from the National Ethics Committee, Ministry of Health (MOH) in Vientiane (reference no. 027/NECHR). Permission for field work was obtained from MoH, the provincial Health Office (PHO) and the District Health Office (DHO). Village meetings were held and village authorities and villagers were given detailed explanations about the aims, procedures, potential risks and benefit of the study. Written informed consent was obtained from the heads of participating households prior to enrolment.

All individuals infected with *O. viverrini*, *S. mekongi*, soil-transmitted helminths and intestinal protozoa were treated according to national guidelines [20]. An anti-spasmodic treatment and oral dehydration was provided in case of side effects following drug administration.

7.4.3 Study design and population surveyed

Our cross-sectional surveys were carried out between March and May 2006. In each setting, three villages were selected from the available village list in collaboration with the DHO, and 20-25 households were randomly selected in each village. All family members aged ≥6 months were invited to participate. The number of inhabitants per household was recorded. Unique identifiers were assigned to households and study participants.

7.4.4 Field and laboratory procedures

In each village, a house (usually a school or a temple) was designated as area of work for Kato-Katz (K-K) thick smear preparation, microscopic examination of stool samples, etc. Two members of our research team (one interviewer and one general physician) went from house to house and interviewed first the head of household and then the other household members. Two questionnaires and a clinical form were administrated in each household. The household questionnaire (after pre-testing in a neighbouring area) was administered to the heads of household. Data pertaining to household characteristics (e.g. building type and water supply), asset ownership (e.g. farm engine and bicycle) and ownership of animals (e.g. buffalo cow and pig) were collected. The geographical coordinates of each household were collected, using a hand-held global positioning system (GPS) receiver (Garmin Ltd., Olathe, USA).

Next, a pre-tested individual questionnaire was used and all household members were interviewed for demographic data (e.g. age, sex, educational attainment and professional activity) and behavioural risks (e.g. food consumption habits and personal hygiene). Parents or legal caregivers answered for children.

Finally, stool sample containers were prepared for all members of each study household. Participants' names and unique identifiers were marked on the containers and distributed to the heads of household with detailed explanation about how to collect a fresh morning stool sample. After filled containers were collected, new empty containers were handed out with the goal to obtain 3 stool samples over consecutive days from each participant.

Stool samples were processed on the spot by experienced laboratory technicians. A single K-K thick smear was prepared from each stool sample, using a standard plastic template holding 41.7 mg of stool [21]. Slides were allowed to clear for 30 min prior to examination under a microscope. The number of eggs was counted and recorded for each helminth species separately.

Additionally, exactly 300 mg of stool taken from one sample was fixed in a tube containing 10 ml of sodium acetate acetic-acid formalin (SAF) [22]. SAF-conserved samples were forwarded to the parasitological department of the Faculty of Medicine, National University of Lao PDR. The samples were subjected to the formalin-ethyl acetate concentration technique (FECT) [23] and diagnosed for the presence of intestinal protozoa and helminth species-specific infections and intensities with the assistance of laboratory staff from the Swiss Tropical Institute (Basel, Switzerland).

7.4.5 Statistical analysis

Data were double-entered and cross-checked using EpiData version 3.1 (Epidata Association; Odense, Denmark). Statistical analyses were performed with STATA version 10 (Stata Corporation; College Station, TX, USA). Only those individuals who had at least two K-K thick smear readings and an additional FECT result, and complete questionnaire data were included in the final analyses.

People's socio-economic status was determined using a household-based asset approach according to methodologies presented by HNP/World Bank [24], and stratified into five wealth quintiles, namely (i) poorest, (ii) very poor, (iii) poor, (iv) less poor and (v) least poor. Details of this approach have been presented elsewhere [25]. In brief, a principal component analysis (PCA) was used to define the asset weights after missing values were replaced by the mean of the respective asset. The first principal component (PC) explained 17.2% of the total variability. The greatest weights were attached to families living in a wooden house (0.30), a bamboo house (0.29) and the presence of a television at home (0.20). After standardization of these weighted asset variables, families living in a cement house had the highest scores (0.47). Lowest scores were attached to families living in a bamboo house (-0.55). The sum of total asset scores was assigned to each study participant.

The point prevalence of parasitic infections were determined and stratified by study area, sex and age group. Chi-square test was employed to investigate associations between categorical variables (e.g. infection status and sex, age group and study area). Geometric mean and negative binomial regression models were employed to estimate the intensity of helminth egg counts (expressed as gram of stool [EPG]) and to associate with sex and age groups. Bivariate logistic regression models were utilized to test for an association between parasitic infection and risk factors (demographic, socio-economic and behavioural variables). A predictor variable with a significant level bellow 0.15 in the bivariate modeling approach was included in a backward stepwise multiple logistic regression to investigate the associations between the parasitic infections and a particular risk factor.

7.5 Results

7.5.1 Study cohort and socio-economic profile

From 1,213 registered individuals, 1,051 were present during the cross-sectional survey and underwent an interview (Figure 7.2). 314 individuals (29.9%) failed to submit sufficient numbers and/or quantities of stool samples for subsequent diagnosis. Fourteen individuals (1.3%) had no SAF-conserved stool sample and 192 individuals (18.3%) were absent during the household-based interviews, and hence their socio-economic status could not be determined. Overall, 669 individuals (63.7%) had complete data records (at least 2 K-K thick smears, 1 FECT result and complete questionnaire data).

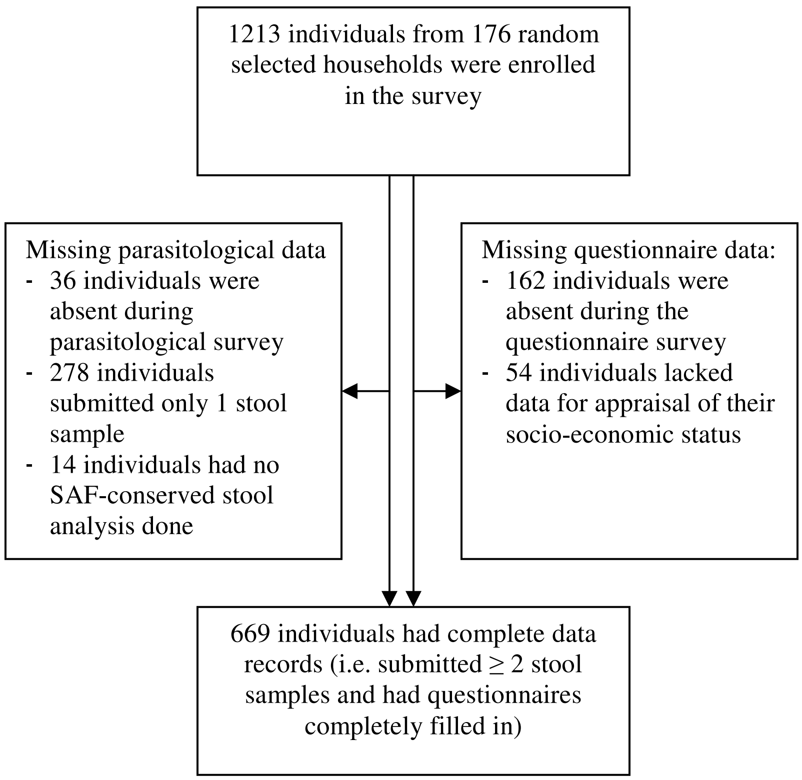

Figure 7.2: Study participant's compliance for parasitological investigation and questionnaire survey in three settings of Champasack, Lao PDR.

Among this cohort, 212 individuals (31.7%) were from Paksong district, 232 (34.7%) from Mounlapamok district and 225 (33.6%) from Khong district. Most study participants belonged to the Lao-loum ethnic groups (68.5%), whereas the Lao-theung minority accounted for the remaining 31.5%. There were slightly more females (n=347, 51.9%). The median age was 15 years (range: 6 months to 87 years). The age structures was as follows: ≤ 5 years (17.3%), 6-10 years (17.3%), 11-15 years (15.6%), 16-30 years (16.4%), 31-45 years (17.5%), 46-60 years (10.2%) and >60 years (5.7%). Pre-school children, school-aged children and elderly accounted for 17.3%, 20.4% and 8.8% of the population sample, respectively. Most adult study participants were engaged in subsistence farming, while there were only few government employees (1.4%).

With regard to wealth, we observed that most study participants from Paksong district belonged to the poorest group (53.5%), whereas none of them were classified into the group of the least poor. In Khong and Mounlapamok districts, the combined percentage of less poor and least poor was 40.4% and 29.3%. Only a few individuals (Mounlapamok: 3.0% and Khong: 2.2%) were stratified into the poorest group (Figure 7.3).

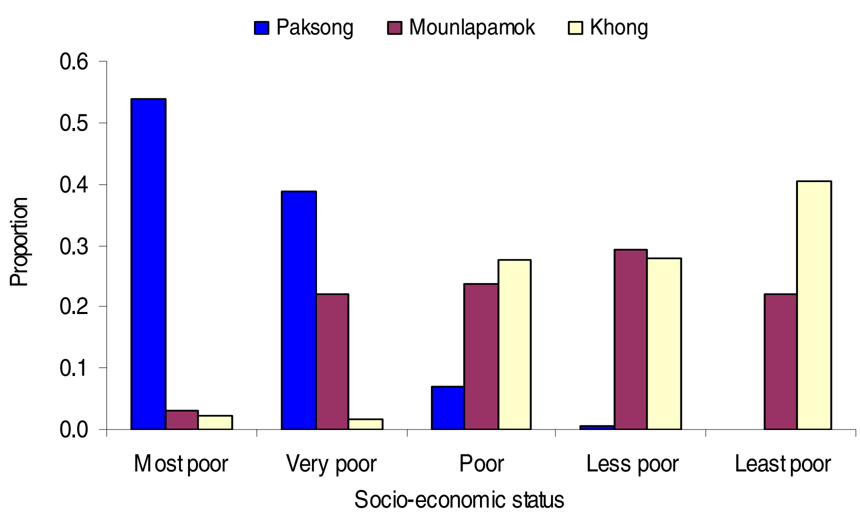

Figure 7.3: Proportion of socio-economic status among 669 individuals from Champasack province, Lao PDR, stratified by study setting

7.5.2 Helminth and intestinal protozoa infections

Table 7.1 summarizes the results from the cross-sectional parasitological surveys, stratified by eco-epidemiological setting, sex and age group.

Analysis of at least two stool samples using the K-K technique, supplemented with an additional FECT result revealed overall infection prevalences of '*O. viverrini*'-like, *S. mekongi* and *Echinostoma* spp. of 64.3%, 24.2% and 6.0%, respectively. The former two trematode infections were particularly prevalent in Khong district ('*O. viverrini*'-like: 92.0%, *S. mekongi*: 68.0%). Whereas a similarly high prevalence of '*O. viverrini*'-like infections was observed in the Mounlapamok district (90.9%), the observed prevalence of *S. mekongi* was only 3.9%. In the Paksong district, only few cases of '*O. viverrini*'-like infections were observed (5.7%), owing to a highly significant difference among study location ($P < 0.001$).

The prevalence of '*O. viverrini*'-like infections increased with age and reached the highest levels in the 46-60 years age group ($P < 0.001$). *S. mekongi* infections were also significantly associated with age, with the peak prevalence observed in the 11-15 years age group ($P = 0.039$). Neither *O. viverrini*'-like nor *S. mekongi* infections were significantly associated with sex. The prevalence of *O. viverrini*-like infections was significantly higher in Lao-loum ethnic group compared to Lao-theung (91.1% vs. 6.2%, $\chi^2 = 453.3$, $P < 0.001$).

The overall infection prevalence of hookworm, *A. lumbricoides* and *T. trichiura* was 76.8%, 31.7% and 25.0%, respectively. There was significant variation from one district to another ($P < 0.001$). The highest prevalences were found in Paksong district (hookworm: 94.8%, *A. lumbricoides*: 85.9% and *T. trichiura*: 55.7%) and the lowest prevalences were observed in the Mounlapamok district (hookworm: 66.0%, *A. lumbricoides*: 6.0% and *T. trichiura*: 8.2%). There were no significant difference between sex and age groups for any of the three main soil-transmitted helminth infections.

Blastocystis hominis (13.6%) was the most common intestinal protozoa diagnosed, followed by *Entamoeba coli* (7.2%), *Giardia lamblia* (4.9%) and *Endolimax* *nana* (0.6%). There was a significant variation in the observed prevalence ($P < 0.001$) for *B. hominis* and *E. coli* according to study location.

7.5.3 Infection intensities and multiparasitism

Table 7.2 shows the intensity rate ratio (IRR) of helminth egg counts expressed in EPG for the most prevalent intestinal parasites investigated. The overall intensity ratio of EPG for '*O. viverrini*'-like infection increased with age and reached the highest intensity in the 31-45 years age group (IRR = 4.03, 95% confidence interval (CI) = 2.57-6.32) with no significant sex difference. School-aged children (6-10 years) were at an elevated risk of higher infection intensity with *S. mekongi* (IRR = 2.50, 95% CI = 1.27-4.94) and hookworm (IRR = 2.00, 95% CI = 1.53-2.63) than their older counterparts. Males had a significantly lower *S. mekongi* intensity compared to females (IRR = 0.49; 95% CI = 0.32-0.75). With regard to *A. lumbricoides* and *T. trichiura*, pre-school children were at highest risk of high infection intensities.

In only 13 (1.9%) individuals no intestinal parasites were diagnosed. Mono-infections were observed in 77 individuals (11.5%). Hence, most of the study participants had a multiple species intestinal parasite infection; 32.9% were infected with two different parasite species, 53.5% harboured 3-6 parasite species concurrently and in one individual seven different parasites were observed. Figure 7.4 shows that over a third of the study participants living in Paksong and Khong districts harboured three different parasite species concurrently and almost half of the surveyed Mounlapamok residents were concurrently infected with two or more parasite species.

Table 7.1: Prevalence of intestinal parasitic infections, diagnosed by Kato-Katz (at least 2 samples) plus formalin-ethyl-acetate concentration (1 sample) among 669 study individuals, stratified by study setting, sex and age group

Parasites	**Prevalence (95% CI)**	**Study settings**			**Sex**		**Age groups (years)**						
	(n = 669)	Khong (n = 225)	Mounlapamok (n = 232)	Paksong (n = 212)	Female (n = 347)	Male (n = 322)	≤ 5 (n = 116)	6 – 10 (n = 116)	11 – 15 (n = 104)	16 – 30 (n = 110)	31 – 45 (n = 117)	46 – 60 (n = 68)	>60 (n = 38)
Trematodes													
‘Opisthorchis’-like	64.3 (60.6 – 67.9)	92.0	90.9	5.7**	65.1	63.4	44.0	56.9	73.1	69.1	67.5	80.9	71.1**
S. mekongi	24.2 (21.0 – 27.5)	68.0	3.9	0.0**	24.2	24.2	9.9	19.8	21.0	15.4	15.4	11.7	6.8*
Echinostoma spp.	6.0 (4.2 – 7.8)	12.9	4.7	0.0**	6.1	5.9	3.5	8.6	8.7	4.6	6.0	4.4	5.3
Nematodes													
Hookworm	76.8 (73.6 – 80.0)	71.1	66.0	94.8**	76.7	77.0	39.7	35.3	26.0	31.8	29.9	23.5	31.6
A. lumbricoides	31.7 (28.2 – 35.2)	7.1	6.0	85.9**	31.7	31.7	39.7	35.3	26.0	31.8	29.9	23.5	31.6
T. trichiura	25.0 (21.7 – 28.3)	13.3	8.2	55.7**	23.6	26.4	19.0	31.9	27.9	26.4	26.5	16.2	21.1
S. stercoralis	4.6 (3.0 – 6.0)	9.8	3.9	0.0**	14.9	4.4	4.3	3.5	3.9	1.8	9.4	4.4	5.3
E. vermicularis	3.6 (2.2 – 5.0)	3.1	4.4	3.3	5.2	1.9*	2.6	2.6	2.9	3.6	3.4	2.9	13.2
Cestodes													
Taenia spp.	3.7 (2.3 – 5.2)	1.8	4.3	5.2	3.1	4.7	0.9	1.7	3.9	3.6	7.7	2.9	7.9
H. diminuta	2.7 (1.5 – 3.9)	0.0	0.0	8.5**	2.9	2.5	1.7	2.6	1.9	2.7	4.3	1.5	5.3
D. latum	0.5 (< 0.1 – 0.9)	0.5	0.9	0.0	0.6	0.3	0.9	0.9	0.0	0.9	0.0	0.0	0.0
Protozoa													
B. hominis	13.6 (11.0 – 16.2)	19.6	6.5	15.1**	13.0	14.3	7.8	19.0	10.6	13.6	13.7	17.7	15.8
E. coli	7.2 (5.2 – 9.1)	3.0	8.4	10.4**	7.8	6.5	6.0	7.8	6.7	9.1	4.3	8.8	7.2
G. lamblia	4.9 (3.3 – 6.6)	3.0	5.9	6.1	2.6	7.6*	5.2	6.9	5.8	8.2	1.7	1.5	2.6
E. nana	0.6 (< 0.1 – 1.2)	0.6	0.4	0.5	0.3	0.9	0.9	1.7	0.0	0.0	0.0	1.5	0.0

CI: conficence interval; * *P*-value < 0.05; ** *P*-value < 0.001; *P*-value based on χ^2 test

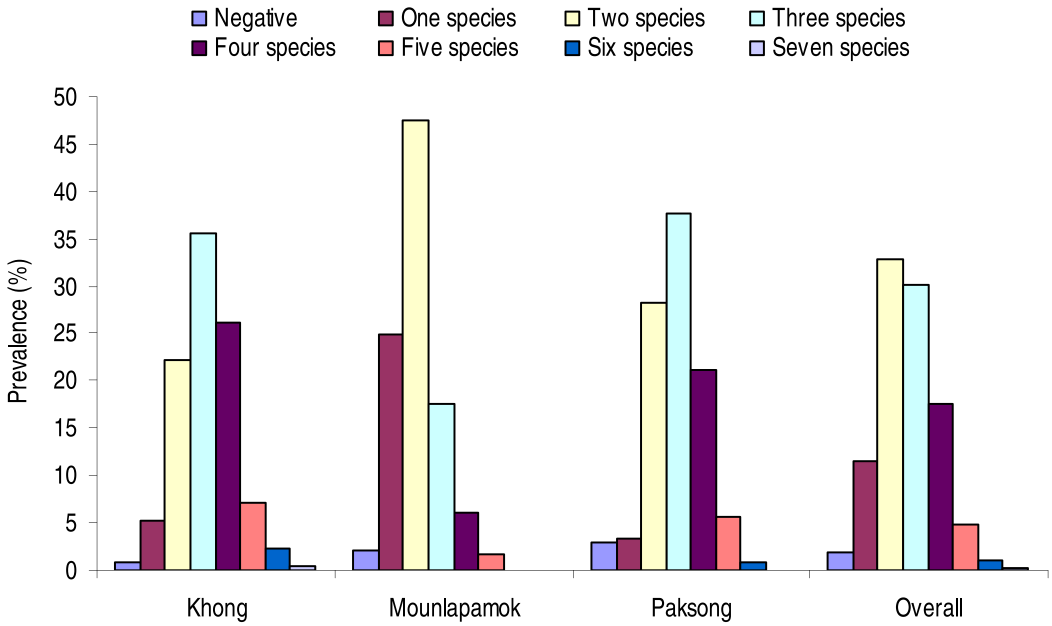

Figure 7.4: Extent of multiparasitism as assesses by stool examination using two methods (at least 2 Kato-Katz thick smears plus 1 FECT sample), stratified study settings (n = 669)

7.5.4 Parasite associations

Table 7.3 summarizes the significant association observed between a selected intestinal parasite and the remaining ones. An '*O. viverrini*'-like infection showed a significant positive association with *S. mekongi* (odds ratio (OR) = 5.09, 95% CI = 2.49-10.42), but negative association with both *A. lumbricoides* (OR = 0.05, 95% CI = 0.03-0.07) and *T. trichiura* (OR = 0.34, 95% CI = 0.20-0.58). Conversely, *S. mekongi* showed a significant positive association with an '*O. viverrini*'-like infection (OR = 5.64, 95% CI = 2.75-11.56). Moreover, there were significant positive associations between *S. mekongi* and *Echinostoma* spp. (OR = 3.19, 95% CI = 1.58-6.45) and between *S. mekongi* and two intestinal protozoa, namely *B. hominis* (OR = 2.19, 95% CI = 1.26-3.79) and *E. coli* (OR = 2.20, 95% CI = 1.01-4.83). An infection with hookworm was significantly associated with *S. mekongi* (OR = 1.70, 95% CI = 1.04-2.79), as well as the other common soil-transmitted helminths (*A. lumbricoides* and *T. trichiura*).

7.5.5 Risk factors for parasitic infections

More than half of our study participants (n = 345, 51.6%) reported to have consumed at least once raw fish dishes within the past 7 days prior to the interview. The habit of raw fish consumption was particularly frequent among the Lao-loum ethnic groups (85.7%), and significantly less common among the Lao-theung ethnic groups (14.3%; $\chi^2 = 98.04$, $P < 0.001$). Consumption of raw meat dishes was reported by 12.3% of our study population. Of those, 80.7% belonged to the Lao-loum and 19.3% to the Lao-theung ethnic groups.

Table 7.4 shows the results from the bivariate logistic regression analyses, emphasising associations between parasitic infections and risk factors. The results obtained from stepwise logistic regression models are summarized in Table 7.5. Lao-loum ethnic groups were more likely to have '*O. viverrini*'-like infections than Lao-theung ethnic groups (OR = 355.8, 95% CI = 149.2-848.6). The Lao-loum were at lower risks of hookworm (OR = 0.11, 95% CI = 0.05-0.21), *A. lumbricoides* (OR = 0.02, 95% CI = 0.01-0.05) and *T. trichiura* infections (OR = 0.03, 95% CI = 0.01-0.05). Swimming (bathing) in the Mekong River was a key risk factor for acquiring a *S. mekongi* infection. With regard to socio-economic status, wealthier people were at a higher risk of a *S. mekongi* infection (very poor: OR = 0.17, 95% CI = 0.07-0.41; most poor: OR = 0.09, 95% CI = 0.03-0.30). Infections with two of the common soil-transmitted helminths were more common in poorer population segments (*A. lumbricoides*: very poor: OR = 2.19, 95% CI = 1.05-4.60; most poor: OR = 3.53, 95% CI = 1.47-8.47; *T. trichiura*: poor: OR = 2.44, 95% CI = 1.24-4.37).

Table 7.2: Negative binomial logistic regression analyses for parasite eggs count (EPG) for '*O. viverrini*'-like, *S. mekongi,* hookworm, *A. lumbricoides* and *T. trichiura* diagnosed by Kato-Katz (at least 2 samples) plus formalin-ethyl-acetate concentration (1 sample) in the three settings in Champasack province, Lao PDR (n = 669)

	***'O. viverrini'*-like**		***S. mekongi***		**Hookworm**		***A. lumbricoides***		***T. trichiura***	
	IRR (95% CI)	*P*-value	IRR (95% CI)	*P*-value	IRR (95% CI)	*P*-value	IRR (95% CI)	*P*-value	IRR (95% CI)	*P*-value
Sex										
Female	1.00		1.00		1.00		1.00		1.00	
Male	0.88 (0.69 – 1.12)	0.305	0.49 (0.32 – 0.75)	0.001	1.15 (0.93 – 1.43)	0.205	1.10 (0.72 – 1.69)	0.652	0.59 (0.40 – 0.86)	0.006
Age groups (years)										
≤ 5	1.00		1.00		1.00		1.00		1.00	
6 – 10	1.53 (0.95 - 2.45)		2.50 (1.27 - 4.94)		2.00 (1.53 - 2.63)		0.51 (0.25 - 1.03)		0.44 (0.23 - 0.84)	
11 - 15	1.28 (0.81 - 2.01)		0.38 (0.19 - 0.74)		1.74 (1.17 - 2.59)		0.50 (0.24 - 1.04)		0.39 (0.19 - 0.79)	
16 - 30	2.51 (1.60 - 3.95)		0.24 (0.12 - 0.48)		1.58 (1.07 - 2.33)		0.29 (0.14 - 0.60)		0.62 (0.31 - 1.24)	
31 - 45	4.03 (2.57 - 6.32)		0.56 (0.28 - 1.14)		1.13 (0.76 - 1.67)		0.24 (0.12 - 0.48)		0.47 (0.24 - 0.94)	
46 - 60	2.74 (1.70 - 4.43)		0.23 (0.11 - 0.50)		1.55 (1.00 - 2.40)		0.08 (0.03 - 0.18)		0.42 (0.17 - 1.01)	
> 60	2.49 (1.39 - 4.46)	< 0.001	0.23 (0.10 - 0.55)	< 0.001	1.14 (0.66 - 1.98)	0.023	0.47 (0.17 - 1.29)	< 0.001	0.40 (0.15 - 1.13)	0.109

IRR: Intensity rate ratio, CI: confidence interval

Table 7.3: Stepwise multiple logistic regression analyses the association between a particular parasite investigated and any of the remaining parasite among 669 study individuals in three settings of Champasack province, Lao PDR

	Association	OR (95% CI)	*P*-value
Trematodes			
"*O. viverrini*"-like	*S. mekongi*	5.09 (2.49 - 10.42)	< 0.001
Hookworm	*A. lumbricoides*	0.05 (0.03 - 0.07)	< 0.001
	T. trichiura	0.34 (0.20 - 0.58)	< 0.001
	'O. viverrini'-like	5.64 (2.75 – 11.56)	< 0.001
A. lumbricoides	*Echinostoma* spp.	3.19 (1.58 - 6.45)	0.001
	B. hominis	2.19 (1.26 - 3.79)	0.005
	E. coli	2.20 (1.01 - 4.83)	0.049
	S. stercoralis	2.07 (1.55 – 11.03)	0.005
T. trichiura			
	S. mekongi	1.70 (1.04 - 2.79)	0.034
	A. lumbricoides	10.64 (4.29 – 26.36)	< 0.001
	T. trichiura	5.68 (2.32 - 13.87)	< 0.001
	Hookworm	3.52 (3.64 – 19.05)	< 0.001
	Entamoeba coli	3.51 (1.33 - 9.26)	0.011
E. vermicularis	*T. trichiura*	2.52 (1.45 - 4.39)	0.001
S. stercoralis	'*O. viverrini'*-like	0.05 (0.03 – 0.08)	< 0.001
	A. lumbricoides	2.55 (1.50 – 4.34)	0.001
	Hookworm	2.38 (2.17 – 12.76)	< 0.001
	S. stercoralis	4.59 (1.24 - 16.99)	0.022
	Taenia spp.	4.09 (1.61 - 10.36)	0.003
	G. lamblia	2.61 (1.11 - 6.15)	0.028
	'*O. viverrini*'-like	0.08 (0.18 – 0.51)	< 0.001
	E. coli	4.03 (1.24 - 13.02)	0.020
	Echinostoma spp	8.60 (2.23 – 33.23)	0.002
	T. trichiura	4.29 (1.15 – 15.90)	0.030
Cestodes			
Taenia spp.	*T. trichiura*	3.40 (1.52 – 7.62)	0.003
H. diminuta	*A. lumbricoides*	19.69 (2.59 – 149.61)	0.004
Protozoa			
B. hominis	*S. mekongi*	2.19 (1.26 – 3.80)	0.005
	E. coli	3.78 (1.93 - 7.38)	< 0.001
	'*O. viverrini*'-like	0.59 (0.35 – 0.99)	0.044
E. coli	*S. mekongi*	2.57 (1.23 – 5.37)	0.012
	A. lumbricoides	3.91 (1.94 – 7.90)	< 0.001
	E. vermicularis	4.14 (1.26 – 13.61)	< 0.019
	B. hominis	4.03 (2.05 – 7.92)	< 0.001
G. lamblia	*T. trichiura*	2.69 (1.21 - 6.00)	0.016

Table 7.5: Stepwise multiple logistic regression analyses the association between underlying factors and the common intestinal parasites investigated among 669 study individuals in three settings of Champasack province, Lao PDR

Parasites	Association	OR (95% CI)	*P*-value
Trematodiasis			
'O. viverrini'-like	Age groups (year)		
	≤ 5	1.00	- ---
	6 - 10	3.05 (1.30 – 7.17)	0.010
	11 - 15	5.59 (2.14 – 14.61)	< 0.001
	16 - 30	22.44 (6.69 – 75.29)	< 0.001
	31 - 45	12.62 (4.08 – 39.05)	< 0.001
	46 - 60	25.44 (5.87 – 110.27)	< 0.001
	> 60	13.44 (2.45 – 73.79)	0.003
	Ethnic group		
	Lao-theung	1.00	- ---
	Lao-loum	355.8 (149.2 – 848.6)	< 0.001
S. mekongi	Socio-economic status		
	Least poor	1.00	- ---
	Very poor	0.17 (0.70 – 0.41)	< 0.001
	Most poor	0.09 (0.03 – 0.30)	< 0.001
	Swimming or bathing in Mekong River	11.2 (6.7 – 18.5)	< 0.001
	Regularly consuming raw water plants	2.42 (1.51 – 3.88)	< 0.001
Soil-transmitted helminths			
Hookworm	Study area		
	Paksong	1.00	- ---
	Mounlapamok	0.10 (0.05 – 0.23)	< 0.001
	Kong	0.12 (0.06 – 0.23)	< 0.001
	Ethnic groups		
	Lao-theung	1.00	- ---
	Lao-loum	0.11 (0.05 – 0.21)	< 0.001
	Professional activity		
	Preschool children	1.00	- ---
	School pupil	2.19 (1.28 – 2.95)	0.004
	Rice farmer	1.19 (1.21 – 2.95)	0.005

Continue: Table 7.5	**Association**	**OR (95% CI)**	***P*-value**
A. lumbricoides	Study area		
	Paksong	1.00	- ---
	Kong	0.03 (0.01 – 0.07)	< 0.001
	Mounlapamok	0.02 (0.01 – 0.05)	< 0.001
	Ethnic groups		
	Lao-theung	1.00	- ---
	Lao-loum	0.02 (0.01 – 0.05)	< 0.001
	Socio-economic status		
	Least poor	1.00	- ---
	Very poor	2.19 (1.05 – 4.60)	0.037
	Most poor	3.53 (1.47 – 8.47)	0.005
T. trichiura	Study area		
	Paksong	1.00	- ---
	Mounlapamok	0.02 (0.01 – 0.05)	< 0.001
	Kong	0.03 (0.02 – 0.07)	< 0.001
	Ethnic groups		
	Lao-theung	1.00	- ---
	Lao-loum	0.03 (0.01 – 0.05)	< 0.001
	Professional activity		
	Preschool children	1.00	
	School pupil	2.47 (1.51 – 4.05)	< 0.001
	Socio-economic status		
	Least poor	1.00	- ---
	Poor	2.44 (1.24 – 4.37)	0.011

7.6 Discussion

Helminth infections are widespread in Lao PDR and the Great Mekong sub-region. *S. mekongi, O. viverrini*, various MIFs and soil-transmitted helminths are prevalent and there is extensive geographical overlap of various helminth infections [7,8,17,26,27]. However, there is a paucity of high-quality data to elucidate the extent of multiparasitism and underlying risk factors [28]. We conducted a cross-sectional study in three distinct eco-epidemiological settings of Champasack province situated in the southern part of Lao PDR. We employed a rigorous diagnostic approach, i.e. at least two stool samples were collected within three days and examining by the K-K method, supplemented with the FECT on one of these stool samples.

Our data confirmed that multiple species parasitic infections are the norm; more than 4 out of 5 study participants with complete data records harboured at least two different intestinal parasite species concurrently. '*O. viverrini'*-like infections were found in more than 90% of the study subjects in the two low-land settings (Khong and Mounlapamok districts). In Khong district, we found a *S. mekongi* infection prevalence as high as 68.0%. Soil-transmitted helminths, i.e. hookworm, *A. lumbricoides* and *T. trichiura*, were common in the highland of Paksong district; overall prevalences were 94.8%, 85.9% and 55.7%, respectively. On the other hand, intestinal protozoa infections (*B. hominis, E. coli, G. lamblia* and *E. nana*) were far less prevalent (< 14.0%).

Highest infection intensities of *A. lumbricoides* and *T. trichiura* were observed in pre-schoolers (aged ≤ 5 years), whereas the peak infection intensities of *S. mekongi* and hookworm were observed in school-aged children (aged 6-10 years). Adults aged 31-45 years were at highest risk of elevated '*O. viverrini'*-like infection intensities.

The high prevalence of *S. mekongi* observed in the Kong district is important to emphasise. This finding suggests that schistosomiasis is re-emerging in southern Lao PDR. Once schistosomiasis had been recognized as a major public health issue in southern Lao PDR and Cambodia in the early 1980s and early 1990s, respectively [29,30,31], community-based control programmes were launched. The aims of these control programmes were to reduce schistosome-related morbidity with large-scale administration of praziquantel utilized as the strategy of choice [7,30]. Multiple rounds of praziquantel reduced the prevalence of *S. mekongi* in the endemic areas to very low levels (2.1% in Khong district and 0.4% in Mounlapamok district) and was considered a successful public health control programme [7].

However, interruption of chemotherapy-based morbidity control in the face of inadequate sanitation, lack of clean water and continued human water contacts are at the root of rapid re-infection and re-emergence of schistosomiasis. In 2006, chemotherapy-based control has been re-established. Failure of neglecting to improve access to clean water and adequate sanitation should be avoided as otherwise sustainable schistosomiasis control will remain a distant goal. In 2007 in our study villages of the Kong district, only 14.5% of the households possessed latrines and 76.0% reported daily use of the Mekong River for bathing (Dr. K. Phongluxa, pers. comm.). Hence, there is also a need for more vigorous health education to avoid risky water contacts as a means of lowering the transmission of schistosomiasis and to thoroughly cook fish and other aquatic products to break the transmission cycle of opisthorchiasis and other food-borne trematode infections.

Our findings underscore that intestinal multiparasitism is very common throughout Champasack province. The same observations have been made in other part of Lao PDR [8,12,13] and neighbouring countries of Vietnam [26,27,32] and southern China [33]. Indeed, multiparasitism is the rule rather than exception in the developing world [34,35], and hence it is surprising that the topic has received only token attention [36]. Our data showed that '*O. viverrini*'-like and hookworm co-infections were highly prevalent in the plain area of Khong and Mounlapamok districts, whilst multiple species soil-transmitted helminth infections were common among the study participants in the highlands of Paksong district.

From a clinical point of view, co-infection of *S. mekongi* and *O. viverrini* is of particular concern. Indeed, an infection with *O. viverrini* leads to severe clinical manifestations such as hepato-biliary pathologies, including hepatomegaly, jaundice obstructive, gallbladder stones, cholecystitis and cholangitis and most importantly the development of a fatal liver cancer (cholangiocarcinoma) [9,37,38]. Chronic infection with *S. mekongi* contributes to a formation of hepatomegaly, periportal fibrosis and portal hypertension [29,39,40,41]. Co-infections of these two trematodes might further aggravate the host-organ pathology, especially the liver. In fact, the results of a subsequent purgation study in a sub-sample of our cohort (i.e. those individuals found positive for *'O. viverrini'*-like infection) after a single oral dose of praziquantel (40 mg/kg) revealed that most study participants were infected with *O. viverrini* rather than other trematodes.

Another interesting finding of our study was the significant association observed between *S. mekongi* and hookworm. Interestingly, previous studies carried out in Côte d'Ivoire found a significant association between *S. mansoni* and hookworm [34,35,42]. Whilst the distribution of single species infection and co-infections have been mapped [43] and risk factors elucidated, there is a lack of epidemiological investigations focusing on symptoms and morbidities due to co-infections.

Soil-transmitted helminths were also found to be highly prevalent in the present study, particularly among those living in the highlands of Paksong district. An infection with soil-transmitted helminths can lead to nutritional deficiencies and may impair growth and cognitive development in children [3,44,45]. It is widely acknowledged that children aged below 5 years are at high risk of mortality in developing countries [46]. Although the causes of death are multi-factorial, malnutrition is a key factor and parasites contribute to a substantial fraction of this under-nourishment [46,47]. In Lao PDR, a national de-worming programme is currently being implemented at the school level in collaboration with the MoH and the Ministry of Education [48]. However, pre-school children are currently not part of the project. Our findings of high prevalence and infection intensity of soil-transmitted helminths among pre-school children should be considered for future control activities. It is conceivable that including under-5 year-old children into the de-worming programme might improve their health status.

Epidemiological studies have shown that prevalence and infection intensity of several parasitic infections are governed by behavioural, socio-economic and environmental characteristics [25,28]. In the current study, we observed that the consumption of raw or insufficient cooked fish through traditional dishes (i.e. Lap-pa and

Koy-pa) was commonly practiced among the Lao-loum ethnic groups. This is the most likely explanation why the Lao-loum was at a significantly higher risk of *'O. viverrini'*-like infection than Lao-theung. This behavioural practice is known to be a potential risk for acquiring *O. viverrini* and other fish-borne trematode infections [12,49], particularly in an area where several food-borne trematodes co-exist, such as Lao PDR [8,13,14,15].

It is also important to note that intestinal parasites varied according to people's socio-economic status. Interestingly, *S. mekongi* was significantly more prevalent among better-off study participants who live along the lower Mekong islands. On the other hand, as expected, the highest prevalence of soil-transmitted helminths, particularly *A. lumbricoides* and *T. trichiura,* were observed among the poorest living in the highlands. The latter finding is in line with observations reported from southern China [25] and other parts of the developing world [50]. People belonging to the poorest wealth quintiles are at higher risk of infection with soil-transmitted helminths.

Finally, we found a very low prevalence of intestinal protozoa in our study cohort. These findings strongly support the previous observations, which have shown low prevalence of pathogenic intestinal protozoa in Southeast Asia [33,51].

We conclude that multiparasitism is the rule in different eco-epidemiological settings of Champasack province, and most likely elsewhere in Lao PDR. The extent of multiparasitism and the high infection prevalence and intensity with a host of intestinal parasites, most importantly *S. mekongi* and *O. viverrini* are of major public health concerns. Consequently, a chemotherapy-based morbidity control programme should be re-implemented without delay. To consolidate progress and ascertain sustainability, other control measures such as health education, improving access to clean water and sanitation in an intersectoral fashion must be considered.

7.7 Acknowledgements

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8. Repeated stool sampling and use of multiple techniques enhance the sensitivity of helminth diagnosis in southern Lao PDR

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8.1 Summary

OBJECTIVE To assess the diagnostic performance of the Kato-Katz (K-K) technique in relation to varying stool sampling efforts, and to determine the effect of concurrent use of a quantitative formalin-ethyl acetate concentration (FECT) method for helminth diagnosis.

METHODS The study was carried out between March and May 2006 in Champasack province, Lao PDR. Overall, 485 individuals aged ≥6 months who submitted three stool samples were included in the final analysis. Each stool sample was subjected to the K-K technique and one of these samples was additionally processed by the FECT method. Diagnosis was done under a light microscope by experienced laboratory technicians.

RESULTS Analysis of three stool samples with K-K plus a single FECT – considered as our diagnostic 'gold' standard – resulted in prevalence estimates of hookworm, *Opisthorchis viverrini*-like infection, *Ascaris lumbricoides, Trichuris trichiura* and *Schistosoma mekongi* of 77.9%, 65.0%, 33.4%, 26.2% and 24.3%, respectively. As expected, a single K-K and a single FECT test missed a considerable number of infections. Whilst our diagnostic 'gold' standard produced similar results than those obtained by a mathematical model for most helminth infections, the 'true' prevalence predicted by the model for *S. mekongi* (28.1%) was considerably higher than after multiple K-K plus a single FECT examination (24.3%).

CONCLUSION Triplicate K-K thick smears supplemented with a single FECT diagnose helminth infections with reliable accuracy. This diagnostic approach produces high-quality baseline data and might be used for anthelminthic drug efficacy studies.

Keywords

Helminths, *Opisthorchis viverrini*, *Schistosoma mekongi*, diagnosis, Kato-Katz, formalin-ethyl acetate concentration, Lao PDR

8.2 Introduction

Food-borne trematodiases, schistosomiasis and soil-transmitted helminthiases continue to exert negative impact on human health and well-being, and drain the social and economic development of affected countries (de Silva *et al.* 2003; Hotez *et al.* 2008; King 2008). Hundreds of millions of people are infected with common soil-transmitted helminths, especially the poorest of the poor (Bethony *et al.* 2006; Keiser & Utzinger 2008). Of note, several parasite species are often highly prevalent in the same eco-epidemiological setting, i.e. intestinal protozoa, cestodes, nematodes and trematodes (Marti & Koella 1993; Raso *et al.* 2004; Sachs & Hotez 2006; Montresor *et al.* 2008; Sayasone *et al.* 2008).

In Lao People's Democratic Republic (Lao PDR), multiparasitism is common (Sayasone *et al.* 2007; 2008). Two of the common soil-transmitted helminths (i.e. *Ascaris lumbricoides* and *Trichuris trichiura*) are more prevalent in the mountainous and highland areas of the country, particularly in the north, whilst hookworm is highly prevalent across the country (Rim *et al.* 2003). The liver fluke *Opisthorchis viverrini* and five species of small intestinal trematodes (i.e. *Haplorchis taichui, Haplorchis yogokawai, Haplorchis pumilio, Phaneropsolus bonnei* and *Prosthodendrium molenkampi*) are highly endemic in the lowlands of the Mekong River basin (Rim *et al*. 2003; Chai *et al.* 2005, 2007; Sayasone *et al*. 2007, 2008). *Schistosoma mekongi* is endemic in the lower Mekong River, in the southern most part of country (Urbani *et al.* 2002).

Concurrent helminth infections challenge epidemiological surveys in tropical countries in two ways. Firstly, the community prevalence of parasitoses is underestimated, since no single standard technique is capable of diagnosing all parasite infections with a high level of precision. Therefore, a combination of different diagnostic techniques on repeatedly collected stool samples is warranted to obtain a reliable estimate of the 'true' prevalence of different helminth infections (Brown *et al.* 2003; Knopp *et al.* 2008; Marti & Koella 1993; Raso *et al*. 2004; Steinmann *et al.* 2008). However, combining of tests is costly and not always possible because some techniques need a various set of materials or efforts, e.g. Koga agar plate and Baermann method for the diagnosis of *Strongyloides stercoralis*, or serological diagnosis of parasitic infections. Secondly, the impact of concurrent infections on health is underestimated. It is generally thought that light infection with a single parasite is associated with no or little morbidity. However, there is growing evidence that infections from multiple parasite species, even at the light infection intensity may contribute to substantial morbidity to the host (Drake & Bundy 2001; Ezeamama *et al.* 2005; King & Bertino 2008).

Two simple standard techniques are widely used in parasitological surveys: the Kato-Katz (K-K) technique (Katz *et al.* 1972) and the formalin-ethyl acetate concentration (FECT) method (Elkins *et al.* 1991). The latter method is a routine technique often performed in specialized laboratories on stool samples conserved with e.g. sodium acetate acetic-acid formalin (SAF) (Marti & Escher 1990). This technique allows for assessment of helminth infections, as well as intestinal protozoa. The K-K method is widely used in community-based epidemiological survey using simple equipment. It is recommended by the World Health Organization (WHO) as a suitable tool for diagnosis of helminthic infections such as *S. mansoni*, *S. japonicum*, *S. mekongi* and the common soil-transmitted helminths (WHO 1993). However, this technique fails to detect *S. stercoralis* (arguably the most neglected of the soil-transmitted helminths) and intestinal protozoa. Moreover, the K-K technique is known to have low sensitivity because it is usually performed on a single stool sample processing only a small amount of faeces (41.7 mg). Light infections may be missed, consequently, underestimating the 'true' community prevalence (de Vlas & Gryseels 1992; Kongs *et al.* 2001; Utzinger *et al.* 2001; Booth *et al.* 2003; Steinmann *et al*. 2008). Analysis of multiple stool samples and combination of different techniques increase the sensitivity of helminth diagnosis (Mullen & Prost 1983; Marti & Koella 1993; Brown *et al*. 2003; Raso *et al*. 2004; Knopp *et al*. 2008; Steinmann *et al*. 2008)

The aim of this study was to investigate the effect of varying sampling effort on the diagnostic performance of the K-K technique and the combination of two diagnostic assays (K-K plus FECT) for detecting helminth infections in rural communities in southern Lao PDR.

8.3 Materials and methods

8.3.1 Study area and population surveyed

A cross-sectional survey was carried out between March and May 2006 in Champasack province, the southern region of Lao PDR. Details of the study area and the population surveyed have been presented elsewhere (Sayasone *et al.* submitted). In brief, the study was conducted in three different eco-epidemiological settings, namely (i) the plains along the Mekong River basin where *O. viverrini* and hookworm infections are endemic, (ii) the small islands in the Mekong River in the southern part of the province where S*chistosoma mekongi*, opisthorchiasis and hookworm disease are endemic, and (iii) the mountainous and highland areas of the eastern part of province where hookworm and other soil-transmitted helminth infections are widespread. In each setting, three villages were selected in collaboration with the District Health Office (DHO), and 20-25 households were randomly selected in each village from readily available census data. All family members aged ≥6 months were invited to participate.

8.3.2 Study design and procedures in the field and at the bench

All study participants were asked to submit three stool specimens collected in the early morning over consecutive days. Details of laboratory procedures have been described previously (Sayasone *et al*. 2008). In summary, stool samples were delivered by the heads of household to a designated area in the village where the research team was located. Stool samples were processed on the spot by experienced laboratory technicians. A single K-K thick smear was prepared from each stool sample, using a standard template holding 41.7 mg of stool (Katz *et al.* 1972). Slides were allowed to clear for 30 min prior to examination under a microscope. The number of eggs was counted and recorded for each helminth species separately.

In addition, exactly 300 mg of stool derived from one sample was fixed in a small tube containing 10 ml of SAF (Marti & Escher 1990). SAF-conserved stool samples were forwarded to the parasitological department of the Faculty of Medicine, National University of Lao PDR. The samples were processed using the quantitative FECT method. The number of helminth eggs found in total sediment was counted and recorded separately for each species (Elkins *et al*. 1991) with the assistance of laboratory staff from the Swiss Tropical Institute in Basel, Switzerland.

8.3.3 Ethical issues

The study was approved by the Ethics Committee of the University and the State of Basel (EKBB; reference no. 255/06). Ethical clearance was obtained from the National Ethics Committee, Ministry of Health in Vientiane (reference no. 027/NECHR). The purpose and procedures of the study were explained to the heads of households and they were invited to sign a written informed consent sheet prior to enrolment. Participation was on a voluntary basis and individuals could withdraw at any point in time. All participants found positive for '*O. viverrini'*-like eggs, *S. mekongi* and/or soil-transmitted helminths were treated with anthelminthic drugs according to Lao national guidelines (MoH 2004).

8.3.4 Statistical analysis

Data were double-entered and cross-checked in EpiData version 3.1 (EpiData Association; Odense, Denmark). Statistical analyses were performed with STATA version 10 (Stata Corporation; College Station, TX, USA). Individuals were included in the final analyses if they had submitted three stool samples that were examined by the K-K technique and had a single stool sample additionally analysed by the FECT method.

For each individual the infection intensity was calculated for '*O. viverrini'*-like, *S. mekongi,* hookworm, *A. lumbricoides* and *T. trichiura* infection. According to Maleewong and colleagues (1992) and adopting from WHO guidelines published in 2002, the infections were classified into no, light, moderate and heavy infection intensities, as summarized in Table 8.1.

Table 8.1: Stratification of helminth infection intensities investigated in the Champasack province, southern Lao PDR

Parasite	Infection intensity (EPG)			
	No	Light	Moderate	Heavy
Trematodes				
'O. viverrini'-like [a]	0	1 - 999	1000 - 9999	≥ 10000
S. mekongi [b]	0	1 - 100	101 - 399	≥ 400
Nematodes				
Hookworm [b]	0	1 - 1999	2000 - 3999	≥ 4000
A. lumbricoides [b]	0	1 - 4999	5000 - 49999	≥ 50,000
T. trichiura [b]	0	1 - 999	1000 - 99999	≥ 10,000

a: Maleevong et al., (1992)

b: WHO (2002)

Geometric means of helminth eggs per gram of stool (EPG) for the entire population (infected and non-infected individuals) diagnosed by each method were calculated, using the natural logarithm of the EPG plus 1, i.e. $\log_{10}$ (EPG + 1).

Finally, a mathematical model developed by Marti and Koella (1993) was used to calculate the sensitivity and the negative predictive value of the two diagnostic tests. The model was also used to estimate the 'true' prevalence of each helminth parasite species in the study population. The procedure follows an approach developed by Mullen and Prost (1983) and has been used before to predict the 'true' prevalence for helminth infections in other Asian (Steinmann *et al*. 2008) and African settings (Knopp *et al*. 2008). For all statistical analyses, our significance level was set at a p-value of 0.05.

8.4 Results

8.4.1 Study compliance and final cohort

From 1213 individuals invited to participate in this study, 485 individuals submitted three stool samples of sufficient quantity to generate a 41.7 mg K-K thick smear from each sample and one SAF-conserved sample for subsequent processing with the FECT method. The overall compliance was therefore low; i.e. 40.0%. The remaining 728 individuals were excluded from the final analysis due to missing personal data (n = 162), absence during parasitological surveys (n = 36), or failure to submit a second (n = 278), or a third stool sample for K-K (n = 184) or insufficient stool quantity for SAF conservation (n = 68).

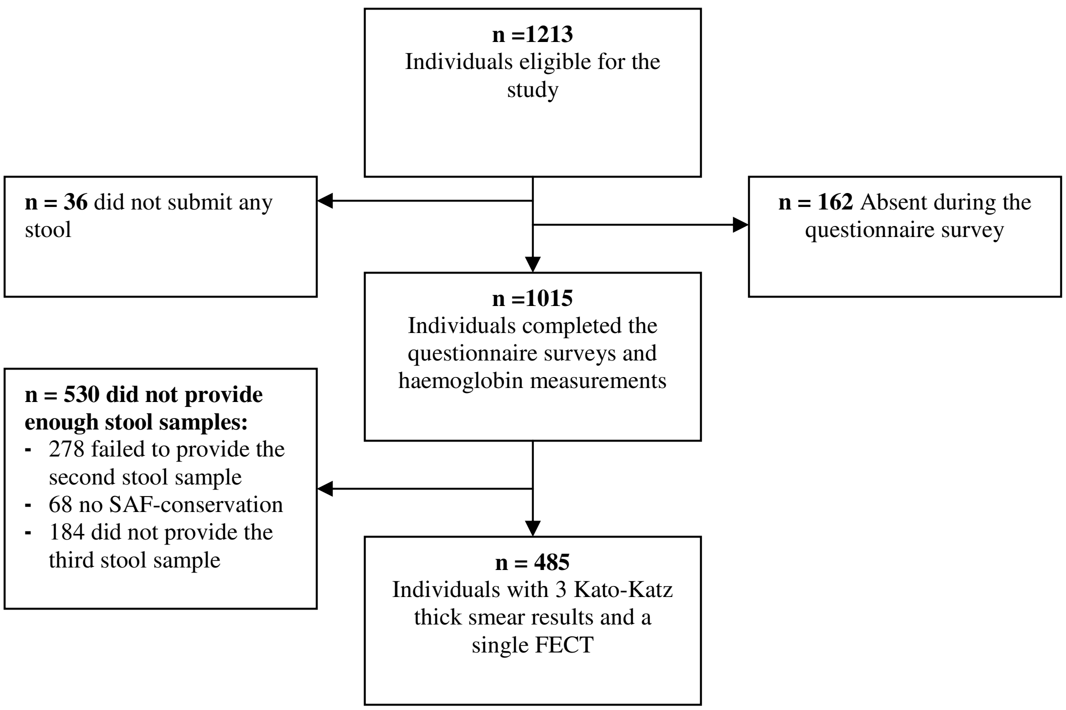

Figure 8.1: Chart detailing the study participation and compliance for assessment of multiple stool sampling effort

The age and sex profile of our final study cohort (n = 485 individuals) is shown in Table 8.2. For comparison, those 728 individuals with incomplete data records are also shown; there was no significant difference for sex; however a significant difference was found in age group ≤5 year (χ^2 = 59.42, P < 0.001) and age group of 31-45 year (χ^2 = 16.54, P < 0.001). In our final study cohort, there were 252 (52.0%) females. Approximately two-third of the study participants belonged to the Lao-loum ethnicity (n = 333, 68.7%). Most of study participants were illiterate or only finished primary education (n = 418, 86.2%). Only few participants attended secondary school or attained higher educational level (n = 67, 13.8%). Subsistence agriculture (mainly rice farming) was the main source of income (50.1%). Only seven study participants (1.4%) were state employees.

Table 8.2: Study participants, stratified by sex and age groups, among 485 individuals in the final cohort and 728 individuals excluded from final analysis

Indicator	**Final cohort (n = 485)**	**Excluded (n = 728)**	χ^2	***P*-value**
Sex	**% (n)**	**% (n)**		
Female	52.0 (252)	53.3 (388)		
Male	48.0 (233)	46.7 (340)	1.43	0.232
Age groups (years)				
≤ 5	15.3 (74)	35.4 (258)	59.42	< 0.001
6 – 10	16.9 (82)	16.3 (119)	0.07	0.789
11 – 15	16.9 (82)	10.2 (74)	11.87	0.001
16 – 30	16.5 (80)	16.0 (116)	0.07	0.787
31 – 45	19.0 (92)	10.7 (78)	16.54	< 0.001
46 – 60	10.1 (49)	7.5 (55)	2.43	0.119
> 60	5.3 (26)	3.9 (28)	1.58	0.208

8.4.2 Helminth infections according to sampling effort and diagnostic techniques

Analysis of a single stool sample by the K-K technique compared to a single test using FECT showed significantly higher prevalence of *S. mekongi* (χ^2 = 20.96, P <0.001) and hookworm (χ^2 = 68.95, P <0.001). On the other hand, both techniques performed similarly well for diagnosis of '*O. viverrini'*-like, *A. lumbricoides, T. trichiura, Taenia* spp. and *Hymenolepis diminuta* (P > 0.10).

Figure 8.2 shows the observed prevalence of different helminths according to sampling effort (single vs. multiple K-K thick smears). The combined results of the K-K technique (3 samples) plus a single stool sample additionally analysed by FECT were considered as diagnostic 'gold' standard. Stool analysis of three samples with the K-K method plus a single FECT showed a significant increase in the prevalence of '*O. viverrini*'-like infection by 27.2% based on the first K-K thick smear (from 51.1% to 65.0%) and by 22.6% based on a single FECT(from 53.0% to 65.0%). For diagnosis of *S. mekongi,* similar observations were made. Whilst a single K-K revealed a prevalence of 12.2%, the 'gold' standard found a prevalence that was nearly doubled (24.3%). A single FECT only revealed a prevalence of 4.1%, which was a several-fold underestimation compared to the diagnostic 'gold' standard.

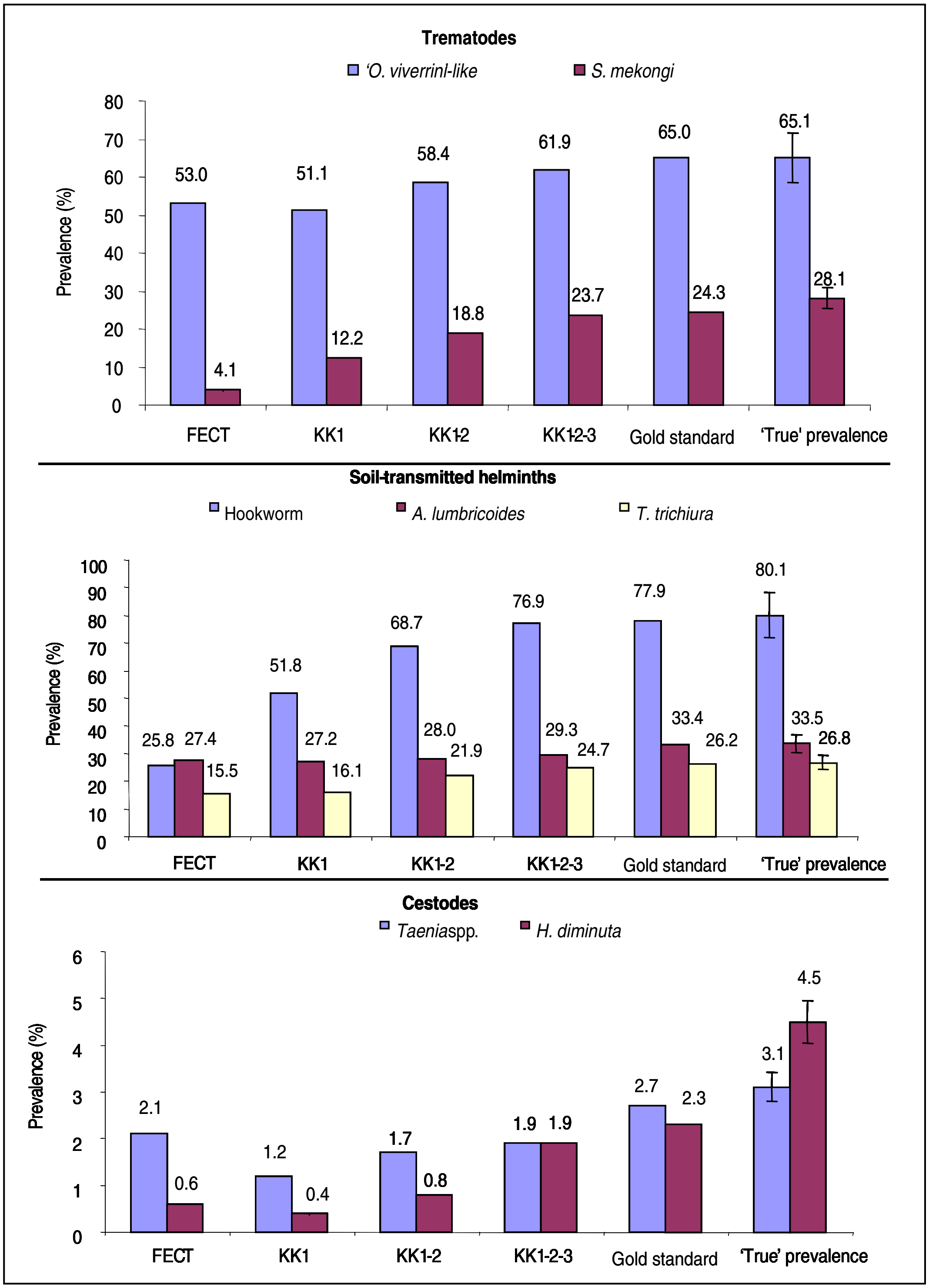

Figure 8.2: Difference in observed and 'true' prevalence in a stool sample and 3 stool samples for K-K technique and K-K technique plus a FECT for diagnosis of helminth infection in 3 districts of Champasack province, southern Lao PDR (n = 485)

Table 8.3 shows a comparison of parasite egg counts (EPG) between K-K and FECT for detection of '*O. viverrini*'-like eggs, *S. mekongi*, hookworm, *A. lumbricoides* and *T. trichiura* infections. Egg counts were significantly higher when using K-K rather than a single FECT for all parasites investigated (P <0.001).

Table 8.3: Comparison of parasite egg counts per gram (EPG) of stool detected by K-K and FECT in a single stool sample, and intensity classification for combining result among 485 study participants

	Geometric mean $\log_{10}$ (EPG+1)			**Intensity classification**		
	$K\text{-}K_1$	$FECT_1$	*P*-value	Light	Moderate	Heavy
Trematodes						
'*O. viverrini*'-like	2.54	1.95	< 0.001	55.7	41.2	3.1
S. mekongi	1.71	0.90	< 0.001	62.1	27.8	10.1
Nematodes						
A. lumbricoides	3.65	1.90	< 0.001	35.8	53.0	11.2
Hookworm	2.20	1.01	< 0.001	93.6	6.4	0.0
T. trichiura	1.91	1.22	< 0.001	96.0	4.0	0.0

Table 8.4 summarizes the infection intensity of '*O. viverrini'*-like, *S. mekongi*, hookworm, *A. lumbricoides* and *T. trichiura,* stratified by sex and age group. Overall, more than half of study participants harboured light intensity helminth infections with the exception of *A. lumbricoides*. No significant difference of infection intensity for all helminths was observed by sex (P >0.20). Highest prevalence of '*O. viverrini'*-like heavy infection (9.4%) was found in participants aged 31-45 years, whereas heavy infection with *S. mekongi* (28.6%) was mainly observed in school-aged children (6-10 years), and in pre-school children (≤5 years) for *A. lumbricoides* (29.0%).

Table 8.4: Percentage of infection intensity of helminthiasis among 485 study participants, stratified by sex and age groups

	‘*O. viverrini*’-like (n = 315)			*S. mekongi* (n = 118)			Hookworm (n = 378)			*A. lumbricoides* (n = 162)			*T. trichiura* (n = 127)		
	Light	Moderate	Heavy	Light	Moderate	Heavy	Light	Moderate	Heavy	Light	Moderate	Heavy	Light	Moderate	Heavy
Sex															
Female	53.1	43.9	3.0	58.1	32.3	9.6	94.5	5.5	0.0	34.9	57.8	7.3	95.1	4.9	0.0
Male	58.3	38.4	3.3	66.1	23.2	10.7	92.7	7.3	0.0	36.7	48.1	15.2	97.0	3.0	0.0
Age groups (year)															
≤ 5	83.9	16.1	0.0	33.4	44.4	22.2	95.8	4.2	0.0	22.6	48.4	29.0	100.0	0.0	0.0
6-10	68.0	30.0	2.0	38.1	33.3	28.6	93.9	6.1	0.0	21.4	71.4	7.2	96.1	3.9	0.0
11-15	61.3	38.7	0.0	64.3	28.6	7.1	90.9	9.1	0.0	28.6	52.4	19.0	96.0	4.0	0.0
16-30	52.8	45.3	1.9	73.3	26.7	0.0	92.5	7.5	0.0	51.6	45.2	3.2	95.2	4.8	0.0
31-45	39.1	51.5	9.4	65.0	25.0	10.0	96.0	4.0	0.0	40.7	59.3	0.0	95.5	4.5	0.0
46-60	39.5	55.3	5.2	75.0	25.0	0.0	90.0	10.0	0.0	71.4	28.6	0.0	90.9	9.1	0.0
> 60	52.9	47.1	0.0	88.9	11.1	0.0	100.0	0.0	0.0	20.0	60.0	20.0	100.0	0.0	0.0

8.4.3 Diagnostic performance

The analysis of three stool samples with the K-K technique increased the sensitivity of diagnosis for all helminth infections compared to a single stool sample with negative predictive values being close to 100%. Table 8.5 summarizes the sensitivity of the K-K technique on a single stool sample and, finally, all three stool samples for diagnosis of helminth infections.

Table 8.5: Sensitivity and negative predictive value of a stool sample and 3 stool samples for Kato-Katz technique given by mathematical model for diagnosis of helminth infections in 3 districts of Champasack province, southern Lao PDR (n = 485)

Helminth infections	**Kato-Katz technique, n (%)**			**Sensitivity of test, %**		**Negative predictive value, %**
	1 sample	2 samples	3 samples	1 single sample	3 samples	
Trematodes						
'O. viverrini'-like	46 (9.5)	50 (10.3)	204 (42.1)	83.9 (±2.5)	99.6	99.3
S. mekongi	60 (12.4)	30 (6.2)	25 (5.2)	49.0 (±6.7)	86.8	95.3
Nematodes						
Hookworm	101 (20.8)	105 (23.7)	157 (32.4)	69.7 (±3.0)	97.0	97.4
A. lumbricoides	11 (2.3)	11 (2.3)	120 (24.7)	92.2 (±2.6)	99.9	99.9
T. trichiura	37 (7.6)	40 (8.3)	43 (8.9)	65.5 (±5.6)	95.9	98.6
Cestodes						
Taenia spp.	4 (0.8)	5 (1.0)	0	41.4 (±24.8)	79.9	99.5
H. diminuta	7 (1.4)	2 (0.4)	0	19.4 (±23.1)	47.7	97.9

8.5 Discussion

We investigated the effect of varying stool sampling effort and the combination of two techniques (K-K and FECT) for diagnosis of helminth infections. Study participants were invited to submit three early morning faecal samples over three consecutive days. These samples were first examined on the spot by the K-K method with minimal time delays between stool production and microscopic analysis, whereas from a single sample exactly 300 mg was conserved in SAF for subsequent FECT analysis approximately six months later. It is important to note that a single stool sample examined by the K-K technique revealed a *S. mekongi* prevalence of 12.2%, whereas the cumulative prevalence after examination of three K-K thick smears was 23.7%. A single stool sample subjected to the FECT method revealed a prevalence of only 4.1%, and hence this method is clearly not indicated for diagnosis of *S. mekongi*. However, the single FECT still discovered 3 cases with a *S. mekongi* infection that otherwise would have been missed. Hence, the overall prevalence of *S. mekongi* was 24.3%. The observed prevalence of three K-K plus a single FECT was still considerably lower than the 'true' prevalence predicted by a mathematical model (28.1%).

Large increases in the observed prevalence when combining multiple K-K thick smear results and a single FECT result were also observed for hookworm, *T. trichiura, Taenia* spp., and *H. diminuta.* On the other hand, only a relatively small increased prevalence resulted for '*O. viverrini'*-like infections (i.e. one KK: 51.1%; one FECT: 53.0%, versus 65.0% for our diagnostic 'gold' standard) and *A. lumbricoides* (i.e. 27.2% and 27.4%, versus 33.4%). Furthermore, the examination of three stool samples with the K-K technique resulted in a considerably higher sensitivity of the tests for diagnosis of all helminths. The negative predictive values were close to 100%, compared to analysing a single sample.

Our findings confirm that analysis of a single stool sample underestimates the 'true' prevalence of helminth infections (de Vlas & Gryseels 1992; Booth *et al*. 2003). Light infections are particularly prone to be missed (Knopp *et al*. 2008; Steinmann *et al*. 2008). Examination of three stool samples using only the K-K technique showed a considerably increased test sensitivity with a negative predictive value above 90% for the observed helminths as function of sampling effort (Marti & Koella 1993; Raso *et al*. 2004; Steinmann *et al*. 2008). In fact, adding a FECT into the analysis of three stool samples by the K-K technique showed a higher prevalence and sensitivity than analysis with K-K alone for all helminths investigated, except *S. mekongi*. These findings support observations made elsewhere (de Vlas & Gryseels 1992; Marti & Koella 1993; Raso *et al*. 2004; Knopp *et al*. 2008; Steinmann *et al*. 2008). Furthermore, the observed prevalence of *S. mekongi* and hookworm was significantly higher in the K-K than the FECT method when a single stool sample was analysed. In addition, we also observed that the K-K technique resulted in significantly higher EPG when compared to the FECT method. Previous studies have shown that the performance of the FECT method is higher or equal to the K-K technique for detection of small trematode eggs if stool samples were preserved in 7 ml of 10% formalin-solution (Sithithaworn *et al.* 1994). The reduction of sensitivity when combining both methods for *S. mekongi* diagnosis and the low observed EPG when using FECT method for all helminths is of concern and might be due to the light formalin proportion in the SAF-solution. In fact, the SAF is ideal to preserve intestinal protozoa (Marti & Escher 1990), but it might be less effective to preserve relatively large helminth eggs such as *S. mekongi* and other helminths over prolonged periods (i.e. ~ 6 months in the present study).

We found a high prevalence of hookworm infection in rural dwellers in three different settings of Champasack province, southern Lao PDR. This finding suggests that prevalence of *S. stercoralis* might also be elevated in these areas as both parasites have the same route of infection (Keiser & Nutman 2004; Vadlamudi *et al.* 2006; Steinmann *et al.* 2007). Previous studies have shown that *S. stercoralis* can be fatal, particularly among immunocompromised individuals (Keiser & Nutman 2004; Vadlamudi, Chi, & Krishnaswamy 2006; Jeyamani *et al.* 2007). Analysing of SAF-conserved stool sample by FECT in the current study identified 4.6% of study participant infected with *S. stercoralis.* However, FECT is not the recommended technique for *S. stercoralis* diagnosis, and hence the ‘true’ prevalence might be several-fold higher. More reliable techniques are the Koga agar plate and the Baermann technique (Koga *et al.* 1991; Steinmann *et al*. 2007; Knopp *et al*. 2008; Stothard *et al.* 2008). Most recently a promising polymerase chain reaction (PCR) method has been described (Hasegawa *et al.* 2009). Further investigations are warranted, using a sensitive diagnostic approach to estimate the extent of *S. stercoralis* infection in Lao PDR.

We conclude that analysis of multiple stool samples by the K-K method, supplemented with a single FECT allows reasonably accurate diagnosis of helminth infections. In our view, the combination of these two methods – if resources allow – performed on at least two stool samples, provides reasonable sensitive helminth diagnosis. The combination of different diagnostic assays and an enhanced sampling effort is mandatory for generating high-quality baseline data, and should be considered for evaluation of anthelminthic drug efficacy studies and for rigorous monitoring of helminth control programmes.

8.6 Acknowledgments

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9. *Opisthorchis viverrini*: relationship between infection intensity and morbidity in Lao PDR

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9.1 Summary

Background: Infection with *Opisthorchis viverrini* is highly prevalent in Lao People's Democratic Republic. High intensity of infection is significantly associated with observed hepato-biliary morbidities. We conducted two cross-sectional studies in central and southern Lao PDR to relate intensity of infection with *O. viverrini* and co-infection between *O. viverrini* and *S. mekongi* with hepato-biliar and abdominal morbidity.

Methodology / Principal Findings: Two cross-sectional studies were conducted in eco-epidemiological distinctly different settings of Lao PDR. Each study participant received a purgative after single oral dose of praziquantel treatment (40 mg/kg). All stools produced within 24 hours after treatment were collected and examined for the presence of the *O. viverrini* and other parasitic adult worms. The adult worms collected were counted and recorded separately in each individual. Finally the detailed clinical data (e.g. physical examination, ultrasound, liver function test and whole blood count) was obtained from each study participant. Ultrasonographically assessed liver pathologies were detected in high frequencies: common bile duct dilatation and cholecystitis was seen in 10.5% of patients, and liver fibrosis and gall bladder stones were diagnosed in 8.4% and 7.3%, respectively. Infection of *O. viverrini* and *S. mekongi* concurrently was 75.0 (95% CI: 7.5 – 752.0) times and 29.1 (95% CI: 6.9 – 121.9) times higher risk for a marked enlarged left liver lobe and liver fibrosis, respectively.

Significance: The concomitant infections are abundant and lead to an increase in morbidity. In particular, *S. mekongi* induced morbidity is of importance and needs urgent control.

Keywords: *Opisthorchis viverrini*, *Schistosoma mekongi*, multiparasitism, morbidity, Lao PDR

9.2 Introduction

Food-borne parasitic infections, particularly those caused by trematodes, are an emerging public health threat around the world [1,2]. In Lao People's Democratic Republic (Lao PDR), *Opisthorchis viverrini*, a liver fluke, is the most important food-borne trematode infection. Nationwide over 2 million people are estimated to be infected [3]. Previous studies showed that *O. viverrini* is endemic in all provinces of Lao PDR [4]. The highest prevalence rates were found in villages of central and southern Lao PDR, exceeding two-third of the general population [5,6,7,8].

The impact of *O. viverrini* on health is considerable. Morbidity due to *O. viverrini* infection varies from asymptomatic infection to severe disease including severe hepato-biliar morbidity i.e. hepatomegaly, jaundice, cholecystitis, cholangitis and gall bladder stone [9,10,11], depending on the infection intensity [11]. *O. viverrini* infection has been recognized as main risk factor for cholangiocarcinoma (CCA), a malignant liver cancer [12,13]. The worldwide highest incidence of CCA occurs in *O. viverrini* endemic areas of northern Thailand [14]. As the habit of eating raw fish is similarly frequent in Lao PDR as in northern Thailand the public health impact of *O. viverrini* in Laos must be similar. Recent studies showed that in many parts of the country, the prevalence of infection is several folds higher than that in northeast Thailand [4,5,6,7]. Little information on the *O. viverrini* related morbidity is available for Lao PDR. In particular, it has not been shown whether the current endemic level of *O. viverrini* in Lao PDR is related to severe morbidity. This study addressed this knowledge gap. We conducted two cross-sectional studies in central and southern Lao PDR to relate intensity of infection with *O. viverrini* with hepato-biliar and abdominal morbidity.

9.3 Materials and methods

9.3.1 Study areas and population

Two cross-sectional studies were conducted in eco-epidemiological distinctly different settings of Lao PDR. The first and second study was conducted in central (Vientiane and Savannakhet provinces) and southern Lao PDR (Champasack province), respectively.

1.5.1.1 Study in Central Lao PDR (Vientiane and Savannakhet province)

Details of the cross-sectional study procedures are provided elsewhere [6]. In brief, the study was conducted in two referral hospitals and two villages in the central part of the country where opisthorchiasis is highly endemic. The hospital work was carried out at the infectious disease wards of the Mahosot hospital (largest hospital in Vientiane) and the Savannakhet provincial hospital (referral hospital for central provinces of Lao PDR) in September and October 2005. The community field work was carried out in Khamsida (Champhone district, Savannakhet province) and Thamouangkao (Saravane district, Saravane province) village in June 2006.

All patients hospitalized in the infectious disease departments and habitants in the villages aged ≥ 15 years who reported hepato-biliary or intestinal symptoms such as icterus, stomach-ache, abdominal pain (right hypochondrial quadrant), nausea, vomiting, and abdominal irritation were invited to provide a stool sample for parasitological (helminth) infection using a Kato-Katz technique [15]. Patients with '*O. viverrini*-like' infection with intensity of higher than 1000 eggs per gram stool (epg) were enrolled in the study.

1.5.1.2 Study in Southern Lao PDR (Champasack province)

Details of the study procedures are provided elsewhere [7]. In brief, this cross-sectional study was conducted between January and May 2006 in 9 villages of three districts (3 villages per district) of Champasack province (Khong, Mounlapamok and Paksong). Opisthorchiasis, schistosomiasis and soil-transmitted helminthiasis are endemic. In each village 5-7 adults, aged ≥ 15 years and positive for '*O. viverrini*'-like eggs with or without *S. mekongi* co-infection, were randomly selected from all study participants and enrolled in this study. Adult non-infected villagers (aged ≥ 15 years, negative for "*O. viverrini*"-like and *S. mekongi*) were used as reference population.

9.3.2 Field and laboratory procedures

Demographic data (i.e., age, educational attainment and professional activity) and behavioural data (i.e. food consumption habits) were obtained with a pre-tested questionnaire from all enrolled individuals. Information on morbidity were gained from each study individuals by interview (i.e. right upper quadrant pain [RUQ]), abdominal discomfort [ADC], diarrhoea and vomiting), by physical examination (i.e. hepatomegaly, jaundice, splenomegaly, pale conjunctiva and RUQ pain at palpation) conducted by a general physician and by ultrasound examination (i.e. liver fibrosis, common bile duct dilatation [CBD] and intra-hepatic bile duct dilatation [IHBD]) carried out by an experienced radiologist. In the study in Central Lao PDR, a blood sample of 10 ml was taken from each individual. Cell blood counts (CBC), phosphatase alkaline (PAL), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and HBs-antigen were examined. Haemoglobin level was measured in each individual from finger-pick blood, using a haemoglonometer (HemoCue®). Level of PAL above 120 U/L, AST and SLT above 35 U/L was considered abnormal [16].

Three stool specimens, collected over consecutive days, were obtained from each individual. One Kato-Katz thick smear, using 41.7 mg templates, was prepared from each specimen according to a standardized, quality-controlled method [15]. Slides were allowed to clear for 30 minutes prior to examination under a light microscope. The number of eggs was counted and recorded for each parasite species separately.

9.3.3 Purgation, consent and ethical clearance

All study participants were enrolled in a purge procedure. Each individual received a single 40 mg/kg oral dose of praziquantel after dinner. Albendazole 400 mg was added if the patient was co-infected with soil-transmitted helminths. The following morning, 45 ml of monosodium-sulphate solution was administered to patients (Swiff®, Berlin Pharmaceutical Industry Co, Ltd.; Berlin, Germany). All stools produced within 24 hours after treatment (usually 6-8 bowel movements) were collected and examined for the presence of the *O. viverrini* adult worms. The adult worms collected were counted and recorded separately in each individual.

The study was approved by the Ethics Committee of the University and the State of Basel (EKBB; reference no. 255/06). Ethical clearance was obtained from the National Ethics

Committee, Ministry of Health in Vientiane (reference no. 027/NECHR). A signed informed consent was obtained from heads of participating household and study participant. All individuals infected with *O. viverrini* or *S. mekongi* were treated with praziquantel (40 mg/kg, single oral dose). Soil-transmitted helminth infections were treated with albendazole (400mg, single oral dose), according to national treatment guidelines of Lao PDR [17]. Among individuals who participated in the purge an anti-spasmodic and oral dehydration was provided in case of side effects due to drug administration (e.g. abdominal pain and diarrhoea).

9.3.4 Data management and statistical analysis

Data were double-entered using EpiData version 3.1 (Epidata Association; Odense, Denmark). Statistical analyses were performed with STATA version 10 (Stata Corporation, College Station, TX). Only individuals with complete data records were retained for the final analysis. Age was subdivided into five groups: (i) 15-25 years, (ii) 26-35 years, (iii) 36-45, (iv) 46-55 years, and (v) > 55 years. Frequency was used to describe the proportion of *O. viverrini* infection, stratified by sex, age and study settings. Arithmetic mean and negative binomial regression was applied to calculate the intensity rate ratio (IRR) between the number of adult *O. viverrini* worms recovered in purged stool and the presence and absence of the clinical indicators. Chi^2-test and multinomial logistic regression were used to compare clinical outcomes of non-infected individuals with categories of infected individuals. In the Southern Lao PDR cross-sectional study, the infection status was classified into non-infection (reference population), single infection with *O. viverrini* and dual infection with *O. viverrini* and *S. mekongi.* For all analysis the significant level was P-value ≤ 0.05.

9.4 Results

9.4.1 Study population

A total of 243 persons were enrolled in the two studies. Of those, 95 patients participated in the study in Central Lao PDR (female: 44.8%; mean age: 40 years). Hundred-forty-eight (148) persons were in the study in Southern Lao PDR. Of those, 49 were patients and 99 were healthy, non-infected individuals (reference group). Female and male participated equally in this study (female: 50.0%; median: 36 years).

Table 9.1 summarizes the intensity of *O. viverrini* infection among the study participants in both settings based on the number of adult *O. viverrini* worms recovered in the purged stool samples. In both studies the intensity of infection with *O. viverrini* was significantly higher in males than females (IRR: 1.6, 95% CI: 1.1 – 2.6, P = 0.046 and IRR: 2.0, CI: 1.2 – 3.4, P = 0.010, respectively). In Central Lao PDR the intensity of infection increased clearly with age while in Southern Lao PDR the highest intensity of infection was found in the 26-35 years old age group (IRR: 3.0, CI: 1.2 – 7.4, P = 0.015).

Table 9.1: Intensity of *O. viverrini* infection (number of worms recovered in purged stool sample) in Central and Southern Lao PDR, stratified by sex and age groups

Indicators	**Mean worms (95%, CI)**	**IRR (95%, CI)**	***P*-value**
Central Lao PDR (n = 95)			
Study settings			
Community	181.8 (117.3 – 146.3)	1.0	
Hospital	206.2 (53.3 – 359.1)	1.1 (0.6 – 2.2)	0.703
Sex			
Female	139.1 (83.2 – 195.1)	1.0	
Male	223.4 (127.2 – 319.5)	1.6 (1.1 – 2.6)	0.046
Age groups (years)			
15 – 25	90.5 (8.8 – 181.5)	1.0	
26 – 35	149.6 (52.7 – 246.4)	1.7 (0.7 – 3.8)	0.241
36 – 45	198.2 (112.4 – 284.0)	2.2 (1.1 – 4.7)	0.042
46 – 55	203.9 (96.0 – 311.8)	2.3 (1.1 – 5.0)	0.047
> 55	243.3 (47.2 – 486.2)	2.7 (1.2 – 6.0)	0.017
Mean intensity (range)	243.3 (47.2 – 486.2)		
Southern Lao PDR (n = 49)			
Sex			
Female	106.5 (73.9 – 139.1)	1.0	
Male	214.0 (91.1 – 336.9)	2.0 (1.2 - 3.4)	0.010
Age groups (years)			
15 – 25	83.0 (23.7 – 142.3)	1.0	
26 – 35	250.6 (6.4 – 507.6)	3.0 (1.2 – 7.4)	0.015
36 – 45	191.5 (101.3 – 281.8)	2.3 (1.1 – 5.3)	0.004
46 – 55	57.2 (23.7 – 90.7)	0.7 (0.3 – 1.7)	0.432
> 55	124.5 (4.9 – 298.4)	1.5 (0.5 – 4.7)	0.483
Mean intensity (range)	161 (5-1373)		

Intensity Rate Ratio (IRR) and *P*-value were obtained from binomial logistic regression model

9.4.2 Morbidity due to O. viverrini infection in Central Lao PDR

Table 9.2 summarizes morbidity data of Central Lao PDR. Overall, an abdominal discomfort was commonly reported by the study participants (84.5%), and right upper quadrant pain (32.5%) and diarrhoea (12.5%) was reported with considerable frequencies. On physical examination the most frequent sign was a hepatomegaly (9.5%). Ultrasonographically assessed liver pathologies were detected in considerable frequencies: common bile duct dilatation and cholecystitis was seen in 10.5% of patients, and liver fibrosis and gall bladder stones were diagnosed in 8.4% and 7.3%, respectively. In three patients (3.2%) from the communities an intra-hepatic bile duct dilatation was diagnosed. Abnormally increased liver function tests were found in every 5th patient (i.e. increased phosphatase alkalkine in 22.1%). There were significant differences between men and women (i.e. skin rash, P = 0.012). Acute symptoms such as diarrhoea (P = 0.041), vomiting (P = 0.011) and severe symptoms such as jaundice (P < 0.001) and increased liver test level (i.e. phosphatase alkaline: P = 0.026) were significantly more frequent in hospitalized participants.

Table 9.3 summarizes the association between morbidity and intensity of *O. viverrini* infection. Overall, morbidity increased with *O. viverrini* worm burden. Study participants who reported a RUQ pain had 1.9 times higher *O. viverrini* worm burden than those without this report (IRR = 1.9, 95% CI = 1.2 - 3.0, P = 0.010). Jaundice was significantly associated with an increased worm burden (IRR = 1.6, 95% CI = 1.4 – 6.0, P = 0.004). Ultrasonographically assessed hepto-biliar pathology was significantly associated with intensity of *O. viverrini* infection. Patients with intra-hepatic bile duct dilatation, liver fibrosis and common bile duct dilatation had a 7.7 (95% CI: 2.2 – 26.9) 3.1 (95% CI: 1.4 – 6.9) and 2.4 (95% CI: 1.1 – 5.0) times higher intensity of infection, respectively, than those without the pathology. Study participants with increased phosphatate alkaline and aminotransferase aspartate had a 2.3 (95% CI: 1.4 – 4.0) and 2.2 (95% CI: 1.3 – 3.8) times higher *O. viverrini* worm burden than those with a normal liver function test.

Table 9.2: Morbidity in study participants in Central Lao PDR, stratified by sex and study settings

Key findings	Overall, n (%) (n = 95)	Female, n (%) (n = 43)	Male, n (%) (n = 52)	χ2	*P*-value	Community, n (%) (n = 82)	Hospital, n (%) (n = 13)	χ2	*P*-value
Self-reported morbidity									
Abdominal discomfort	85 (89.5)	41 (95.3)	44 (84.6)	2.23	0.136	72 (87.8)	13 (100.0)	1.60	0.206
Right upper quadrant pain	31 (32.6)	17 (39.5)	14 (26.9)	0.28	0.589	26 (31.7)	4 (30.8)	< 0.01	1.000
Diarrhoea	12 (12.6)	3 (7.0)	6 (11.5)	0.15	0.698	7 (8.5)	5 (38.5)	5.42	0.041
Vomiting	8 (8.4)	5 (11.6)	3 (5.8)	0.18	0.665	4 (4.9)	4 (30.8)	9.82	0.011
Physical examination									
Hepatomegaly	9 (9.5)	5 (11.6)	4 (7.7)	1.22	0.726	7 (8.5)	2 (15.4)	0.61	0.606
Jaundice	3 (3.2)	2 (4.7)	1 (1.9)	0.16	0.685	0	3 (23.1)	16.72	< 0.001
Abdominal collateral	1 (1.1)	1 (2.3)	0	1.24	0.264	1 (1.2)	0	0.18	0.665
Skin rash	5 (5.3)	5 (11.6)	0	6.26	0.012	5 (6.1)	0	0.84	0.357
Pale conjunctiva	5 (5.3)	5 (11.6)	0	6.26	0.012	5 (6.1)	0	0.84	0.357
Ultrasound examination									
Common bile duct dilatation	10 (10.5)	4 (9.3)	6 (11.5)	0.11	0.747	10 (12.2)	0	2.07	0.150
Intra-hepatic bile duct dilatation	3 (3.2)	0	3 (5.8)	0.46	0.495	3 (3.7)	0	0.57	0.449
Cholecystitis	10 (10.5)	6 (14.0)	7 (13.5)	1.12	0.290	8 (9.8)	2 (15.4)	0.36	0.550
Liver fibrosis	8 (8.4)	2 (4.7)	6 (11.5)	1.38	0.240	5 (6.1)	3 (23.1)	4.20	0.075
Gall bladder stones	7 (7.4)	3 (7.0)	4 (7.7)	0.01	0.915	6 (7.3)	1 (7.7)	< 0.01	1.000
Blood test									
Phosphatase alkaline increased	21 (22.1)	6 (14.0)	15 (28.8)	3.21	0.073	15 (18.3)	6 (46.2)	4.93	0.026
Aminotransferase aspartate increased	20 (21.1)	5 (11.6)	15 (28.8)	4.00	0.045	16 (19.5)	4 (30.8)	0.09	0.879
Aminotransferase alanine increased	11 (11.6)	4 (9.3)	7 (13.5)	0.72	0.393	9 (11.0)	2 (15.4)	0.21	0.644
HBs-Ag positive	4 (4.2)	2 (4.7)	2 (3.8)	0.05	0.827	4 (4.9)	0	0.79	0.373

Table 9.3: Association between morbidity and intensity of *O. viverrini* infection (mean worm burden) in Central Lao PDR (n = 95) as calculated by negative binomial logistic regression analysis

Indicator measurements	Findings	Mean worms	IRR (95%, CI)	*P*-value
Self-report morbidity	Right upper quadrant pain			
	No	144.6	1.0	
	Yes	272.0	1.9 (1.2 - 3.0)	0.010
	Abdominal discomfort			
	No	162.7	1.0	
	Yes	188.0	1.2 (0.5 - 2.6)	0.724
	Diarrhea			
	No	185.0	1.0	
	Yes	188.0	1.0 (0.5 – 2.1)	0.954
	Vomiting			
	No	187.0	1.0	
	Yes	171.0	0.9 (0.4 – 2.1)	0.834
Physical examination	Jaundice			
	No	182.2	1.0	
	Yes	291.7	1.6 (1.4 – 6.1)	0.044
	Hepatomegaly			
	No	178.0		
	Yes	247.0	1.4 (0.6 – 3.0)	0.405
	Skin rash			
	No	187.0	1.0	
	Yes	160.0	0.9 (0.3 – 2.5)	0.772
	Pale conjunctiva			
	No	191.4	1.0	
	Yes	81.4	0.4 (0.2 – 1.2)	0.111
Ultrasound examination	Cholecystitis			
	No	171.7	1.0	
	Yes	293.5	1.7 (0.8 – 3.5)	0.150
	Common bile duct dilatation			
	No	163.0	1.0	
	Yes	383.0	2.4 (1.1 – 5.0)	0.013
	Intra-hepatic bile duct dilation			
	No	153.0	1.0	
	Yes	1188.0	7.7 (2.2 – 26.9)	< 0.001
	Liver fibrosis			
	No	159.0	1.0	
	Yes	484.0	3.1 (1.4 – 6.9)	0.002
Blood test	Phosphatase alkaline increased			
	No	143.1	1.0	
	Yes	328.9	2.3 (1.4 – 4.0)	0.002
	Aminotransferase aspartate increased			
	No	149.0	1.0	
	Yes	326.0	2.2 (1.3 – 3.8)	0.006
	Aminotransferase alanine increased			
	No	173.0	1.0	
	Yes	232.0	1.5 (0.8 – 3.1)	0.275

9.4.3 Morbidity due to O. viverrini infection in Southern Lao PDR

Table 9.4 displays the prevalence rates of morbidity in study participants infected with *O. viverrini* alone or with dual infections of *O. viverrini* and *S. mekongi* in comparison to the control group (non-infected).

Table 9.5 shows the risks for morbidity associated with *O. viverrini* single-infection and *O. viverrini* and *S. mekongi* dual-infection. Study participants with a single-infection with *O. viverrini* had 7.0 (95% CI: 1.6 – 31.2) times and 6.4 (95% CI: 1.4 – 29.1) times higher risk of having a liver fibrosis and marked left liver lobe enlargement, respectively. In addition they were 4.0 (95% CI: 1.1 – 15.0) times more likely to suffer from RUQ pain than the uninfected control group.

Study participants with a dual-infection of *O. viverrini* and *S. mekongi* had a substantially increased risk for morbidity. Compared with the uninfected reference group, they had a 75.0 (95% CI: 7.5 – 752.0) times and 29.1 (95% CI: 6.9 – 121.9) times higher risk for a marked enlarged left liver lobe and liver fibrosis, respectively. On physical examination in the dual infected group, 8.1 (95% CI: 2.3 – 29.5) times and 7.5 (95% CI: 2.7 – 20.8) times higher risk for a hepatomegaly and splenomegaly, respectively

Table 9.4: Morbidity in non-infected, *O. viverrini* infected and *O. viverrini* and *S. mekongi* co-infected study participants in Southern Lao PDR (n = 148)

Morbidity	Non infected reference group (n = 99)	Any infection (n = 49)	Single infection *O. viverrini* (n = 28)	Dual infection *O. viverrini* + *S. mekongi* (n = 21)
Self-report morbidity				
Abdominal discomfort	45 (45.5)	29 (59.2)	18 (64.3)	11 (52.4)
Diarrhea	10 (10.1)	2 (4.1)	2 (7.1)	0
Right upper quadrant pain	5 (5.1)	8 (16.3)	5 (17.9)	3 (14.3)
Vomiting	5 (5.1)	6 (12.2)	4 (14.3)	2 (9.5)
Physical examination				
Hepatomegaly	42 (42.4)	34 (69.4)	16 (57.1)	18 (85.7)
Pale conjunctiva	28 (28.3)	6 (12.2)	3 (10.7)	3 (14.3)
Splenomegaly	15 (15.2)	16 (32.7)	4 (14.3)	12 (57.1)
Right upper quadrant pain at palpation	5 (5.1)	2 (4.1)	2 (7.1)	0
Ultrasound examination				
Liver fibrosis	3 (3.0)	15 (30.6)	5 (17.9)	10 (47.6)
Size of left liver lobe > 2SD	4 (4.0)	16 (32.7)	6 (21.4)	10 (47.6)
Portal vein diameter > 2 SD	10 (10.1)	4 (8.2)	2 (7.1)	2 (9.5)
Gallbladder stone	4 (4.0)	4 (8.2)	2 (7.1)	2 (9.5)
Gallbladder length (mean in cm)	6.1*	6.5*	6.4	6.7*
Haemoglobin level				
Anemia (Hb < 12.0g/dl)	26 (26.3)	10 (20.4)	7 (25.0)	3 (14.3)

* $P < 0.020$

.

Table 9.5: Risk of morbidity associated with any infection, single-infection with *O. viverrini* and dual-infection with *O. viverrini* and *S. mekongi* in Southern Lao PDR (n = 148)

	Reference	Any infection		Single infection *O. viverrini*		Dual infection *O. viverrini* + *S. mekongi*	
	OR (95%, CI)	**OR (95%, CI)**	***P*-value**	**OR (95%, CI)**	***P*-value**	**OR (95%, CI)**	***P*-value**
Self-report morbidity							
Diarrhoea	1.0	0.4 (0.1 – 1.8)	0.217	0.7 (0.1 – 3.3)	0.628	n.a.	n.a.
Abdominal discomfort	1.0	1.7 (0.8 – 3.7)	0.116	2.2 (0.8 – 5.8)	0.078	1.3 (0.5 – 3.8)	0.563
Vomiting	1.0	2.6 (0.8 – 9.1)	0.128	3.1 (0.8 – 12.6)	0.107	2.0 (0.4 – 11.0)	0.435
Right upper quadrant pain	1.0	3.6 (1.1 – 11.6)	0.033	4.0 (1.1 – 15.0)	0.040	3.1 (0.7 – 14.0)	0.148
Physical examination							
Hepatomegaly	1.0	3.1 (1.5 – 6.4)	0.002	1.8 (0.8 – 4.2)	0.170	8.1 (2.3 – 29.5)	0.001
Splenomegaly	1.0	2.7 (1.2 – 6.1)	0.016	0.9 (0.3 – 3.1)	0.910	7.5 (2.7 – 20.8)	< 0.001
Pale conjunctiva	1.0	0.4 (0.1 – 0.9)	0.034	0.3 (0.1 – 1.1)	0.067	0.4 (0.1 – 1.6)	0.193
Right upper quadrant pain at palpation	1.0	0.8 (0.2 – 4.2)	0.775	1.4 (0.3 – 7.7)	0.688	n.a.	n.a.
Ultrasound examination							
Liver fibrosis	1.0	14.1 (3.9 – 51.8)	< 0.001	7.0 (1.6 – 31.2)	0.011	29.1 (6.9 – 121.9)	< 0.001
Size of left liver lobe							
Mark enlarged > 2SD	1.0	15.0 (3.9 – 57.6)	< 0.001	6.4 (1.4 – 29.1)	0.016	75.0 (7.5 – 752.0)	< 0.001
Portal vein diameter							
Dilated > 2SD	1.0	0.8 (0.2 – 2.7)	0.705	0.68 (0.1 – 3.3)	0.638	0.9 (0.2 – 4.6)	0.936
Gallbladder stone	1.0	2.1 (0.5 – 8.8)	0.306	1.8 (0.3 – 10.5)	0.500	2.5 (0.4 – 14.6)	0.310
Haemoglobin level							
Anemia (Hb < 12.0g/dl)	1.0	1.4 (0.6 – 3.2)	0.436	1.1 (0.4 – 2.8)	0.893	2.1 (0.4 – 7.9)	0.253

n.a. not applicable

9.5 Discussion

In our study conducted in two settings of central and southern Lao PDR we found severe morbidity associated with the currently observed *O. viverrini* infection intensities. In the first cross-sectional study we included 95 study participants from highly endemic areas in the central part of the country. In the second study 148 study participants from southern Lao PDR were enrolled where opisthorchiasis and schistosomiasis are concurrently present.

Our analysis of first cross-sectional study showed that the reported symptom of RUQ, the hepato-biliar pathologies (i.e. CBD, IHBD and liver fibrosis) and the mild liver dysfunction (i.e. increased PAL and AST) obtained from interview, ultrasound examination and blood testing, respectively were significantly associated with the infection intensity of *O. viverrini* infection. These findings are similar to those observations made in the northeast Thailand over the last decade where epidemiological studies have shown that severe liver morbidity due to *O. viverrini* infection were observed in higher frequency among people with heavy infection [18,19,20]. In this setting the worldwide highest incidence of CCA was recorded [21,11,22]. In many settings of Lao PDR the prevalence and intensity of infection with *O. viverrini* exceeds the area of northern Thailand [23,5,6,7]. It must be concluded that the CCA incidence in highly endemic area of Lao PDR is as high as in Northern Thailand. During our study a patient with an electrogenic-dense, tumor-like body (7^{th} segment of left liver lobe) was diagnosed in Mahosot hospital. Suspected cases of CCA patients are regularly seen at referral hospital. But final diagnosis can not be made in the country. Today no population-based information is available on incidence of CCA and their pre-cursors. Hence, there is a pressing need to close this knowledge gap and address the public health problem with adequate action.

In addition, an analysis of second cross-sectional study shows that the reported symptom of RUQ was significantly observed in higher prevalence among the study participants harboured with only *O. viverrini* than those non-infected persons.

Interestingly, two important findings in the physical examination e.g. hepatomegaly and splenomegaly were strongly significantly observed among study participants who were infected with *O. viverrini* and *S. mekongi* concurrently, whilst the latter parasite combination was also significantly associated with the liver fibrosis and left liver lobe enlargement. Studies showed that infection with *O. viverrini* is associated with a number of hepato-biliary tract abnormalities including chronic inflammation of bile duct system, jaundice obstructive, cholecystitis and cholangitis [11]. *S. mekongi* single-infection results in liver (advanced liver fibrosis and portal hypertension) and spleen morbidity [24,25,26,27]. In our study we examined *O. viverrini* concurrently infected with *S. mekongi.* Our findings suggested that the combination of infection aggravates the spleen and hepato-biliar abnormalities rather. This observation supports earlier reports suggesting that multiparasitism even in low intensities may inflict important morbidity [28,29,30].

In southern Lao PDR opisthorchiasis, schistosomiasis and STH are highly prevalent. Thus multiple parasite infection is highly abundant, i.e. in our study all patients infected with *S. mekongi* were also infected with *O. viverrini.* Multiparasitism complicates the assessment of morbidity attributable to each specific parasite. Hence, in our case we were not able to assess morbidity attributable to single-infection with *S. mekongi*. However, our study documents the hepato-biliar morbidity rise from a single *O. viverrini* infection to a double-infection with *O. viverrini* and *S. mekongi.*

In our study we used a rigorous assessment of the intensity of infection status. Our enrolled patients were purged after a treatment allowing determining the number of adult flukes with hepato-bilar and intestinal tropism. In general the intensity of eggs shed in the stools is taken as proxy for the number of adult fluke parasite. In fact, in a context like Lao PDR where several minute intestinal flukes (MIF) are abundant [31,32,6] this approach is not possible. MIF have very similar eggs morphology as *O. viverrini.* With general parasitological techniques such as the Kato-Katz thick smear diagnosis [15] the distinction can not be made and can there not be employed.

Our study documents the infection status and intensity of *O. viverrini* in Lao PDR. Similar *O. viverrini* endemic settings are known in Thailand and Cambodia [33,34]. We showed that substantial hepato-bilar pathologies are induced by this level of endemicity. Therefore, in Southeast Asia, community morbidity attributable to

O. viverrini is much larger than currently known. Further, community-based investigations, including the assessment of precursors of CCA are warranted. In addition, concomitant infections are abundant and lead to an increase in morbidity. In particular, *S. mekongi* induced morbidity is of importance and needs urgent control.

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10. Discussion

This Ph.D. thesis addresses the infection status, morbidity and geographical distribution of *O. viverrini* and underlying determinants such as environmental and socio-economic risk factors, in the provinces of Champasack, Saravane and Savannakhet in southern and central Lao PDR. In addition, it furthers our understanding on the frequency and importance of concomitant infections with other trematodes and soil-transmitted helminths and their underlying factors between the parasitic infections and between environmental, socio-economic and behavioural factors and multiparasitism. Finally, an attempt is made to explore the importance of multiparasitism in terms of morbidity.

The data presented in this work were obtained from a series of epidemiological studies. Data pertaining to the epidemiology were primarily obtained from a cross-sectional study carried out in February and March 2004 in Saravane district, province of Saravane. In this starting point this work aimed to collect the particular epidemiological indicators for opisthorchiasis, other intestinal helminthiasis and multiparasitism in a specific eco-epidemiological setting in the country (Sayasone *et al.* 2007). In March to May 2006 a next cross-sectional study was carried out in three distinct eco-epidemiological settings of Champasack province (Sayasone *et al.* 2009a). This study pertained to the broader epidemiological aspects (e.g. demographic, behavioural, socio-economic and environmental data) related to opisthorchiasis and other concomitant intestinal helminthiases as well as the assessment of multiparasitism in these distinctly different settings and their determinants.

Finally, an in-depth study (hospital- and community-based) carried in the year of 2005 and 2006 in the two referral hospitals (Mahosot and Savannakhet provincial hospital) and in communities of three provinces in central and southern Lao PDR (Savannakhet, Saravane and Champasack) addressed the diversity of human intestinal helminthiasis (Sayasone *et al.* 2008a, 2008b) and to assess their association with hepato-biliar and intestinal morbidity. Especially morbidity-related to infection with *O. viverrini* alone compared with *O. viverrini* and *S. mekongi* co-infection was of major interest (Sayasone S et al. 2009b).

Within this framework, we evaluated a combined approach of standard parasitological diagnostic tools such as KK technique and FECT method each of which is widely used in parasitological diagnosis in the health services and in field investigations. The examination of three stool samples over consecutive days with the KK technique, supplementing with a FECT method on one of those stool samples were considered as rigorous approach for the parasitological investigations in our study.

Furthermore, we added the purgation after treatment (praziquantel, 40 mg/kg BW single oral dose) in our in-depth study allowing recovering of adult worms, and thus to identify the specific food-borne trematode species, placing emphasis on *O. viverrini* and MIF. Since the eggs of those flukes are similar in shape and size, distinguishing their eggs in the simple standard parasitological techniques (KK and FECT) is challenging. The detailed clinical examinations were additionally obtained from study participants enrolled in this in-depth study (e.g. physical examination, ultrasound, liver function test and whole blood count), which in turn allowed us to quantify the relationship between infection status and morbidity and co-morbidity related to infection.

It is important to note that our studies have several limitations. Our epidemiological investigations were predominantly conducted in the central and southern provinces of Lao PDR, and hence did not include the mountainous northern part the country. Generalization across the country is therefore not possible. Further parasitic infections which are associated with these environmental conditions such as e.g. paragonimiasis were not considered in our investigations, although these are important features of the Lao parasite-landscape (Strobel *et al.* 2005; Odermatt *et al.* 2007b). In Lao PDR several *Paragonimus* species (Odermatt *et al.* 2007a; Yahiro *et al.* 2008) are known to be endemic, particularly in high-lands of the north such as the province of Luang Prabang (Odermatt *et al.* 2007a; Song *et al.* 2008; Sohn *et al.* 2009).

In the initial epidemiological investigation of the province of Saravane, we used one single stool sample analysed by KK. In this study, the limitations of single-stool and single-technique analysis in field surveys were recognized, in particular the underestimation of the prevalence rates of helminths in the region (e.g. opisthorchiasis, STH and multiparasitism) (de Vlas & Gryseels 1992; Kongs *et al.* 2001; Utzinger *et al.* 2001; Steinmann *et al.* 2008; Knopp *et al.* 2008).

10.1 Infection with *O. viverrini,* soil-transmitted helminths, *S. mekongi* and risk factors

Our first study on the epidemiology of *O. viverrini* infection carried out in Saravane province showed a high prevalence rate of 58.5% among 814 study participants. The infection occurred in all age groups, including pre-school children of less than 6 years of age. The highest prevalence and infection intensity was observed among adults of middle age between 30 and 45 years, indicating an accumulation over time of this food-borne trematode infection. Examination of *cyprinoid* fish species in the local rivers showed high rate of infection with metacercariae. From 98 fish samples out of 23 *cyprinoid* species, almost two-third were found to harbour metacercariae. In the same setting, soil-transmitted helminths were found at lower rates, e.g. hookworm 46.1%, *A. lumbricoides* 15.7% and *T. trichiura* 11.1%.

In our investigations in Champasack, we employed a rigorous diagnostic approach. Two stool samples were collected of each study participant and the examination of stools was repeated. The overall prevalence of *O. viverrini* infection in the three settings of Champasack province was 64.3%. There were remarkable differences found between the settings. While high prevalence rates were observed in the low-lands of the Mekong River basin e.g. in the districts of Khong (92.0%) and Mounlapamok (90.9%), in the Paksong district the prevalence was only 5.7%, approximately 16 times lower than in the low land. Paksong district is located on the Bolaven plateau at an altitude of about 800 to 1,200 m above sea level. Geographically, this area compounds by the secondary forests, where small river streams with strong flows are abundant. From an epidemiological point of view, these would be the natural disadvantage for *O. viverrini* development.

In addition, this area is mainly populated by the Lao-theung minority, who consume raw or undercooked fish less frequently than the Lao-loum ethnic group. The results of the present study showed that only one-quarter of the Lao-theung minority (49/210) consumed uncooked fish in one week prior to the survey, while almost two-third of Lao-loum ethnic group (294/445) practiced so in the same period. This would be a key that explains why people living the Paksong district harboured with the low prevalence of *O. viverrini.*

In Lao PDR the consumption of raw food-stuff is deeply culturally rooted. There are mainly two fish dishes namely “koy-pa” and “lap-pa” which are associated with *O. viverrini* infection. They are a sort of fish salad prepared with minced fish mixed with herbs, chilli, lemon juice or other sauces. There are numerous local adaptations in the different parts of the country but all are made of raw or only briefly cooked fish (Strandgaard *et al.* 2008). In communities of high prevalence, these dishes were most frequently consumed. Frequently exposed to raw or uncooked fish consumption was significantly associated to a high prevalence of opisthorchiasis in the community. The infection intensity increased with age indicating an accumulation of infection over time. This finding corresponds to observations made in a neighbouring country (Thailand), which have shown that opisthorchiasis is highly prevalent in the areas where the traditional dishes containing raw or insufficient cooked fish are widely consumed (Haswell-Elkins *et al.* 1991; Sithithaworn & Haswell-Elkins 2003; Sripa *et al.* 2003).

In Champasack province the occurrence of soil-transmitted helminth infections was distinctly different in the three settings. All three major soil-transmitted helminths were highest in the mountainous Paksong district compared to the settings in the plain (e.g. hookworm 94.8%, *A. lumbricoides* 85.9% and *T. trichiura* 55.7%). The highest prevalence and infection intensity was observed in the young children aged below 5 years. An infection with soil-transmitted helminths can lead to nutritional deficiencies and may impair growth and cognitive development in children (Al-Mekhlafi *et al.* 2005; Bethony *et al*. 2006; Hotez *et al.* 2008). It is widely acknowledged that children aged below 5 years are at high risk of mortality in developing countries (Bryce *et al.* 2005). Although the causes of death are multi-factorial, malnutrition is a key factor and parasites contribute to a substantial fraction of this under-nourishment (Albonico *et al.* 2008; Bryce *et al*. 2005).

With regarding to *S. mekongi* infection, a patchy distribution was observed. The study participants living in Kong district (68.0%: 153/68) were highly infected, whilst only 3.9% (9/232) of study participants were positive in Mounpamok district and there was no *S. mekongi* detected in Paksong district. These finding further document previous observations made in the country, showing that *O. viverrini* infection is highly prevalent in the low-land Lao communities, and is particularly frequent in the Mekong River plains, whereas the STH is prevalent in the high-lands of northern provinces and *S. mekongi* (Giboda *et al.* 1991; Kobayashi *et al.* 2000; Urbani *et al.* 2002; Rim *et al.* 2003).

Furthermore, our results show that several parasites were significantly associated to multi-risk factors after adjustment. Unprotected disposal of faeces in the environment is a key feature to introduce parasites into the environment (de Silva *et al.* 2003; Bethony *et al.* 2006), particularly parasites for which humans act as a main reservoir such as *O. viverrini* (Saowakontha et al. 1993).

Our examination of *cyprinoid* fish samples from local rivers showed that the high proportion of fish (59.2%) is infected with metacercariae. Similar infection rates in freshwater fish populations has been observed in other parts of Lao PDR (Rim *et al.* 2008), and in neighbouring Vietnam (Thien *et al.* 2007) and Thailand (Boonchot & Wongsawad 2005; Kumchoo *et al.* 2005). This extensive fish infection, in turn, contributes directly to the observed prevalence of opisthorchiasis in the communities.

Indeed, in our study, community latrine facilities were virtually absent, and the study participants reported that they defecate freely in the village environment. Our findings therefore underscore that primary prevention of *O. viverrini* infection through sanitation facilities, and their utilisation must be substantially improved in the country.

Low socio-economic status is a negative predictor for people’s access to clean water, adequate sanitation and anthelminthic medicines, which in turn favours parasitic infections (de Silva *et al.* 2003; Raso *et al.* 2005; Steinmann *et al.* 2007). The results of our present work revealed that *A. lumbricoides* and *T. trichiura* were found to be highly prevalent in the poorest group of the study participants, and thus confirms previous observations made by colleagues in neighbouring countries such as Southern China (Steinmann *et al.* 2007) and from African countries (Raso *et al.* 2005). There the prevalence of soil-transmitted helminths was also rampant among the poorest population living in the deprived rural settings.

Schistosomiasis is re-emerging in Khong district, southern Lao PDR. In 1990s, schistosomiasis, caused by *S. mekongi* was highly prevalent in the lower Mekong River basin (southern Lao PDR and northern Cambodia) (Stich *et al.* 1999; Biays *et al.* 1999). High observed number of severe cases was of medical concern and contributed to the high morbidity and mortality of the region. In northern Cambodia (Sambo and Kratie district), an estimated 10 severe cases were hospitalized each month in the hospitals and 150 out-patients were treated due to chronic schistosomiasis (Biays *et al.* 1999; Keang *et al.* 2007). A similar situation was observed in the southern part (Khong and Mounlapamok district) of Lao PDR.

A community-based control programmes was later launched, using praziquantel-based morbidity control as strategy of choice in such areas. Multiple rounds of praziquantel reduced the prevalence of *S. mekongi* to very low levels (2.1% in Khong district and 0.4% in Mounlapamok district) and were considered a successful public health control programme (Urbani *et al*. 2002). However, interruption of chemotherapy-based morbidity control in the face of inadequate sanitation, lack of clean water and continued human water contacts are at the root of re-emergence of schistosomiasis.

Up to today, severe schistosomiasis cases, not only among adults but also among schoolchildren were regularly found in our cohort study villages of Khong district (P. Soukhathammavong, Personal communication). This urgently calls for concerted action to remedy the morbidity and mortality due to this disease in the region.

10.2 Assessment of multiparasitism and *O. viverrini* infection

Multiple parasitic infections are a common phenomenon noted in populations living in socio-economically disadvantaged settings. They represent a common fact in the daily realities of clinical health services (Petney & Andrews 1998; Drake & Bundy 2001). Despite these realities, relatively little attention have been given to systematically document this phenomenon and determine underlying risk factors. To close these knowledge gaps, a number of studies have been carried out in Africa (Keiser *et al.* 2002; Raso *et al.* 2004; Matthys *et al.* 2007), China (Steinmann *et al.* 2008) and Southeast Asian countries (Ezeamama *et al.* 2005).

In Lao PDR, only concurrent infections with *O. viverrini* and small intestinal trematode have been described (Chai *et al.* 2005, 2007). To our knowledge, no data on co-infections with different intestinal helminths as well as intestinal protozoa were available prior to out studies for Lao PDR. However, several parasites have been reported, starting from food-borne (e.g. *O. viverrini,* MIF, *Paragonimus,* and *F. buski*), to soil-transmitted helminths, *S. mekongi,* and intestinal protozoa, together with the absence of sanitary facility, low socio-economic status and raw food consumption behaviour. A nationwide survey among schoolchildren has shown a high geographical overlap of different helminth prevalence, hence co-infection must occur (Rim *et al.* 2003).

Our studies showed that people living in the rural areas of the country were currently suffering from multiparasitism rather than single-species infection. For example, in Saravane province (Sayasone *et al.* 2007) almost two-third of study participants harboured two or more parasite species. In Champasack province we could confirm rampant multiparasitism (Sayasone *et al.* 2009a). Fifteen different intestinal parasite species were discovered belonging to the family of Trematodiae, Nematodiae and Cestodiae and to intestinal protozoa, and 4 out of 5 study participants harboured two or more intestinal parasite species. A strong positive association was observed for co-infection between *'O. viverrini'*-like and *S. mekongi,* hookworm and *A. lumbricoides,* hookworm and *T. trichiura* and *E. coli* and *A. lumbricoides.* This is the first study pertaining to co-infection of different intestinal parasites starting from trematodiasis to intestinal protozoa infections in the country, and assessing the underlying risk factors. Furthermore, a positive association between '*O. viverrini'*-like and *S. mekongi* was described, to our knowledge for the first time ever. The positive association between soil-transmitted helminths and intestinal protozoa have been investigated before for Côte d'Ivoire and China (Raso *et al.* 2004; Steinmann *et al.* 2008), and have now been extended to Lao PDR.

The presence of extensive multiparasitism is challenging the epidemiological study in the country; until today there is no a single standardized tool capable to detect the multiple intestinal parasitic infections with the high sensitivity (Bergquist *et al*. 2009). The assessment of infection is therefore based on direct parasitological techniques. Two parasitological techniques such as KK and FECT are widely used in the parasitological survey. However, those techniques have some limitations on their performance (Bergquist *et al*. 2009). The KK technique has been commended by WHO for epidemiological survey related to STH (WHO 1993). It equips with basic materials, allows screening a large sample size of population and is applicable in the field work. However, it does not allow screening the infection with *S. stercoralis* and intestinal protozoa. Most importantly, it could not differentiate the *O viverrini* eggs from those of MIF. Moreover, the KK-technique is also known as having the low sensitivity detecting the parasite's eggs in the stool if only one sample is analyzed (de Vlas & Gryseels 1992; Kongs *et al.* 2001; Utzinger *et al.* 2001; Booth *et al.* 2003). Due to the relatively small amount of faeces (41.7 mg), light infections may be missed, consequently, underestimating the 'true' community prevalence and it has additionally noted as having a low sensitivity for hookworm diagnosis (Booth *et al.* 2003; Raso *et al.* 2004; Steinmann *et al.* 2008). A prolongation time from stool sample collection and smear slide preparation and reading results in a substantial reduction of eggs visualized under a light microscopy.

The FECT method is usually a standard parasitological tool of a laboratory. It is equipped with the basic materials e.g. tubes, formalin, ether, pipettes, normal saline and centrifuge (WHO 1998). This technique is able to screen a large sample size of conserved-stool samples. It is capable to detect the helminth eggs, cyst of intestinal protozoa and larvae of *S. stercoralis*. However, FECT is not the recommended technique for *S. stercoralis* diagnosis and hence the more reliable techniques are commended such as Koga agar plate and Baermann technique (Koga *et al.* 1991; Stothard *et al.* 2008; Steinmann *et al.* 2008; Knopp *et al.* 2008). Utility of FECT method for distinguishing the eggs of *O. viverrini* from eggs of those MIF has been described in the literature (Elkins *et al.* 1991; Sithithaworn *et al.* 1991; Tesana *et al.* 1991). The *O. viverrini* diagnosis demonstrates the presence of rough eggshells, knob and prominent shoulder, which are more or less prominent in eggs of MIF. With a 400 times magnification these characters are clearly visible (Tesana *et al.* 1991).

With these limitations, the quantitative assessment of multiple parasitic infections in the population is only possible by the combination of different diagnostic techniques. In case of Lao PDR, a particular technique is additionally required to differentiate the abundant food-borne parasitic infections. The particular quantitative assessment of different species of FBT, including species assessments of liver and intestinal flukes makes this endeavour challenging. In our present study, we introduced the purgation of study participants after treatment as an additional technique to the routine stool examination, i.e. the examination of the entire stool collected after treatment. The adult flukes were collected, identified and counted. The results showed that 83% of the examined person harboured at least 2 parasites species (Sayasone *et al.* 2008b).

In addition to the stool sample analysis, six different species of small intestinal trematodes and one of Echinostomatidae were identified only in the purging study. These findings further document that multiparasitism is indeed the norm rather than the exception in the developing world and underscores the methodological challenges which need to be tackled when addressing quantitative research questions related to multiple parasitic infections in a specific setting (Raso *et al.* 2004; Sachs & Hotez 2006; Dung *et al.* 2007; Steinmann *et al.* 2008).

Furthermore, the evaluation of FECT performance in considering the purging results as the diagnostic ‘gold’ standard showed a correct *O. viverrini* egg diagnosis (e.g. sensitivity 96.8% and specificity 100.0%). A weaker performance (e.g. sensitivity 85.0% and specificity 70.6%) of later test was addressed for the MIF egg diagnosis. In fact, in the dissection of *O. viverrini* worms, we observed that a small proportion of *O. viverrini* eggs (5%) is very similar to MIF eggs, i.e. without prominent shoulders and knobs (Tesana *et al.* 1991), therefore a rigorous examination with FECT technique is necessary in order to correctly classify these eggs. In addition, the purgation underestimate the prevalence of MIF, since the collection of tiny heterophyid flukes sized 0.3-0.7 mm wide and 1.0-2.5 mm long (Radomyos *et al.* 2004; Garcia 2007) in the purging stool samples under a stereo-microscope is challenging, particularly in the cases with light infections.

Most recently a promising polymerase chain reaction (PCR) method has been described (Stensvold et al. 2006; Duenngai et al. 2008; Sato et al. 2009; Lovis et al. 2009) for accurate differentiation of *O. viverrini* and MIF infection. These techniques need sophisticated and costly laboratory equipments; therefore in many areas where resources are limited FECT method remains the method of choice for distinguishing *O. viverrini* infection from those of MIF.

The FLoTAC technique has recently been developed (Cringoli 2004, 2006; Utzinger *et al.* 2008; Knopp *et al.* 2009). This method is capable to detect the concomitant infection of helminths and intestinal protozoa in SAF-conserved stool samples with high sensitivity. The screening of stool sample with highest microscopic magnification (100x) results in an accurate diagnosis of helminth species (Cringoli 2006; Utzinger *et al.* 2008; Knopp *et al.* 2009). If this is also a case for food-borne trematode infections, in particular *O. viverrini* and those MIF, then it will offer a new means for assessment the diversity of FBT and in turn, it contributes to provide the comprehensive data on multiparasitism in places, where several food-borne trematode species are endemic.

10.3 Multiple stool samples and multiple examination of stool

Examination of repeated stool sample collection and repeated examination of stools with one or different techniques have previously shown a considerably increased sensitivity of diagnostic tools for several parasitic infections (Marti & Koella 1993; Utzinger *et al.* 2001; Booth *et al.* 2003; Steinmann *et al.* 2008; Knopp *et al.* 2008). Analysis of three stool samples by KK-technique, supplemented with FECT method analyzed on one of those stool samples were considered as 'gold' standard for the parasitological investigations. In our present data, the combination of technique 'gold' standard resulted in the considerable increased observed prevalence of helminth infections. Of note, an increase in the diagnosis of *S. mekongi* and hookworm, for example the prevalence of *S. mekongi* was only observed in 4.1% and 12.2% if a single stool sample was analyzed by FECT and KK, respectively, while the 'gold' standard had a 6 times and 2 times higher prevalence compared to FECT and KK alone, respectively. Similar findings were observed for hookworm. The observed prevalence was 3 times and 1.5 times higher in the 'gold' standard than the single FECT and the single KK, respectively. However, it is important to note that the collection of multiple stool samples is not always easy in the field. Analysis of our present data showed that 43.7% of enrolled study participants failed to submit the stool samples as study requirements.

A low compliance rate indicates that a large number of sampling populations should be considered to assure, having enough study sample, hence the evaluation of multiple stool sampling effect of diagnostic tools could be performed with high data quality. However, a large number of sampling populations interprets in an increase of resources, materials and budget, which are always limited in the developing countries. Experiences from field show that prolongation the time of field work resulted in minimizing the low compliance in the final cohort; this could alternatively be an option in the place, where human resources are limited.

In our sample, although a relatively low compliance was seen, our final examined cohort was large enough to perform the sampling effect on the repeat stool sample analyzed by KK-technique for helminth diagnosis, using a mathematical model (Marti & Koella 1993). In addition, it corresponded well in age and sex distribution to the enrolled persons. Except young children aged < 6 years, 11-15 years and 31-45 years was significantly lower compliant. The results suggest a high increased sensitivity of diagnostic tool in relation to the examination of three stool samples. The increased sensitivity was particularly marked for *S. mekongi, T. trichiura*, hookworm and '*O. viverrini'*-like eggs, respectively. Our findings are in an agreement with the recommended sampling frequency (van Gool *et al.* 2003) and the results previously observed elsewhere (Knopp *et al.* 2008; Steinmann *et al.* 2008) in the repeated stool sample examination.

10.4 Helminth infections and related morbidity

With our research findings on multiple parasitic infections, stimulated by previous studies (Raso *et al.* 2004; Matthys *et al.* 2007; Steinmann *et al.* 2008) and combining purging methods which was used also by others (Chai *et al.* 2005), we could add to the growing body of literature of multiple parasitic infection including underlying risk factors in various settings of the tropical world. However, from a public health perspective it is essential to understand in how far multiple parasitic infections are shaping the morbidity burden in the population.

It is well acknowledged that infection with intestinal helminths has a considerable impact on the public health and human well-being (de Silva *et al.* 2003; Utzinger &

Keiser 2004; Hotez *et al.* 2007). For example, chronic infection with schistosomiasis kills approximately 200,000 people every year. It follows that the global disability-adjusted life year lost (DALYs) is as high as 4.5 million (van der Werf *et al.* 2003; Utzinger & Keiser 2004). STH might be responsible for the global burden of 39.0 million DALYs (WHO 2002; Utzinger & Keiser 2004; Hotez *et al.* 2007). Chronic *O. viverrini* infection is significantly associated with the development of CCA, a fatal hepato-biliar cancer. The worldwide highest CCA incidence occurs in the high *O. viverrini* endemic areas of northeast Thailand (Sripa *et al.* 2007; Andrews *et al.* 2008; Kaewpitoon *et al.* 2008). The CCA-related *O. viverrini* infection results in the estimated annual economic cost of US$ 120 million in the *O. viverrini*-endemic areas of Thailand alone (Andrews *et al.* 2008).

Today there is still a gap in the understanding on how multiparasitism influences morbidity. Only few data on morbidity induced by multiparasitism are available. Some authors have shown that concurrent infections with different parasite species, even in light intensity infections, might aggravate morbidity (Ezeamama *et al.* 2005).

We have attempted to address this gap by combining multiparasitic infections and extended morbidity assessments with clinical and laboratory examinations, questionnaire reported signs and symptoms and ultra-sonographical assessment of hepato-biliar morbidity. In particular we addressed the question in how far *O. viverrini* induced morbidity is aggravated by a co-infection with *S. mekongi*. In our study we noted a variation of intensity of infection with *O. viverrini* worms in our patients. Hepato-biliary morbidity such as liver fibrosis, common and intra-bile duct dilatation in the ultrasound examination were associated with the intensity of the infections. The high intensity of *O. viverrini* infection is usually observed among people of middle age of age-group 30-45 years. This finding was similar to results observed during the community-based studies conducted in the *O. viverrini*-endemic areas of northeast Thailand, which have shown that heavy infection with *O. viverrini* were significantly associated to development of the above-mentioned hepato-biliary pathologies (Mairiang *et al.* 1992; Sithithaworn *et al.* 1994).

A significant positive association between *O. viverrini* and *S. mekongi* infection observed in our present study is of particular concern from a clinical point of view.

Indeed, an infection with *O. viverrini* leads to severe clinical manifestations such as hepato-biliary pathologies, including hepatomegaly, liver fibrosis, jaundice obstructive, gallbladder stones, cholecystitis and cholangitis and most importantly the development of a fatal liver cancer (cholangiocarcinoma) (Sripa & Kaewkes 2002; Sripa *et al.* 2007; Andrews *et al.* 2008). Chronic infection with *S. mekongi* contributes to a formation of hepatomegaly, periportal fibrosis and portal hypertension (Biays *et al*. 1999; Hatz 2001; Keang *et al.* 2007; Chiavaroli *et al.* 2008). Co-infections of these two trematodes could further aggravate the host-organ pathology, especially the liver.

Our present data underscore that heavy infection with *O. viverrini* was significantly associated to the observed hepato-biliary pathologies in the ultrasound examination such as liver fibrosis, common bile duct dilatation and intra-bile duct dilatation. The co-infection with *S. mekongi* aggravated significantly the liver morbidity, for example the study participants infected with *O. viverrini* and *S. mekongi* concurrently were at 2 and 6 times and 30 and 75 times higher risk of having an observed liver fibrosis and hepatomegaly in the ultrasound examination compared with mono-*O. viverrini* infection and non-infected individuals, respectively.

The carcinogenic nature *O. viverrini* infection has been recognized (Vainio & Kleihues 1994; Mayer & Fried 2007). Chronic infection is significantly associated to development of CCA (Haswell-Elkins *et al.* 1994; Sripa *et al.* 2007; Andrews *et al.* 2008). In our in-depth study, we excluded patients diagnosed with suspected severe liver morbidity due to ethical reasons. In Lao PDR the cancer treatments are not available in the hospitals, and thus we could not guarantee an adequate management upon diagnosis. It follows that the morbidity assessment in the community linked with *O. viverrini* was underestimated.

10.5 Conclusion

This Ph.D. thesis contributes to a better understanding of the epidemiology and morbidity of *O. viverrini,* a common food-borne trematode infection in Lao PDR. Moreover, multiple species trematode, nematode and intestinal protozoa infections have investigated and hence the issue of multiparasitism has been advanced. The following conclusions can be drawn from the results of the work presented in this thesis.

- The consumption of raw or insufficiently cooked food stuffs i.e. vegetables, meet and fish, is widely and frequently practiced and culturally deeply routed in the Lao community, particularly among the Lao-loum ethnic groups. In addition, in most rural settings of the country access to safe water supply is limited and adequate sanitation virtually absent.
- *O. viverrini* is highly prevalent in rural Lao PDR. In some villages, prevalence rates exceed 90.0% of the general population. Highest intensity of infections is observed among the adult people aged between 30 and 45 years i.e. 4.0 times higher than in young children aged below 6 years, indicating an accumulation over time.
- Today observed intensity of infection with *O. viverrini* is associated with significant hepato-biliar pathology, i.e. bile duct dilatation, liver fibrosis and liver dysfunction. From experience in Thailand, it is known that this level chronic infection, infection intensity with *O. viverrini* and morbidity is associated with the development of CCA.
- *S. mekongi* is highly prevalent in some of the Mekong islands in the Khong district. Over two-third of the study population in this area was found positive with *S. mekongi* eggs during stool sample examination. Severe cases are frequently observed in the community.
- Co-infection of *O. viverrini* and *S. mekongi* is a common phenomenon in Khong district and has a significant higher risk hepato-pathology than single infection, i.e. liver enlargement and liver fibrosis compared to non-infected individuals and individuals infected with only *O. viverrini*.
- Soil-transmitted helminths are highly prevalent, particularly in highlands. Pre-chool children and schoolchildren are affected with high infection intensities.
- Multiparasitism is a common phenomenon. Four out of five study participants enrolled in our studies harboured at least two intestinal parasite species. Concomitant infection of several food-borne trematodes, in particular liver fluke and MIF are also common.
- Rare human food-borne parasitic infections, i.e. *Echinochasmus japonicus* and *Capillaria philippinensis* are also observed in the communities. *C. philippinensis* leads to severe gastro-intestinal morbidity.

Our findings demand for the design and implementation of sustained helminth control efforts in the southern part of Lao PDR. Access to treatment must assure morbidity control. In these areas of high prevalence rates, regular mass drug administration (MDA) of treatment is warrant. Combination of anthelminthic medicines (i.e. praziquantel and albendazole) is essential to address widespread multiparasitism and co-morbidity. A particular attention is required for pre-school children. To alter underlying risk factors such as absence of clean water, inadequate sanitary facilities and insufficient health knowledge will result in sustainable and cost effective situation as transmission will be affected. Training of health staff is fundamental to diagnose and manage severe cases at health facilities and perform appropriately community control activities. Integration into existing national health programmes i.e. school health promotion, vitamin-A supplementation and enlarged program of immunization are potential options to implement a cost effective control and cover a large proportion of people in the endemic areas.

10.6 References

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11. Curriculum vitae

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Education

2005-2009 PhD in Epidemiology at the Swiss Tropical Institute, University of Basel, Switzerland.

PhD thesis: Epidemiology and morbidity of food-borne trematodiasis in Lao People's Democratic Republic, with particular consideration to opisthorchiasis

2001-2003 Study of Master's degree in tropical health at the "Institut de la Francophonie pour Médecine Tropicale", Vientiane, Lao PDR. To be awarded the Diploma of Master degree in 2003.

M.Sc. thesis: Bladder stones in children bellow 15 years in Lao PDR

1995-2001 Study at the Faculty of Medical Sciences, Department of Medicine, National University of Laos, Vientiane. To be awarded the Diploma in Medical Doctor (MD), major in general medicine in 2001.

1984- 1995 Study at the primary and secondary School at Khammouane province and Vientiane Municipality. To be awarded the Baccalaureate in 1995.

Professional experiences

Jan-Jun 2007	Field study on risk prediction and assessments of food-and water-trematodiasis in Southern Lao PDR
Mar-May 2006	Field study on infection and morbidity due to food- and water-borne trematodes in the districts of Champasack province, southern Lao PDR
Sept-Dec 2005	Field study on diversity of intestinal helminthiasis: infection status and identification of parasite species and related morbidity
Oct 2004-Mar 2005	Consultation: analysis of baseline health data of Namtheun Hydroelectric Project (NTPC) in collaboration with the Institut de la Francophonie pour la Médicine Tropicale and the Swiss Tropical Institute
Dec 2003-Dec 2004	Research assistant at the Institute de la Francophonie pour la Médecine Tropicale, Vientiane, Lao PDR
May- June 2003	Field study on the economic impact of urinary tract calculi in three southern provinces of Lao PDR
May- June 2002	Field study on rapid identification of *Tuberculosis* and *Paragonimus* cases in Luang Prabang province, northern Lao PDR

Oral presentation at scientific meetings

10 Sept 2007	Joint meeting 7th RNAS+Workshop / 1st International Symposium on Geospatial Health (Regional Network of Asian Schistosomiasis and other Helminth Zoonoses; Global Network for Geospatial Health), Lijiang, People's Republic of China (05-10 Sept 2007): Diversity of intestinal human helminthiasis in Lao PDR
25 Sept 2007	National Health Research Forum, Vientiane Lao PDR (24-26 Sept 2007): *Opisthorchis viverrini*: relationship between infection intensity, co-infection and morbidity in Lao PDR

Publications

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