Morbidity and treatment of *Opisthorchis viverrini, Schistosoma mekongi*, hookworm and other helminth infections in Lao People's Democratic Republic

INAUGURALDISSERTATION

Zur

Erlangung der Würde eines Doktors der Philosophie

Vorgelegt der

Philosophisch-Naturwissenschaftlichen Fakultät der

Universität Basel

Von

Phonepasong Ayé Soukhathammavong

aus Laos

Basel 2013

Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakultät auf Antrag von Prof. Dr. Marcel Tanner, Prof. Dr med. Christoph Hatz, Assoc. Prof. Dr. Banchob Sripa, und PD Dr. Peter Odermatt

Basel den 13. Dezember 2011

Prof. Dr. Martin Spiess
Dekan

Dedicated to my beloved family

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List of Abbreviations

ABZ	Albendazole
ADB	Asian development bank
AFP	Alpha-fetoprotein serum
APF	Advanced periductal fibrosis
AST/SGOP	Aspartate aminotransferase
ALT/SGPT	Alanine aminotransferase
ALP	Alkaline phosphatase
CCA	Cholangiocarcinoma
CT scan	Computed tomography scan
CA 19-9	Carbohydrate antigen 19-9 or cancer antigen 19-9
EMAE	European agency for the evaluation of medicinal products
EH-CCA	Extrahepatic Cholangiocarcinoma
EPG	Egg per gram
FDA	Food drug admistration
FECT	Formalin ether contentration technique
G-G	Gamma-glutamyl transpeptidase
HBsAg	Surface antigen of the hepatitis-B-Virus
HCC	Hepatocarcinoma
HBcAg	Surface antigen of the hepatitis-C-Virus
IEC	Information, education and communication
IH-CCA	Intrahepatic Cholangiocarcinoma
IL-6	IL-6-Interleukin
K-K	Kato-Katz thick smear technique
Lao PDR	Lao People's Democratic Republic
MBZ	Mebendazole
MDA	Mass drug administration
MoH	Ministry of Health
NIOPH	National Institute of Public Health
ERCP	Endoscopic retrograde cholangiopancreatography
MRI	Magnetic resonance imaging
PZQ	Praziquantel
PAI-1	Endothelial plasminogen activator inhibitor or serpin E1
SEA	Southeast Asia
STHs	Soil-transmitted helminths/helminthiasis
Swiss TPH	Swiss Tropical and Public Health Institute
UNDP	United Nation and Development programme
US	Ultrasonography/Ultrasound
WCB	White blood cell count
WHO	World Health Organization
WPRO	World Health Organization Regional Office for the Western Pacific

I. Acknowledgements

This PhD thesis was jointly undertaken within the research collaboration on helminth control of the two main research institutions: the *National Institute of Public Health* (NIOPH), Vientiane, Lao People's Democratic Republic (PDR) and the *Swiss Tropical and Public Health Institute* (Swiss TPH), Basel, Switzerland. Additionally, the work of this PhD was in close collaboration with various other Lao institutions of the Lao Ministry of health: the *National Center of Malariology, Parasitology and Entomology* (CMPE) and *National Center for Laboratory and Epidemiology* (CLE), Vientiane, Lao PDR.

First of all, I am very grateful to my supervisor, PD Dr. Peter Odermatt from the Department of Public Health and Epidemiology at the Swiss TPH, whose guidance and support was very helpful from the initial to the final level of my Ph.D. The foreword of this thesis would not exist without his dedication. Absolutely, I would not have been able to conduct scientific studies without his encouragement. I deeply appreciate all his contributions, sharing time and ideas to fulfil my Ph.D. thesis.

I am indebted to Assoc. Prof. Dr. Kongsap Akkhavong, acting general director of NIOPH, Prof. Dr. Boungnon Boupha, Vice-President of Foreign Affaires, National Assembly of Lao PDR (a former general Director of NIOPH), Prof. Dr. Marcel Tanner, Director of Swiss TPH, my co-supervisors Prof. Dr med. Christoph Hatz and Prof. Dr. Jennifer Keiser, Prof. Dr. Jürg Utzinger and Dr. med. Oroth Rasphone, who contributed with their excellent expertise and skills to proposals to publications. This thesis, as well as all the papers it includes, could not have been written without them. I will never forget Prof. Dr med. Hans Peter Rohr, who introduced me to my supervisor, PD Dr. Peter Odermatt.

I would like to express my sincere gratitude to Dr. Bouasy Hongvanthong, the acting Director of CMPE in Vientiane, Dr. Samlane Pompida, the former Director of CMPE in Vientiane, and Prof. Dr. Sithat Insisiengmai, head of National Ethics Committee, Ministry of Health, Vientiane, Lao PDR for kind support and recommendations. I am indebted to the wonderful and fantastic staff and collaborators from Khong District, Malaria station and Provincial Health Department in Champasack Province, Center Malaria, Entomology and Parasitology in Vientiane, Laboratory personnel in Attapeu province, Provincial Health Department in Saravane. I shall never forget their outstanding effort to dedicate days and nights to finish our work in time during the field work and always willing to assist tackling any problem together; my studies could not have been completed without these people.

I. Acknowledgements

I express my sincere gratitude to all medical staff and nurses from Radiology Department at Mahosot hospital, Vientiane capital, Lao PDR, and Radiology Department, Srinagarind hospital, Khon Kaen University, Thailand, for their professionalism and for my training in sonography. Also my sincere gratitude goes to Dr. Eimon Mairiang for sharing her experience and expertise. Through this connection, I have an opportunity to know Prof. Dr. Banchob Sripa, a liver fluke expert who accompanied me to the field study in Khon Kean and shared his experiences on how to implement the community-based study on cholangiocarcinoma in the endemic areas. Also I will never forget the fantastic hospitality from the Tropical Disease Research Khon Kean, Thailand during my visit in Khon Kean.

Respectful thanks go to all scientists from Swiss TPH who shared with me their academic excellence, in particularly Prof. Nino Künzli, head of Department of Public health and epidemiology Prof. Christian Lengeler, Dr. Daniel Mäusezahl, Dr. Peter Steinmann. I acknowledge PD Dr. Penelope Vounatsou, Dr. Jan Hattendoff, Drs. Laura and Dominic Gosoniu for statistical advice whenever I needed help.

I am deeply indebted to the secretary team at Swiss TPH, led by Christine Mensch, Margrit Slaoui, Chirstine Walliser, Susi Gyoerffy and Maya Zwygart. Without their kind support, my stay in Basel and my trips back and forth from Laos to Switzerland would have been really difficult.

Big thanks go to my colleagues, followed by Dr. Khampheng Phongluxa, Dr. Somphou Sayasone, Dr. Youthanavanh Vonghachack, Dr. Dalouny Buakhasith, Dr. Virasack Rajpho, Dr. Tippi Mak, Leonore Lovis, Dr. Sakountala Binbandith, Dr. Vilavanh Xayaseng, Dr. Syda Sayavong, for their kind effort and for sharing all the good and difficult moments together in the field.

I wish to thank the IT and library team at Swiss TPH for their kind assistance on any technical issues related to software, database and books, journals, references, computer and so on.

I am very happy and enjoyed my PhD with all my dear friends from Swiss TPH: Mercy Ackumey, Nadine Schur, Virak Khieu, Sarah Rajkumar, Stella Hartinger, Pham-Duc Phuc, Nadine Schur, Claudia Schmutz, Stephanie Mauti, Fabian Schär, Phillip Bless, Christian Schaetti, Isabelle Grilli, Susan Rumisha, Verena Jurgens, Sandro Schmidlin, Thomas Fuerst, Angelina Lutambi, Lu Shang, Joldoshbek Kasymbekov, Vasudeo Paralika, Karin

Gross, Judith kahama, Angel Dillip, Bernadett Huho, Dr. Hung Nguyen Viet, Damiano Urbinello, Ombek Amek and Simon Kasasa. I would like to extend my appreciation to Dr. Sophie Odermatt-Biays for her great hospitality in Basel. My gratitude goes to my dear close friends, notably, Dr. Viengmany Boukham, Dr. Koukeo Phommasone, Dr. Phetsavanh Chanthavilay, Dr. Vilada Chansamouth, Dr. Viengvaly Phommanivong, Dr. Viraseng Rajpho, Dr. Anan Vannavong, Dr. Phonepadith Xangsayarath, Dr. Daovieng Douangvichit, Dr. Vilaysone Rajpho, Dr. Vatthanaphone Latthaphasaveng, Dr. Sivilay Thammasack, Dr. Sackmone Sychaleun without whose love, encouragement, I would not have finished this thesis.

Special thanks to my family, especially my mum Thoum Soukhathammavong, my auntie Khamphouang Soukhathammavong and my uncle Souvanh Sananikhom who dedicated their life and led me through this Ph.D. I sincerely thank my family in Laos, France and Australia who always support and encourage me. I thank my beloved husband, Raffael Ayé, who has contributed a lot all along my thesis and precise subediting whenever I need help.

I recognize that these research studies would not have been possible without the financial support granted by various funders and institutions. I would like to express my sincere thank to University of Basel, the City of Basel, the Swiss National Science Foundation and the Swiss Agency for Development and Cooperation (project no. 3270B0-110020), the commission of research partnership with developing countries (KFPE), the UNDP/WB/WHO Special Programme for Research and Training in Tropical Diseases, the Joint Small Grants Programme for Operational Research in Communicable Diseases, Manila, Philippines (2010/92660-0), and the Rudolf Geigy Foundation.

Lastly, I offer my regards and blessings to all of those who supported me in any respect during the completion of this thesis.

II. Summary

Background. Opisthorchiasis, schistosomiasis and soil-transmitted helminthiasis (STH) caused by *Opisthorchis viverrini*, *Schistosoma mekongi* and hookworm are neglected diseases in Southeast Asia. An estimated 40 million people are infected with opisthorchiasis, 207 million with schistosomiasis and more than 740 million individuals are infected with hookworm worldwide. There is growing recognition of substantial morbidity and mortality induced by these parasites. Nevertheless, the burdens of trematodes and hookworm are difficult to gauge due to their long subclinical disease and often insidious morbidity. While Cholangiocarcinoma (CCA) has been attributed to chronic *O. viverrini*, *S. mekongi* is the main cause of portal hypertension, liver cirrhosis and oesophageal varices. Hookworm infections are responsible for iron-deficiency anemia, physical and cognitive retardation, premature newborns, and low birth weights in at-risk populations (i.e., pre- and school-aged children; pregnant women). While *O. viverrini* and *S. mekongi* are mainly endemic in the central and southern Lao PDR, STH are ubiquitous, especially infections with hookworm, which is the most common STH species in Lao PDR. Health education on changing human eating behaviour, access to clean water as well as hygiene and sanitation remain challenging.

No evidence for the appropriate interval between community-wide praziquantel treatments against *O. viverrini*, *S. mekongi* and mixed infections are available. Although regular deworming programmes have been emphasized as a pillar of helminth control in Lao PDR for a few decades already, the importance of quality drugs is still neglected. There is an urgent need for safe and efficacious drugs against helminth infections.

***Aim and Objectives*.** This Ph.D. had the aim to deepen our understanding on the importance of helminthic parasitic infections in Lao PDR and challenges of their treatments by documenting severe morbidity due to parasitic infections in Lao PDR and assessing the effects of available and new treatments on infection and morbidity status of common helminth infections and pursued three main objectives. First, to document severe morbidity of parasitic infection in Lao PDR: *Capillaria philippinensis* and *S. mekongi*. Second, to assess the burden of parasitic infections due to *S. mekongi*, *O. viverrini* and co-morbidity of *S. mekongi* and *O. viverrini* and resolution of morbidity after treatment. Third, to assess efficacy of present anthelmintic drugs and potentially new drug candidates.

Methods. All data of this Ph.D were obtained from two major epidemiological settings: community and hospital-based studies. The case series reports were identified from community and hospital-based studies. Three cases of *Capillaria philippinensis* were identified from 2005 to 2007 in three referral hospitals (provincial hospital, Savannakhet and Mahosot and Sethathirath hospitals, Vientiane capital, Lao PDR). Severe *S. mekongi* cases were obtained from community helminth survey on Khong district from 2006-2010. Detailed clinical, micro-biological and imaging technique examinations, including ultrasound (US) and intestinal aspiration or biopsy through endoscope (*C. philippinensis*) were performed at baseline and during yearly follow-up (*S. mekongi*).

The cross-sectional study on morbidity due to *S. mekongi* and co-infections with *O. viverrini* was conducted in three villages, Khong district, Champasak province from March 2006 to March 2008. The participants were individuals who were infected with *S. mekongi* over the age of 6 months. Infection status and infection intensity were determined by 3 Kato-Katz thick smears (3 stool samples examined with single Kato-Katz each). The individuals were given a single dose of 40 mg/kg praziquantel (PZQ) and/or Albendazole (ABZ) at the baseline. At midpoint (at 11 months since the last praziquantel) and endpoint (at 23 months post treatment) of the morbidity assessment, patients were evaluated by stool and ultrasound examination. Those individuals who completed at least 2 Kato-Katz and the US examinations at each follow-up were included at the final evaluation. Futhermore, the morbidity induced by *O. viverrini* was addressed with two sub-studies: the community- and hospital-based studies carried out in 2011. a community-based, cross-sectional study in a high prevalence area for *O. viverrini* in which adult community members with parasitologically confonfirmed O. viverrini infection was screened for hepatobiliary morbidity, including suspected lesions of CCA using ultrasonography. Additional data on risk factors etc. were obtained from each study participant. The second hospital-based study was carried out in six referral hospitals across country (Laungprabang, Vientiane capital, Savannakhet, Champasack). All medical records of patients admitted from 2006 to 2010 in Lao PDR included in the hospital-based, retrospective study in which suspected cases of CCA were identified in patient records of all referral hospital in Lao PDR.

A randomized open-label two-arm trial was assessed the efficacy of single albendazole and mebendazole in Bachieng, Champasack province, southern Laos. In total, 200 schoolchildren infected with hookworm (determined by quadruplicate Kato-Katz thick smears derived from two stool samples) were randomly assigned to albendazole (n=100) and mebendazole (n=100) in Bachieng district, Champasak province in 2006. The second study was a randomized, exploratory open-label trial assessing the efficacy and safety mefloquine (25 mg/kg), artesunate (10 mg/kg as 3 split doses within 12 h), mefloquine-artesunate (100 mg artesunate plus 250 mg mefloquine once daily for 3 consecutive days), and tribendimidine (200 or 400 mg single dose) compared to praziquantel (75 mg/kg in 2 divided doses) in schoolchildren, in Attapeu, *O. viverrini*-endemic areas, southern Laos. Primary outcomes of both clinical trials studies were cure rates and egg reduction rates at 21-23 days posttreatment. Adverse events were assessed at 3h, 48h, and 120h after treatment.

***Principle findings/results*.** Our case series report on *C. philippinensis* was conducted at the referral hospital in Savannakhet and Vientiane. The three patients were unrelated previously healthy young men (24-27 years of age). Chronic diarrhea, abdominal pain, edema, and severe weight loss were the symptoms reported. Two of them acquired the infection in Thailand; one patient had acquired it indigenously in Lao PDR. Co-infections with trematode and nematodes were found. All patients recovered with albendazole treatment (400 mg/day for a month). The study on severe *S. mekongi* cases indentified nine patients. Male was the predominant sex, the mean age was 36 years. All patients had dwelled in Khong Island since birth and had previously been treated with several praziquantel rounds. Most of the patients developed hepato-splenic diseases. At the final follow-up, three patients improved, two adult patients remained unchanged or reversal after treatment. Two patients died due to rupture of oesophageal varices. Two were lost to follow-up. Liver regression improved after treatment in particular in young patients.

The study on morbidity due to *S. mekongi* and co-infections with *O. viverrini* showed that there were significant changes after therapy such as reversals of periportal fibrosis among mild and moderate cases ($p<0.001$), as well as decreased size of the left liver lobe, the spleen and splenic veins ($p<0.01$). However, re-infection was common at 11 months after therapy. The community- and hospital-based studies indicated a high

prevalence of hepatobiliary diseases attributable to *O. viverrini* infection. Of 431 individuals with parasitologically confirmed *O. viverrini* infections screened during the community-based ultrasound study, five cases demonstrated lesion suggestive of CCA. Other hepatobiliary lesions were detected (gall bladder and kidney anormalities fatty liver, cirrhosis and so on). In the hospital-based study in *O. viverrini* endemic areas of Laos, lesions suggestive of CCA were found by means of US and CT scan in 274 patients admitted. Males and people aged 50 years and above were more commonly diagnosed with suspected CCA. Of 274 suspected CCA cases, 267 (97.4%) had dilated bile duct, 240 (74.1%) had gallstone(s) or intrahepatic stone(s), but only 33 (12%) were parasitologically confirmed *O. viverrini* cases. Histopathology is not available in Laos and consequently, we could not have the final diagnosis of CCA.

Compared to a recent meta-analysis, single dose of ABZ and MBZ resulted in disappointing efficacy against hookworms in terms of cure rates in our study (36.0% and 17.6% respectively). But single-dose of ABZ cleared significantly more hookworm infections than mebendazole with ERR of 86.7% and 76.3%, respectively. In addition, both ABZ and MBZ showed effects against *O. viverrini* with high egg reduction rates. In the second clinical trial, single dose of tribendimidine at the recommended dosage for the treatment of STH infections achieved higher cure and egg reduction rates compared to PZQ (CRs=70.4%, 56.0% and ERRs= 99.3%, 98.4%, respectively) in schoolchildren infected with *O. viverrini*. No or only very moderate effects were observed with mefloquine, artesunate and mefloquine-artesunate against *O. viverrini*. All children experienced drug-induced adverse events. The most frequent adverse events were among those patients treated with mefloquine and mefloquine-artesunate. Only mild and transient adverse events were observed in the tribendimidine group.

Conclusion/significance. The case reports raises awareness of the re-emergence of *S. mekongi* and potential risk of outbreak of *C. philippinensis* in Lao PDR. Hence, These parasites require more attention. Substantial *S. mekoni*-, *O. viverrini*- and coinfection-induced morbidity were present and caused various hepato-biliary and -splenic lesions, including lesion suggestive of CCA, liver cirrhosis and oesophageal varices. Monitoring on efficacy of current drugs used for mass drug administration campaigns (ABZ, MBZ and Praziquantel) is needed. Tribendimidine could be a potential drug of choice for liver fluke treatment as well as other parasitic infections.

III. Zusammenfassung

Hintergrund. Opisthorchiasis, Schistosomiasis und durch Bodenkontakt übertragene Helminthiasis (STH, Wurmerkrankungen) werden durch *Opisthorchis viverrini*, *Schistosoma mekongi* und Hakenwürmer und anderen verursacht und gehören zu den vernachlässigten Krankheiten. Geschätzte 40 Millionen Menschen sind weltweit mit *Opisthorchis* infiziert, 207 Millionen mit *Schistosoma* und mehr als 740 Millionen mit Hakenwürmern. Zunehmend wird die beachtliche Morbidität und Mortalität, die durch diese Parasiten verursacht wird, anerkannt. Nichtsdestotrotz ist die durch Trematoden und Hakenwürmer verursachte Krankheitslast aufgrund der langen subklinischen Erkrankung und unterschwelligen Morbidität schwierig abzuschätzen. Während Cholangiokarzinoma (CCA) chronischen Infektionen mit *O. viverrini* zugeschrieben wird, ist *S. mekongi* ein wichtigster Kausalfaktor für Pfortaderhochdruck, Leberzirrhosen und Ösophagus-Varizen.

Hakenwurminfektionen sind für Eisenmangelanämie, physische und kognitive Entwicklungsverzögerung, Frühgeburten und geringe Geburtsgewichte in gefährdeten Bevölkerungsschichten (Kinder im Vorschul- und Schulalter, schwangere Frauen) verantwortlich. Während *O. viverrini* und *S. mekongi* mehrheitlich in Zentral- und Süd-Laos endemisch sind, sind STH allgegenwärtig, insbesondere Infektionen mit dem Hakenwurm, der häufigsten STH-Art in Laos. Gesundheitskampagnen zur Änderung der Essgewohnheiten, Zugang zu sauberem Wasser sowie Hygiene bleiben Herausforderungen.

Regelmässige Behandlungen mit Praziquantel ist die wichtigste Bekämpfung-massnahme gegen *S. mekongi* und *O. viverrini.* Jedoch bis heute gibt es keine Angaben für das am Besten geeignete Intervall zwischen Praziquantel-Behandlungen. Obwohl regelmässige Entwurmungsprogramme seit mehreren Jahrzehnten als eine Stütze der Helminthen-Kontrolle in durchgeführt werden, wird die Überwachen der Effizienz Medikamenten vernachlässigt. Sichere und wirksame Medikamente gegen Helminthen-Infektionen werden dringend benötigt.

Ziele. Diese Doktorarbeit hatte das Ziel, unser Verständnis der Bedeutung von Parasiteninfektionen in Laos und Schwierigkeiten in ihrer Behandlung zu verbessern, in dem sie die schwere Morbidität aufgrund der Parasiteninfektionen dokumentiert und die Wirkung von bisher verfügbaren und neuen Behandlungen auf Infektion und Morbidität untersucht. Sie verfolgte deshalb drei Ziele. Erstens, die schwere Morbidität

aufgrund von Parasiteninfektionen, nämlich *Capillaria philippinensis* und *S. mekongi* in Laos zu dokumentieren. Zweitens, die Krankheitslast der Parasiteninfektionen aufgrund von *S. mekongi*, *O. viverrini* und Mischinfektionen der beiden Parasiten zu dokumentieren sowie die Besserung der Morbidität nach der Behandlung zu messen. Drittens, die Wirksamkeit aktuell erhältlicher Medikamente und neuer Medikamenten-Kandidaten zu untersuchen.

***Methoden*.** Alle Daten für diese Doktorarbeit wurden in zwei epidemiologischen Milieus gesammelt: Studien in der Bevölkerung und in Krankenhäusern. Drei Fälle von *Capillaria philippinensis* wurden zwischen 2005 und 2007 in drei Referenzkrankenhäusern identifiziert (Provinzkrankenhäuser, sowie die Krankenhäuser Savannakhet, Mahosot und Sethathirath, in Vientiane). Schwere Fälle von *S. mekongi* wurden zwischen 2006 und 2010 währen einer Querschnittsstudie im Distrikt Khong gefunden. Detaillierte klinische, mikrobiologische und Ultraschall-Untersuchung (Endoskop-Biopsie für *C. philippinensis*) wurden zu Beginn und bei der jährlichen Kontrolle durchgeführt (*S. mekongi*).

Die Querschnittsstudie gefolgt von einer Kohort Studie zur Morbidität von *S. mekongi* und *O. viverrini* Infektionen wurde in drei Dörfern im Khong Distrikt, Provinz Champasak, zwischen März 2006 und März 2008 durchgeführt. Die Studienteilnehmer waren mit *S. mekongi* infiziert und über 6 Monate alt. Die Infektion und ihre Intensität wurden mit 3 Kato-Katz-Ausstrichen (3 Stuhlproben mit jeweils einmaligem Kato-Katz) bestimmt. Die Studienteilnehmer erhielten zu Beginn der Studie eine Einzeldosis von 40mg/kg Praziquantel (PZQ) und/oder Albendazole (ABZ). Zur Halbzeit (11 Monate nach der Behandlung mit PZQ) und am Ende der Studie (23 Monate nach der Behandlung) wurden die Patienten mit einer Stuhlprobe und einer Ultraschalluntersuchung evaluiert. Diejenigen Teilnehmer, von denen zu jedem Untersuchungszeitpunkt mindestens 2 Kato-Katz-Ausstriche und die Ultraschalluntersuchung vorlagen, wurden in die Schlussanalyse einbezogen. Zudem wurde die durch *O. viverrini* verursachte Morbidität in zwei Teilstudien angegangen: den Studien in der Bevölkerung und in den Krankenhäusern, die 2011 durchgeführt wurden. In einer Querschnittstudie in der Saravan Provinz, in einem Gebiet mit hoher *O. viverrini* Prävalenz wurden alle erwachsenen Mitglieder der Dorfgemeinschaft mit parasitologisch bestätigter *O. viverrini*-Infektion auf Leber- Morbidität untersucht, im

speziellen auf mögliche CCA-Läsionen. Zusätzliche Daten über Risikofaktoren wurden von jedem Studienteilnehmer erhoben. Eine zweite Krankenhaus-basierte Studie wurde in 6 Referenzkrankenhäusern im ganzen Land (Luangprabang, Vientiane, Savannakhet, Champasack) durchgeführt. Vermutete CCA-Fälle wurden im Rahmen einer retrospektiven Studie in allen Patientenakten von Patienten, die von 2006 bis 2010 in diesen Referenzkrankenhäusern hospitalisiert worden waren, identifiziert.

Eine randomisierte Studie mit zwei Behandlungsgruppen ohne Verblindung untersuchte 2006 die Wirksamkeit einer Einzeldosis von Albendzole (ABZ) und Mebendazole (MBZ) in Bachieng, Provinz Champasack, Süd-Laos. Insgesamt 200 Hakenwurm-infizierte Schulkinder (durch vierfachen Kato-Katz-Ausstrich basierend auf zwei Stuhlproben bestimmt) wurden zufällig ABZ (n=100) oder MBZ (n=100) zugeordnet. Eine zweite Studie war eine randomisierte, explorative Studie, welche die Wirksamkeit und Sicherheit von Mefloquine (25mg/kg), Artesunat (10mg/kg in 3 getrennten Dosen innerhalb 12 Std), Mefloquine-Artesunat (100mg Artesunat und 250mg Mefloquine einmal täglich während drei aufeinanderfolgenden Tagen) und Tribendimidine (200mg oder 400mg als Einzeldosis) im Vergleich zu Praziquantel (75mg/kg in 2 Dosen) in Schulkindern, in Attapeu, Süd-Laos, untersuchte. Primärergebnisse beider klinischen Studien waren die Heilungsrate und die Eizahl-Reduktionsrate 21-23 Tage nach der Behandlung. Nebenwirkungen wurden 3 Std., 48 Std. und 120 Std. nach der Behandlung untersucht.

Wichtigste Ergebnisse. Unsere Fallreihe von *C. philippinensis* wurde in den Referenzkrankenhäusern von Savannakhet und Vientiane durchgeführt. Die drei Patienten waren nicht verwandte, vormals gesunde junge Männer (24-27 Jahre alt). Die genannten Symptome waren chronischer Durchfall, Bauchschmerzen, Ödema und starker Gewichtsverlust. Zwei von ihnen hatten die Infektion in Thailand aufgelesen; ein Patient hatte sie in Laos angesteckt. Es wurden Koinfektionen mit Trematoden und Nematoden gefunden. Alle Patienten erholten sich unter ABZ-Behandlung (400mg/Tag während 30 Tagen).

In der Reihe von schweren *Schistosoma*-Fällen wurden neun Patienten gefunden. Das vorherrschende Geschlecht war männlich, das durchschnittliche Alter 36 Jahre. Alle Patienten hatten seit ihrer Geburt auf der Khong Insel gelebt und waren bereits zuvor mehrmals mit PZQ behandelt worden. Die meisten Patienten zeigten schwere Leber-

Milz-Pathologien. Bei der letzten Kontrolle waren drei Patienten in besserem Zustand, zwei erwachsene Patienten zeigten unveränderten Zustand oder Rückfall nach Behandlung. Zwei Patienten starben im Verlaufe der Untersuchung aufgrund geplatzter Ösophagus-Varizen. Zwei Patienten konnten nicht mehr gefunden werden. Die Leberpathologien verbesserten sich nach der Behandlung insbesondere in jungen Patienten.

Die Studie der Morbidität aufgrund von *S. mekongi* und Koinfektion mit *O. viverrini* zeigte signifikante Unterschiede nach der Behandlung, wie Heilung periportaler Fibrosen bei leichten und mittelschweren Befall ($p<0.001$) sowie verkleinerte linke Leberlappen, Milz und Kolateralevenen ($p<0.01$). Allerdings wurden Neuinfektionen 11 Monaten nach der Behandlung beobachtet. Die Studien in der Saravabne Bevölkerung und in den Krankenhäusern deuteten auf eine hohe Prävalenz von Leberpathologien hin, welche *O. viverrini* zugeschrieben werden können. Von 431 Patienten mit parasitologisch bestätigter *O. viverrini* Infektion, die in der Ultraschall-Studie in der Bevölkerung untersucht wurden, wiesen fünf Fälle Läsionen auf, die auf CCA deuteten. Weitere Läsionen wurden ebenfalls entdeckt (Gallblasen- und Nieren-Anomalitäten, überfettige Leber, Zirrhose usw.). In der Krankenhaus-basierten Studie in Gebieten in Süd-Laos, in denen *O. viverrini* endemisch ist, wurden mithilfe von Ultraschall und Computertomographie bei 274 Patienten Läsionen gefunden, die auf CCA deuten. Männer und Patienten im Alter von 50 Jahren und mehr wurden gehäuft mit vermutlichem CCA diagnostiziert. Von 274 vermuteten CCA-Fällen hatten 267 (97.4%) einen erweiterten Gallengang, und 240 (74.1%) hatten Gallensteine, aber nur 33 (12%) waren parasitologisch bestätigte *O. viverrini*-Fälle. Histopathologie ist in Laos nicht verfügbar und folglich konnten wir keine definitive Diagnose von vermuteten CCA-Fällen erstellen.

Verglichen mit einer kürzlichen Meta-Analyse, führten Einzeldosen von ABZ und MBZ in unserer Studie zu enttäuschend niedriger Wirksamkeit gegen Hakenwürmer (Heilungsrate 36.0% respektive 17.6%). Aber die Einzeldosis ABZ heilte signifikant mehr Hakenwurm-Infektionen als MBZ mit einer Eier-Reduktionsrate von 86.7% respektive 76.3%. Zusätzlich zeigten ABZ und MBZ Wirkung gegen *O. viverrini* mit hohen Eizahl-Reduktionsraten. In der zweiten klinischen Studie, bewirkte eine Einzeldosis Tribendimidine in der für die Behandlung von STH empfohlenen Dosierung

verglichen mit Praziquantel hohe Heilungs- und Eier-Reduktionsraten (HR=70.4% bzw. 56.0% und ERR=99.3% bzw. 98.4%) in Schulkindern, die mit *O. viverrini* infiziert waren. Keine oder nur sehr mittelmässige Wirkung wurden mit Mefloquin, Artesunat und Mefloquin-Artesunat gegen *O. viverrini* erreicht. Alle Kinder litten unter negativen Nebenwirkungen. Die häufigsten negativen Nebenwirkungen traten in den Patienten auf, die mit Mefloquin und Mefloquin-Artesunat behandelt wurden. Nur leichte und vorübergehende Nebenwirkungen wurden in der Tribendimidine-Behandlungsgruppe beobachtet.

Schlussfolgerung. Die Fallserien erhöhen das Bewusstsein für das Widerauftreten von *S. mekongi* und das potenzielle Risiko eines Ausbruchs von *C. philippinensis* in Laos. Deshalb benötigen Capillariasis und Schistosomiasis mehr Aufmerksamkeit. Substanzielle Morbidität aufgrund von *S. mekongi* und *O. viverrini* war vorhanden und verursachte verschiedene Leber-Gallen- und Leber-Milz-Läsionen, einschliesslich Läsionen, die auf CCA hindeuten. Ein Monitoring der Wirksamkeit der gebräuchlichen Medikamente, die für Massenbehandlungs-kampagnen verwendet werden (ABZ, MBZ und PZQ) ist nötig. Tribendimidine könnte ein potenzielles Medikament der Wahl für die Behandlung von Leberegel und anderen Parasiteninfektionen werden.

IV. Lao Summary

ປະຫວັດຄວາມເປັນມາ: ພະຍາດໃບໄມ້ໃນຕັບ, ພະຍາດໃບໄມ້ດູດເລືອດ, ແລະ ພະຍາດ ກາຝາກ ທີ່ຕິດ ຈາກໜ້າດິນ ຊຶ່ງແມ່ນສາຍເຫດເນື່ອງມາຈາກການຕິດເຊື້ອພະຍາດຊະນິດ *Opisthorchis viverrini, Schistosoma mekongi*, ແລະ ແມ່ທ້ອງປາກຂໍ ເປັນພະຍາດທີ່ຈັດຢູ່ໃນຈຳພວກທີ່ ບໍ່ໄດ້ຮັບ ການ ເອົາ ໃຈໃສ່ຢູ່ໃນພາກພື້ນອາຊີຕາເວັນອອກສ່ຽງໃຕ້. ໃນທົ່ວໂລກ ປະມານ 40 ລ້ານຄົນ ຕິດເຊື້ອ ພະຍາດໃບໄມ້ໃນຕັບ, 207 ລ້ານຄົນ ຕິດເຊື້ອພະຍາດໃບໄມ້ດູດເລືອດ, ແລະ ຫລາຍກວ່າ 740 ລ້ານ ຄົນຕິດເຊື້ອແມ່ທ້ອງປາກຂໍ.ຍິ່ງເປັນທີ່ຮັບຮູ້ກັນຫລາຍຂຶ້ນວ່າພະຍາດແມ່ກາຝາກດັ່ງກ່າວ ແມ່ນເປັນສາຍ ເຫດຕົ້ນຕໍສຳລັບການເຈັບເປັນ ແລະ ການຕາຍ. ແຕ່ເຖິງຢ່າງໃດກໍ່ຕາມພະຍາດ ໃບໄມ້ ແລະ ແມ່ທ້ອງປາກຂໍກໍຍັງເປັນເລື້ອງຍາກທີ່ຈະປະເມີນຄວາມຮຸນແຮງຂອງພະຍາດ ດັ່ງກ່າວ ໄດ້ຢ່າງຊັດເຈນ ເນື່ອງຈາກວ່າມັນເປັນພະຍາດທີ່ເຮັດໃຫ້ຮ່າງກາຍຊຸດໂຊມຍາວນານແລະສ່ວນຫຼາຍເປັນການເຈັບເປັນແ ບບມິດງຽບຫຼືເຊື່ອງຊ້ອນອາການ.ໃນຂະນະດຽວກັນນັ້ນຖ້າມີການຕິດເຊື້ອພະຍາດໃບໄມ້ໃນຕັບແບບຊ້ຳ ເຮື້ອແມ່ນມີໂອກາດທີ່ຈະເປັນມະເຮັງທໍ່ສິ່ງນ້ຳບີ,ແລະເຊັ່ນດຽວກັນກັບພະຍາດໃບໄມ້ດູດ ເລືອດ ຈະເປັນ ສາຍເຫດຕົ້ນຕໍພາໃຫ້ເກີດມີຄວາມດັນ ເລືອດຢູ່ໃນເສັ້ນເລືອດດຳໃນຕັບ (Portal vein), ຕັບແຂງ, ແລະເສັ້ນເລືອດຂອດທາງທໍ່ເດີນອາຫານ.ການຕິດເຊື້ອແມ່ທ້ອງປາກຂໍແມ່ນຈະກໍ່ໃຫ້ເກີດເປັນ ພະຍາດ ເລືອດຈາງຍ້ອນຂາດທາດເຫລັກ, ການຂາຍຫຍາຍຕົວທາງຮ່າງກາຍແລະມັນສະໝອງຊັກຊ້າ, ເດັກເກີດ ກ່ອນກຳນົດ ແລະ ມີນ້ຳໜັກຫລຸດ ຢູ່ໃນກຸ່ມປະຊາກອນທີ່ມີຄວາມສ່ຽງ (ເຊັ່ນ ເດັກກ່ອນໄວຮຽນ ແລະໃນໄວຮຽນຊັ້ນປະຖົມແລະແມ່ຍິງຖືພາ).ພະຍາດໃບໄມ້ໃນຕັບແລະພະຍາດໃບໄມ້ດູດເລືອດສ່ວນ ຫລາຍແມ່ນລະບາດຢູ່ພາກກາງແລະພາກໃຕ້ສ່ວນພະຍາດກາຝາກທີ່ຕິດມາຈາກໜ້າດິນ ໂດຍສະເພາະ ແມ່ທ້ອງປາກຂໍແມ່ນພົບເຫັນຢູ່ທຸກບ່ອນໃນຂອບເຂດທົ່ວປະເທດ.ການສຸຂະສຶກສາເພື່ອປ່ຽນແປງພຶດຕິ ກຳການກິນ, ການເຂົ້າເຖິງນ້ຳສະອາດ ເຊັ່ນດຽວກັນກັບ ການອະນາໄມ ແລະ ສຸຂະອະນາໄມ ຍັງເປັນສິ່ງທີ່ທ້າທາຍ. ປະຈຸບັນຍັງບໍ່ທັນມີຂໍ້ມູນທີ່ເປັນຫລັກຖານພິສູດກ່ຽວກັບການປິ່ນປົວທົ່ວປວງຊົນ ທີ່ຖືກຕ້ອງເໝາະສົມຊຶ່ງນຳໃຊ້ຢາປລາຊິກັງແຕນປິ່ນປົວພະຍາດໃບໄມ້ໃນຕັບ, ພະຍາດໃບໄມ້ດູດເລືອດ ແລະ ການຕິດເຊື້ອແບບປະສົມທັງສອງຊະນິດ.

ເຖິງແມ່ນວ່າ ຢູ່ ສປປ ລາວ ການຢາຍຢາປິ່ນປົວພະຍາດແມ່ກາຝາກທົ່ວປວງຊົນໄດ້ຖືກ ຍົດຖືເປັນ ກິດຈະກຳຕົ້ນຕໍໃນການຄວບຄຸມພະຍາດກາຝາກຢູ່ໃນປະເທດມາໄດ້ຫລາຍທົດສະວັດ ແລ້ວ ກໍ່ຕາມ ແຕ່ຄວາມສຳຄັນດ້ານຄຸນນະພາບຂອງຢາຍັງບໍ່ທັນໄດ້ຮັບການເອົາໃຈໃສ່. ສະນັ້ນ ຈຶ່ງ ເຫັນວ່າມີຄວາມ ຈຳເປັນອັນຮີບດ່ວນທີ່ຈະຕ້ອງປະເມີນຄວາມປອດໄພ ແລະ ຄຸນນະພາບຂອງຢາ ທີ່ໃຊ້ໃນການປິ່ນປົວ ພະຍາດແມ່ກາຝາກ.

ວັດຖຸປະສົງ: ໃນບົດຄວາມທີ່ກ່າວມາຂ້າງເທິງນີ້, ບົດນິພົນປະຣິນຍາເອກສະບັບນີ້ ໄດ້ມີ ສາມຈຸດ ປະສົງຫລັກຄື: 1.) ເພື່ອບັນທຶກຄວາມຮຸນແຮງຂອງພະຍາດແມ່ກາຝາກ ເຊັ່ນ ພະຍາດ ກາຝາກ ຊະນິດ *Capillaria philippinensis*, and *Schistosoma mekongi*; 2.) ປະເມີນ ຄວາມໜັກໜ່ວງຂອງພະ ຍາດ

ແມ່ກາຝາກທີ່ເນື່ອງມາຈາກການຕິດເຊື້ອກາຝາກຊະນິດ *O. viverrini*, *S. mekongi*, ແລະ ການຕິດເຊື້ອກາຝາກແບບປະສົມທັງສອງຊະນິດ ແລະ ເພື່ອຕິດ ຕາມຄວາມຜິດປົກກະຕິພາຍຫລັງ ການປິ່ນປົວ, ແລະ 3.) ເພື່ອປະເມີນປະສິດທິຜົນຂອງຢາປິ່ນປົວ ພະຍາດແມ່ກາຝາກໃນປະຈຸບັນ ກັບ ຕົວຢາໃໝ່.

ວິທີວິທະຍາການຄົ້ນຄວ້າ. ຂໍ້ມູນທັງໝົດທີ່ຢູ່ໃນບົດນິພົນສະບັບນີ້ແມ່ນໄດ້ມາຈາກແຫລ່ງຂໍ້ມູນດ້ານ ລະ ບາດວິທະຍາສອງແຫລ່ງຕົ້ນຕໍ ເຊັ່ນ ການສຶກສາໃນຊຸມຊົນ ແລະ ໂຮງໝໍ ຊຶ່ງກວມເອົາບົດລາຍງານ ກ່ຽວກັບການເກີດພະຍາດຂອງພະຍາດໃບໄມ້ໃນຕັບ, ພະຍາດ ໃບໄມ້ດູດເລືອດ ແລະ ການຕິດເຊື້ອ ແບບປະສົມທັງສອງຊະນິດ, ແລະ ການປະເມີນປະສິດ ທິຜົນຂອງຢາປິ່ນປົວພະຍາດ ແມ່ກາ ຝາກ ປາກ ຂໍໃນປະຈຸບັນ ແລະ ຕົວຢາໃໝ່ເພື່ອໃຊ້ເຂົ້າ ໃນການປິ່ນປົວພະຍາດໃບໄມ້ໃນຕັບ ແລະ ພະຍາດ ແມ່ກາຝາກທົ່ວໄປ.

ບົດລາຍງານກໍລະນີສຶກສາແມ່ນໄດ້ມາຈາກຊຸມຊົນ ແລະ ໂຮງໝໍ. ການສຶກສາໃນສາມ ກໍລະນີ ທີ່ຕິດເຊື້ອແມ່ກາຝາກຊະນິດ *Capillaria philippinensis* ທີ່ພົບເຫັນຢູ່ໃນໂຮງ ໝໍແຂວງ ຄື ແຂວງ ສະຫວັນນະເຂດ ແລະ ໂຮງໝໍສູນກາງ ເຊັ່ນ ໂຮງໝໍມະໂຫສົດ, ແລະ ເສດຖາ ທິລາດ ນະຄອນຫລວງ ວຽງຈັນ ໃນລະຫວ່າງປີ 2005-2007. ການສຶກສາກ່ຽວກັບພະຍາດ ໃບໄມ້ດູດເລືອດທີ່ຕິດເຊື້ອຮຸນແຮງ ແມ່ນໄດ້ມາຈາກການສຳຫລວດຢູ່ໃນຊຸມຊົນ ຊຶ່ງໄດ້ຈັດຕັ້ງ ປະຕິບັດໃນປີ 2006-2010. ລາຍລະອຽດ ຂອງການສຶກສາດ້ານອາການສາດ, ການກວດຈຸລິນຊີ ວິທະຍາ (Microbiology examination), ການກວດລັງສີວິທະຍາຊຶ່ງລວມມີການກວດລັງສີໂດຍ ໃຊ້ຄື້ນສຽງ, ແລະ ການດູດເອົາຊິ້ນສ່ວນ ອະ ໄວຍະວະໄປວິໄຈ (endoscope), ແລະ ການປິ່ນປົວ ພະຍາດກາຝາກຊະນິດ *Capillariasis Philippinenses*, ຊຶ່ງທັງໝົດເຫລົ່ານີ້ແມ່ນໄດ້ເກັບຂໍ້ມູນ ເບື້ອງຕົ້ນ (Baseline survey) ແລະ ຕິດຕາມ ຄົນເຈັບທີ່ເປັນພະຍາດໃບໄມ້ດູດເລືອດໃນແຕ່ລະປີ (yearly follow-up of schistosomiasis cases).

ການສຶກສາແບບຕັດຂວາງ ກ່ຽວກັບການເຈັບເປັນທີ່ເນື່ອງມາຈາກການຕິດເຊື້ອ *S. mekongi* ແລະ ການຕິດເຊື້ອປະສົມກັບ *O. viverrini* ຊຶ່ງໄດ້ດຳເນີນຢູ່ໃນສາມບ້ານຂອງ ດອນໂລງ, ເມືອງໂຂງ, ແຂວງ ຈຳປະສັກ ແຕ່ເດືອນ ມີນາ 2006 ເຖິງ ມີນາ 2008. ຜູ້ທີ່ເຂົ້າຮ່ວມໃນການສຶກສາແມ່ນຜູ້ທີ່ຕິດເຊື້ອ ພະ ຍາດໃບໄມ້ດູດເລືອດຊຶ່ງມີອາຍຸຕ່ຳກວ່າ 06 ເດືອນ, ສະພາບຫຼື ຄວາມຮຸນແຮງຂອງການຕິດເຊື້ອແມ່ນ ໄດ້ມາຈາກການກວດອາຈົມ ໂດຍໃຊ້ເຕັກນິກກາໂຕກາສ (ສາມແຜ່ນກາໂຕກາສຕໍ່ຕົວຢ່າງອາຈົມ 01 ອັນ). ບຸກຄົນທີ່ຕິດເຊື້ອພະຍາດໃບໄມ້ໃນຕັບແມ່ນໄດ້ຮັບການປິ່ນປົວດ້ວຍຢາປລາຊິກັງແຕນ 40ມກ ຕໍ່ກິໂລນ້ຳໜັກຮ່າງກາຍ ແລະ ຢາອານແບັນດາໂຊນ 400ມກ ໜຶ່ງເມັດ ຢູ່ໃນຊ່ວງຂອງການສຳຫລວດ ເບື້ອງຕົ້ນ.

ການສຳຫລວດໄລຍະທີ 2ໄດ້ດຳເນີນພາຍຫລັງທີ່ໄດ້ກິນຢາເປັນເວລາ 11 ເດືອນ, ແລະ ການສຳຫລວດ ຄັ້ງສຸດທ້າຍໄດ້ດຳເນີນພາຍຫລັງໄດ້ກິນຢາເປັນເວລາ 23 ເດືອນ. ການປະເມີນອັດຕາການເຈັບເປັນ

ແມ່ນໄດ້ກວດອາຈົມເພື່ອຊອກຫາພະຍາດໃບໄມ້ໃນຕັບ ແລະ ກວດດ້ວຍຄື້ນສຽງ. ສຳລັບບຸກຄົນທີ່ຖືວ່າສຳເລັດສົມບູນໃນການສຳຫລວດແຕ່ລະຄັ້ງແມ່ນຢ່າງໜ້ອຍຕ້ອງໄດ້ກວດອາຈົມ
ສອງສະໄລ ແລະ ຮັບການກວດເຄື່ອງຊ່ອງໄຟຟ້າທີ່ໃຊ້ຄື້ນສຽງ. ນອກຈາກນັ້ນ ໄດ້ດຳເນີນການສຶກສາຢູ່ຂັ້ນຊຸມຊົນ ແລະ ໂຮງພະຍາບານ ໃນປີ 2011 ເພື່ອຊອກຫາອັດຕາການເຈັບເປັນທີ່ເກີດຈາກພະຍາດໃບໄມ້ໃນຕັບ. ການສຶກສາຊຸມຊົນແມ່ນການສຶກສາແບບຕັດຂວາງ ເພື່ອຊອກຫາອັດຕາການຊຸກຊຸມຂອງການເປັນພະຍາດຢູ່ເຂດທີ່ມີການຕິດເຊື້ອຂອງພະຍາດໃບໄມ້ໃນຕັບສູງ ຊຶ່ງໃນນັ້ນບັນດາບຸກຄົນທີ່ເຂົ້າຮ່ວມການສຶກສາແມ່ນເປັນຜູ້ທີ່ຕິດເຊື້ອພະຍາດໃບໄມ້ໃນຕັບ ແລະໄດ້ດຳເນີນການກວດເພື່ອຊອກຫາອັດຕາການເຈັບເປັນໃນທໍ່ນ້ຳບີ ດ້ວຍການກວດຊ່ອງໄຟຟ້າຄື້ນສຽງ. ນອກຈາກນັ້ນ ຍັງໄດ້ລວບລວມຂໍ້ມູນດ້ານປັດໃຈສ່ຽງຕ່າງໆ ຂອງການນຳໄປສູ່ການຕິດເຊື້ອໂດຍໃຊ້ແບບສອບຖາມຜູ້ທີ່ຕິດເຊື້ອພະຍາດດັ່ງກ່າວນັ້ນ. ການສຶກສາຢູ່ໂຮງພະຍາບານໄດ້ດຳເນີນຢູ່ 06 ແຫ່ງ ໃນທົ່ວປະເທດ ຄື ນະຄອນຫລວງວຽງຈັນ, ຫລວງພະບາງ, ສພຫວັນນະເຂດ, ແລະ ຈຳປະສັກ. ຊຶ່ງໃນນັ້ນໄດ້ເກັບຂໍ້ມູນຍ້ອນຫລັງຂອງຜູ້ປ່ວຍທີ່ສົງໄສວ່າເປັນມະເຮັງທໍ່ສິ່ງນ້ຳບີທີ່ເຂົ້ານອນປິ່ນປົວຢູ່ໂຮງໝໍດັ່ງກ່າວ ລະຫວ່າງ ປີ 2006-2010 ຢູ່ ສປປ ລາວ.
ການທົດລອງດ້ວຍການຊຸ່ມເລືອກແບບເປີດເຜີຍ (randomized open label) ໃນກຸ່ມທົດລອງສອງກຸ່ມ ເພື່ອປະເມີນປະສິດທະພາບຂອງການນຳໃຊ້ຢາ ອານແບັນດາໂຊນ ແລະ ເມແບັນດາໂຊນ ຢູ່ເມືອງບາຈຽງ ແຂວງ ຈຳປະສັກ ໃນປີ 2009. ໃນເດັກນັກຮຽນ ຈຳນວນ 200 ຄົນ ທີ່ຕິດເຊື້ອພະຍາດປາກໍ ຊຶ່ງກວດພົບໂດຍໃຊ້ເຕັກນິກການກວດອາຈົມແບບກາໂຕກາສ ໄດ້ຊຸ່ມໃຫ້ຢາອານແບັນດາໂຊນ 100 ຄົນ ແລະ ເມແບັນດາໂຊນ 100 ຄົນ.
ການສຶກສາທີ່ສອງແມ່ນການສຶກສາໂດຍນຳໃຊ້ວິທີການສຸ່ມທົດລອງແບບເປີດເຜີຍ ສຳລັບການປະເມີນປະສິດທິພາບ ແລະ ຄວາມປອດໄພຂອງຢາ Mefloquine (25mg/kg), artesunate (10mg/kg ໃຫ້ສາມຄັ້ງພາຍໃນ 12 ຊມ), ຢາປະສົມ Mefloquine ແລະ artesunate (artesunate 10mg ກັບ Mefloquine 250mg 1 ຄັ້ງ ຕໍ່ມື້ ໃນສາມມື້ຕິດຕໍ່ກັນ ເພື່ອສົມທຽບກັບຢາ ປລາຊິກັງແຕັນ (75ມກ ຕໍ່ກິໂລນ້ຳໜັກຮ່າງກາຍ ແບ່ງເປັນ ສອງຄາບ) ໃນເດັກນັກຮຽນມັດທະຍົມທີ່ແຂວງອັດຕະປື, ສປປ ລາວ. ເຫັນວ່າເຂດດັ່ງກ່າວແມ່ນມີການຕິດເຊື້ອສູງ. ຜົນການວິໄຈຂອງທັງສອງແບບທົດລອງໄດ້ນຳນິດເອົາອັດ ຕາການ ຫາຍຂາດຈາກການຕິດເຊື້ອ ແລະ ຈຳນວນໄຂ້ຫລຸດລົງພາຍຫລັງການປິ່ນປົວໃນມື້ທີ່ 21 ແລະ 23. ອາການສົນຂອງຢາແມ່ນປະເມີນຢູ່ໃນ 3 ຊມ, 48, ແລະ 120 ຊມ ຫລັງຈາກໄດ້ຮັບຢາປິ່ນປົວ.
ຜົນການສຶກສາ: ກໍລະນີການສຶກສາພະຍາດ *C. philippinensis* ແມ່ນໄດ້ສຶກສາຢູ່ໂຮງໝໍແຂວງ ສະຫວັນນະເຂດ ແລະໂຮງໝໍສູນກາງທີ່ນະຄອນຫລວງວຽງຈັນ, ຊຶ່ງມີທັງໝົດສາມກໍລະນີ ແລະ ຜູ້ປ່ວຍບໍ່ເຄີຍມີບັນຫາດ້ານສຸຂະພາບມາກ້ອນ ທັງໝົດແມ່ນເປັນຊາຍໜຸ່ມ ອາຍຸລະຫວ່າງ 24-27 ປີ ອາການທີ່ປະກົດອອກແມ່ນມີການຖອກທ້ອງແບບຕໍ່ເນື່ອງ, ເຈັບທ້ອງ, ບວມ ແລະ ມີການເສຍນ້ຳໜັກແບບຮຸນ

ແຮງ. ຄົນເຈັບສອງຄົນແມ່ນຄາດວ່າອາຕິດເຊື້ອພະຍາດນີ້ຈາກປະເທດໄທ ແລະ ອີກນຶ່ງກໍລະນີ ຄາດວ່າ ອາດຕິດເຊື້ອຢູ່ ສປປ ລາວ. ໃນນັ້ນພົບວ່າມີການຕິດເຊື້ອແມ່ກາຝາກອື່ນສົມທົບຄື ພະຍາດໃບໄມ້ໃນຕັບ ແລະ ການຝາກໂຕກົມອ່ຽນ. ໃນການສຶກສາກໍລະນີພະຍາດກາຝາກໃບໄມ້ດູດເລືອດ ຢູ່ທີ່ ເມືອງໂຂງ ແຂງຈຳປະສັກ ຈຳນວນ 9 ກໍລະນີພົບວ່າ ສ່ວນຫລາຍ ເປັນເພດຊາຍ ທີ່ມີອາຍຸສະເລ່ຍ ປະມານ 36 ປີ . ທັງໝົດແມ່ນອາໄສຢູ່ດອນໂຂງຕັ້ງແຕ່ກຳເນີດ ແລະຜ່ານມາກໍໄດ້ຮັບການປິ່ນປົວດ້ວຍຢາ ປລາຊິກັງ ແຕນ. ໃນໄລຍະຕິດຕາມຄັ້ງສຸດທ້າຍພົບວ່າ ມີສາມກໍລະນີສຶກສາມີອາການດີຂຶ້ນ ສອງກໍລະນີຍັງມີ ອາການພະຍາດຄືເກົ່າພາຍຫລັງທີ່ໄດ້ຮັບຢາປິ່ນປົວ, ສອງກໍລະນີແມ່ນເສຍຊີວິດຍ້ອນເລືອດໄຫລເນື່ອງ ຈາກເສັ້ນເລືອດດຳຕາມທໍ່ກະເພາະອາຫານຈີກຂາດ, ແລະ ມີສອງກໍລະນີໄດ້ອອກຈາກການສຶກສາ. ສຳລັບອາການຂອງຕັບທີ່ຕິດເຊື້ອເຫັນວ່າດີຂຶ້ນພາຍຫລັງທີ່ໄດ້ຮັບຢາ.

ການສຶກສາກ່ຽວກັບການເປັນພະຍາດຂອງພະຍາດໃບໄມ້ດູດເລືອດ ແລະ ການຕິດເຊື້ອຮ່ວມກັບແມ່ ກາຝາກພະຍາດໃບໄມ້ໃນຕັບພົບວ່າມີການປ່ຽນແປງໃນທາງທີ່ດີຂຶ້ນພາຍຫລັງການກິນຢາ ເປັນຕົ້ນ ການ ຫາຍດີໃນເນື້ອຕັບສຳລັບຜູ້ທີ່ຕິດເຊື້ອລະດັບເບົາບາງ ແລະ ປານກາງ (P<0.001), ລວມທັງຂະ ໜາດ ຕັບຂ້າງຊ້າຍ, ປ້າງ, ແລະ ເສັ້ນເລືອດທີ່ໄປຫລໍ່ລ້ຽງປ້າງແມ່ນຫລຸດລົງ (P<0.01). ແຕ່ການຕິດເຊື້ອແມ່ ກາຝາກຄືນໃໝ່ ໃນເດືອນທີ່ 11 ພາຍຫລັງການປິ່ນປົວແມ່ນຍັງພົບເຫັນໃນຈຳນວນຫລາຍ. ການສຶກສາ ໃນຊຸມຊົນ ແລະ ໂຮງພະຍາບານ ພົບວ່າ ພະຍາດໃບໄມ້ໃນຕັບແມ່ນເປັນສາຍເຫດທີ່ເຮັດໃຫ້ມີການເຈັບ ເປັນໃນຕັບ ແລະ ທໍ່ນ້ຳບີ. ໃນຈຳນວນຄົນເຈັບ 431 ຄົນ ທີ່ຕິດເຊື້ອພະຍາດໃບໄມ້ໃນຕັບພົບວ່າ ມີ ຈຳນວນ 5 ຄົນ ແມ່ນສົງໄສວ່າເປັນພະຍາດມະເຮັງທໍ່ສິ່ງນ້ຳບີ, ພາຍຫລັງການກວດດ້ວຍເຄື່ອງຊ່ອງ ໄຟຟ້າທີ່ໃຊ້ຄື້ນສຽງ ໄດ້ພົບຄວາມຜິດປົກກະຕິຂອງຕັບ ແລະ ຖົງບີ ເຊັ່ນກັນ (ຄວາມຜິດປົກກະຕິຂອງຖົງ ບີ ແລະ ໄຂ່ຫລັງ, ຕັບຊືມນ້ຳມັນ, ຕັບແຂງ, ແລະ ອື່ນໆ). ໃນການສຶກສາຢູ່ໂຮງພະຍາບານໃນເຂດທີ່ມີ ອັດຕາການຊຸກຊຸມຂອງພະຍາດໃບໄມ້ໃນຕັບ ຢູ່ ສປປ ລາວ ພົບວ່າ ມີຈຳນວນ 274 ຄົນ ທີ່ມີການສົງໄສ ໄດ້ເຂົ້ານອນໂຮງໝໍ ຍ້ອນພະຍາດມະເຮັງທໍ່ສິ່ງນ້ຳບີ . ຜູ້ຊາຍ ແລະ ອາຍຸ 50 ປີ ຫຼື ອາຍຸຫລາຍກວ່າ 50 ປີ ແມ່ນພົບເຫັນເປັນຈຳນວນຫລວງຫລາຍທີ່ສົງໄສວ່າເປັນມະເຮັງທໍ່ສິ່ງນ້ຳບີ. ໃນຈຳນວນ 274 ຄົນທີ່ຢູ່ ໃນກໍລະນີສົງໄສ ແມ່ນ 267 ຄົນ ມີທໍ່ນ້ຳບີຕື່ງ, 274 ຄົນ ແມ່ນມີໜ້ອຖົງບີ ຫຼື ໜ້ອທໍ່ທາງເດີນຕັບ ແຕ່ວ່າ ມີພຽງແຕ່ຈຳນວນ 33 ຄົນ ໄດ້ຖືກບົ່ງມະຕິວ່າເປັນພະຍາດໃບໄມ້ໃນຕັບ. ການບົ່ງມະຕິໂດຍການ ຕັດສິ້ນສ່ວນອະໄວຍະວະແມ່ນຍັງບໍ່ມີໃນປະເທດລາວ ແລະ ດ້ວຍເຫດຜົນດັ່ງກ່າວ ພະຍາດມະເຮັງທໍ່ສິ່ງ ນ້ຳບີຈຶ່ງບໍ່ໄດ້ຖືກບົ່ງມະຕິ.

ຖ້າສົມທຽບກັບການສຶກສາແບບ meta-analysis ທີ່ຜ່ານມາ ການປິ່ນປົວດ້ວຍຢາອາແບັນ ດາໂຊນ ແລະ ເມແບັນດາໂຊນ ເຫັນວ່າ ປະສິດທິພາບໃນການປິ່ນປົວແມ່ທ້ອງປາກຂໍ ຍັງເປັນທີ່ບໍ່ເພິ່ງພໍໃຈ. ແຕ່ ສຳລັບການປິ່ນປົວດ້ວຍຢາອານແບັນດາໂຊນ ແມ່ນເຫັນວ່າ ອັດຕາໄຂ່ຂອງພະຍາດແມ່ທ້ອງປາກຂໍ ຫລຸ ດລົງຢ່າງມີຄວາມສຳຄັນທາງສະຖິຕິ ສົມທຽບໃສ່ຢາອານແບັນດາໂຊນ (86,7% ແລະ 76,3% ຕາມ

ລຳດັບ). ນອກນັ້ນ ຍັງພົບວ່າ ຢາອານແປັນດາໂຊນ ແລະ ເມແປັນດາໂຊນ ຍັງມີປະສິດທິຜົນໃນການຂ້າ ເຊື້ອພະຍາດກາຝາກໃບໄມ້ໃນຕັບ ໂດຍເຮັດໃຫ້ຈຳນວນໄຂ່ຫລຸດລົງຢ່າງຫລວງຫລາຍ, ໃນການສຶກສາ ທີ່ສອງ ແບບທົດລອງທາງດ້ານຄລີນິກ ໂດຍນຳໃຊ້ຢາ Tribendimidine ຊຶ່ງໃຊ້ໃນຂະໜາດຄວາມແຮງ ຂອງຢາໃຊ້ປິ່ນປົວພະຍາດແມ່ກາຝາກທີ່ຕິດມາຈາກໜ້າດິນ. ເຫັນວ່າການສຶກສາປະສົບຜົນສຳເລັດໃນ ດ້ານອັດຕາການປິ່ນປົວແລະອັດຕາການຫລຸດລົງຂອງໄຂ່ແມ່ພະຍາດ ຖ້າສົມທຽບຢາທີ່ໄດ້ມາດຖານ ປິ່ນປົວພະຍາດໃບໄມ້ໃນຕັບ ປລາຊີກັງແຕນ (CR 70%, 56% ແລະ ERRs 99.3%, 98.4%). ໃນ ການປິ່ນປົວຢູ່ໃນກຸ່ມເດັກນັກຮຽນ ມັດທະຍົມຕົ້ນ ແລະ ປາຍ ທີ່ມີການຕິດເຊື້ອພະຍາດໃບໄມ້ໃນຕັບ. ການປິ່ນປົວພະຍາດໃບໄມ້ໃນຕັບ ດ້ວຍການນຳໃຊ້ຢາ artesunate, mefloquine-artesunate ແມ່ນບໍ່ໄດ້ ຮັບຜົນ. ພາຍຫລັງກິນຢາເດັກນັກຮຽນທັງໝົດແມ່ນມີອາການສິນ, ໂດຍສະເພາະແມ່ນກຸ່ມເດັກນັກຮຽນ ທີ່ໄດ້ຮັບການກິນຢາ mefloquine, mefloquine-artesunate ແມ່ນມີອາການສິນຫລາຍ ແລະ ຮຸນແຮງ. ສຳລັບກຸ່ມທີ່ໄດ້ຮັບການປິ່ນປົວດ້ວຍຢາ Tribendimidine ແມ່ບໍ່ມີອາການສິນຫລາຍ ແລະ ບໍ່ຮຸນແຮງ.

ສະຫລຸບຜົນການສຶກສ/ຄວາມສຳຄັນ: ການລາຍງານກໍລະນີການສຶກສາແມ່ນຕະໜັກໄດ້ເຖິງການກັບ ຄືນຂອງພະຍາດໃບໄມ້ດູດເລືອດທີ່ເມືອງໂຂງແຂວງຈຳປະສັກ ແລະ ຄວາມສ່ຽງທີ່ອາດເກີດລະບາດ ຂອງພະຍາດ *C philippinensis* ຢູ່ ສປປ ລາວ. ດັ່ງນັ້ນ ຈຶ່ງໃຫ້ຄວາມສຳຄັນພະຍາດດັ່ງກ່າວຫລາຍຂຶ້ນ. ອັດຕາການຊຸກຊຸມທີ່ເກີດຈາກພະຍາດໃບໄມ້ດູດເລືອດ, ພະຍາດໃບໄມ້ໃນຕັບ ແລະ ການຕິດເຊື້ອທັງ ສອງຊະນິດດັ່ງກ່າວແມ່ກໍ່ໃຫ້ເກີດອັດຕາການເຈັບເປັນພະຍາດຕັບ,ຖົງນ້ຳ ແລະ ປ້າງ ຊຶ່ງລວມທັງກໍລະ ນີສິ່ງໄສພະຍາດມະເຮັງທໍ່ນ້ຳບີ, ພະຍາດກະດັ່ນຕັບ ແລະ ຫຼອດເລືອດຊ່ອງພະເພາະອາຫານຕົ່ງ. ການ ຕິດຕາມປະສິດທິພາບຂອງການນຳໃຊ້ຢາປິ່ນປົວພະຍາດແມ່ກາຝາກໃນປະຈຸບັນທີ່ໃຊ້ເຂົ້າໃນການ ປິ່ນປົວທົ່ວປວງຊົນແມ່ນມີຄວາມຮຽກຮ້ອງຕ້ອງການຢ່າງຮີບດ່ວນ Tribendimidine ແມ່ນອາດເປັນຕົວ ຢາທາງເລືອກ ໃຊ້ເຂົ້າໃນການປິ່ນປົວພະຍາດໃບໄມ້ໃນຕັບແລະເຊັ່ນດຽວກັນກັບພະຍາດແມ່ກາຝາກ ອື່ນໆໃນຕໍ່ໜ້າ.

1. Introduction

1.1 Overview of present PhD thesis

The purpose of this Ph.D thesis is to determine morbidity and treatments pertaining to the most common helminth infections in Lao People's Democratic Republic (Lao PDR, Laos), namely food-borne trematode *Opisthorchis viverrini*, water-borne trematode *Schistosoma mekongi* and the soil-transmitted hookworm. Beside the routine stool examination and clinical exam, ultrasound visualization was employed in order to investigate helminthic morbidity. Emphasis is placed on detailed biology, life cycle, mode of transmission; epidemiology and disease burden estimates; clinical manifestations and consequences; diagnosis; and current strategies to control of *O. viverrini, S. mekongi* and hookworm infections, while less common parasitic infection, i.e., *Capillaria philippinensis* and other soil-transmitted helminths (i.e., *Ascaris lumbricoides* and *Trichuris trichiura*) are mentioned.

1.2 Biology, life cycle and mode of transmission of *O. viverrini*, *S. mekongi*, hookworm and *C. philippinensis*

Food and waterborne trematodiasis and soil-transmitted helminthiasis are the most prevalent human parasitic diseases in the developing world (Bethony et al., 2006; Gryseels et al., 2006; Keiser and Utzinger, 2005). Fish, snail, water and soil play an essential role for the transmission of *O. viverrini*, *S. mekongi* and human nematode including hookworm and *C. philippinensis* (Brooker et al., 2004; Gryseels et al., 2006; Odermatt et al., 2010; Sripa et al., 2010a). Fresh water fish is a main staple and daily consumed by people in East and Southeast Asia (SEA), particularly people dwelling along the main tributary and creeks. The behavior of eating raw or uncooked fish is traditionally deep-rooted, which is a prerequisite for the transmission of *O. viverrini* (Sripa, 2003; Sripa et al., 2010a). In areas where the raw or uncooked dishes are a dietary staple, notably in Laos, Northeastern Thailand, and also in Cambodia and Vietnam, the prevalence of food-borne trematodes is rampant (Sayasone et al., 2007; Sithithaworn et al., 2011; Sripa et al., 2010b). The most well-known Lao dishes are cooked with uncooked fish, including *Koi pa*, *Lap pa*, *Pa dek*, *Som pa* (Figure 1.1).

1.2.1 *Opisthorchis viverrini* (*O. viverrini*)

O. viverrini, a hepatobiliary fluke*,* is a member of the family Opisthorchiidae. Four major flukes are known to parasitize humans, namely *Fasciola gigantica*, *F. hepatica, Opisthochis viverrini*, *O. felineus* and *Clonorchis sinensis* (Keiser and Utzinger, 2005; Keiser and Utzinger, 2009; Marcos et al.,2008). The life cycle of *O. viverrini* is complex. It can not replicate in a single host and it needs at least three main hosts. The adult worm lives in the biliary duct of the human liver. Adult worms and their constantly produced eggs impact on host liver tissues (Keiser and Utzinger, 2009). Eggs of the parasite are excreted with faeces and infect two intermediate hosts to complete its life cycle (Sripa, 2003). A fresh water snail of the genus *Bithynia* serves as a first intermediate host, although the infection rate of *O. viverrini* is relatively low in the snail population (Kaewkes, 2003; Kaewpitoon et al., 2008a). Within the snail, the eggs develop in to rediae, sporocysts and cercariae. Once cercariae release from snail, they penetrate the skin of fish and hatch inside the second intermediate host, *Cryprinoid ssp*, especially, young ones are the most susceptible to infective cercariae (Sripa, 2003). An estimated 18 fish species of the family Cyprinidae harbour infective cysts called metacercariae hatching in their muscle and scales with the prevalence of *O. viverrini* infection higher rather than that in the snail population (Kaewkes, 2003; Lun et al., 2005; Muller R. & Wakelin D, 2002). Recent investigation showed trematode metacercariae infects various fish species in Laos (Rim et al., 2003). Humans and acting as the definitive host are of a considerable importance for the transmission (Sripa, 2003). *O. viverrini* adult worms settle successfully in various mammal hosts, including pets such as cats, dogs and wild animals, hence opisthorchiasis may be considered a zoonosis (Conlan et al., 2011; Enes et al., 2010). The infection is acquired by ingesting raw or uncooked fish containing infective metacercariae. The flukes migrate to the liver through the ampullar of Vater and common bile duct and habitat in the bile ducts (biliary phase). Adult flukes can persist for 25 years in the biliary tract of human hosts and cause inflammatory lesions and tissue damage, which can lead to fatal complications (Lim et al., 2008; Sripa and Pairojkul, 2008).

1.2.3 *Schistosoma mekongi* (*S. mekongi*)

Schistosoma mekongi is the causative agent of schistosomiasis mekongi and closely related to *S. japonicum* (McManus et al., 2009; Zhou et al., 2010). It has been first dis-

Figure 1.1 Raw and pickle fish dishes
(A) Pa dek -fermented fish sauce, (B) Som pa-uncooked pickle fish,
(C) Koy Pa -citrus marinated raw fish, (D) Lap Pa -minced raw fish salad

covered in the late 1960ties by VicDupont and colleagues in a Lao migrant admitted at the hospital in Paris, France (VIC, 1957). Foci of *S. mekongi* tend to be geographically restricted in communities along the lower Mekong Basin and its tributaries, namely the northern provinces of Kratié and Stung Treng in Cambodia and Khong Island, Mounlapamok Districts and Champasack province, Lao PDR (Muth et al., 2010). However, the distribution of this disease may stretch to some areas in East of Thailand (Harinasuta and Harinasuta, 1984). The life cycle of the schistosomes includes stages in humans or mammalians and in snails as the intermediate host (Gryseels et al., 2006). The snail species *Neotricula apata* plays an important role acting as the only intermediate host for *S. mekongi* (Attwood, 2001; Attwood et al., 2008). The infections in humans start when they have direct contact with infested fresh water. In addition, the adult worm of *Schistosoma* spp is hermaphroditic; hence, the interruption of the life cycle tends to be challenging (Gryseels et al., 2006).

1.2.4 Hookworm

Hookworm, *Ascaris lumbricoides*, and *Trichuris trichiura* are soil-transmitted helminths. Hookworm (*Necator americanus and Ankylostoma duodenale*) is considered the most widespread species worldwide and causes the most important public health threat

worldwide (Bethony et al., 2006; de Silva et al., 2003). The terms "soil" and "intestine" were commonly used to refer to these parasites, because its mode of transmission and infection are related to the soil and intestinal tract of human (Brooker et al., 2004). Hookworms have a direct life cycle (Hoagland and Schad 1987). They are infecting transcutaneously the human host by active penetration through the skin by larvae (L3). After a migration through heart and lung they reach the intestinal tract. Hookworm attach and then invade intestinal mucosa of humans, where they develop to adult worms (small intestine), and feed on blood and reproduce (Bethony et al., 2006; Hotez et al., 2004; Jex et al., 2011).

1.2.5 *Capillaria philippinensis* (*C. philippinensis*)

Capillaria philippinensis is a rare food-borne nematode causing intestinal disease. It belongs to the family *Trichinelloidae* which is similar to *Trichinella spp.* There are three species, *Capillaria hepatica*, *C. aerophila* and *C. philippinensis* (Saichua et al., 2008). *C. philippinensis* is the most severe species parasitizing human in the genre of *Capillaria* (Chichino et al., 1992). Historically, it was first discovered in a schoolteacher living in Luzon, the Philippines in 1962 (Chitwood et al., 1968). An outbreak occurred during the 1970s. More than 1000 cases and 77 deaths were documented at that time. Subsequently an outbreak appeared in Thailand (Chichino et al., 1992; Chitwood et al., 1968).

1.3 Epidemiology and disease burden estimates

C. philippinensis exhibits a complex life cycle (Figure 1.2), composed of the intermediate host fish, namely *Cyprinus carpio* (Pa Nai), *Puntius gonionotus* (Pa Nin) or *Rasbora boraperensis* (Pa Sew) and human as definitive host (Cross and Basaca-Sevilla 1989; Saichua et al., 2008). Notably, natural reservoir is fish-eating water-birds. In addition, *Capillaria* spp. do not replicate within their mammalian hosts. The direct life cycle or the so-called autoinfection was observed in Gerbil. Autoinfection is when the offspring produced by adults can re-infect the same host, allowing the infestation to multiply within a single host animal.

Since *C. philippinensis* can multiply in human hosts, intensity of infestation can become very high and may lead to death if left untreated (Cross, 1992). Humans get infected by

eating raw fish with infective larvae in their muscle. Consumption of raw fish is the main source of infection (Cross and Basaca-Sevilla 1989; Cross, 1992) (Figure 1.2).

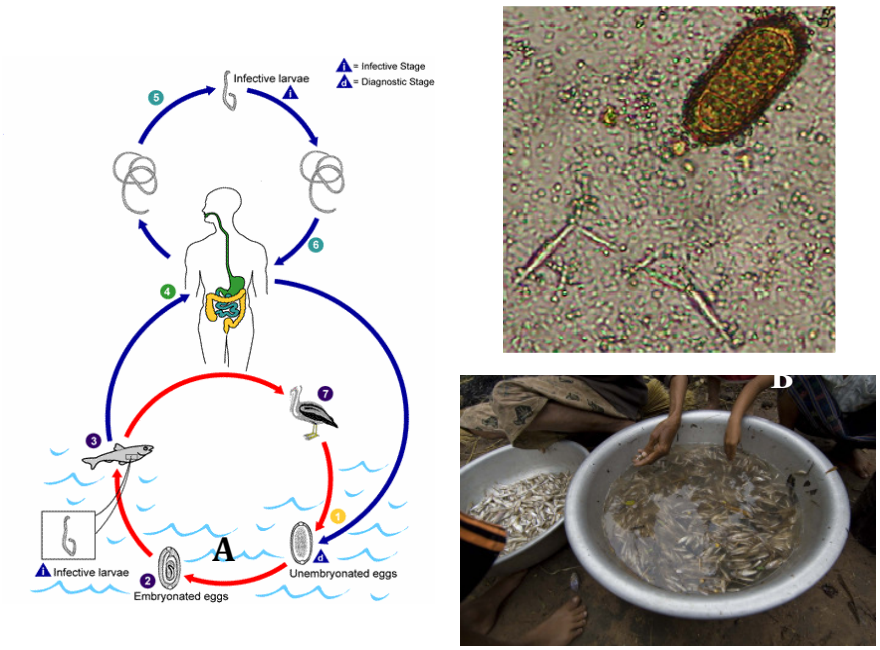

Figure 1.2 (A) Life cycle for intestinal capillariais (adapted from CDC); (B) egg and Chacot-Layden crystal of *Capillaria philippinensis;* (C) Fish (*Rasbora boraperensis*), intermediate host

Opisthorchiasis, schistosomiasis and hookworm belong to the neglected tropical diseases, and cause an extensive socio-economic and public health impact in humans and animals in poor countries (Hotez et al., 2007; Keiser and Utzinger, 2009). The impact of these pathogens occurs in many ways and exact extend of burden is difficult to assess due to various co-infections (e.g. species of Schistosoma and/or opisthorchis trematodes and/or soil-transmitted hlyelminths) (Raso et a., 2004; Steinmann et al., 2008a; Steinmann et al., 2010).

Lao PDR is a tropical, land-locked country in Southeast Asia, which lies along the middle part of the Mekong River and is bordered by China, Myanmar, Thailand, Vietnam and Cambodia. The country has an area of 236,800 square kilometres and more than 6.23 million inhabitants (ADB, 2010). 72.5% were adult literacy, 33.9% of population were living on less than 1.25 dollars a day and the rest on less than 1 dollar per day in the year 2008. ~60% of the national population having access to improved drinking water and less than half of the population having access to improved sanitation facilities

(World Bank, 2008). An estimated per capita gross national income was US$ 740 with a 7.3% economic growth. Culture and ecological factors combined with culinary habits including raw food consumptions and hygiene, intensive agricultural activities and high levels of environmental exposure to human-animal and parasite contact result in the parasitic tropism development and more complex. In these contextual determinants, foodborne trematodiasis (*O. viverrini*, fasciolasis) and nematodiasis *(Paragonemus spp*, trichinellosis, Capillariasis) , waterborne trematode (*S. mekongi),* and the soil-borne hookworm are common in Lao PDR (Barennes et al., 2008; Odermatt et al., 2009; Sayasone et al., 2009a; Sayasone et al., 2009b).

1.3.1 *Opisthorchis viverrini* (*O. viverrini*)

Food-borne liver fluke infection are emerging, neglected and underestimated disease worldwide with an estimated 700 million people are at risk of opisthorchiasis. Opisthorchiasis is caused by *O. viverrini* and *C. sinensis* (Keiser and Utzinger, 2005). It remains of high medical and economic importance in Southeast Asia (Figure 1.3) (Andrews et al., 2008; Sripa, 2008).

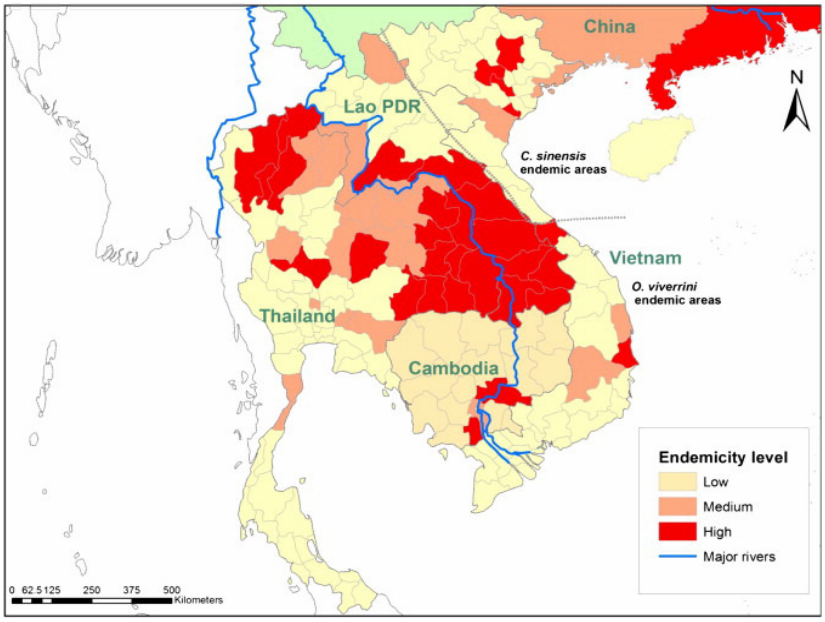

Figure 1.3 the prevalence of *O. viverrini* and *C. sinensis* in Asian countries; endemicity level is defined based on prevalence infections following low (0-5%), medium (5.1-15%), high (>15%) (Sithithaworn et al., 2011).

Although, *O. viverrini* is confined to Northeast Thailand, Lao PDR, Central Vietnam, Cambodia. Currently, an estimated 8 million people are infected in Thailand and 2 million people are infected in Lao PDR (Sithithaworn et al., 2011; Sripa et al., 2010a). Patients positive for eggs of *C. sinensis* have been confirmed recently in Attapeu (Personal communication; PD Dr. Peter Odermatt).

In Lao PDR prevalence of *O. viverrini* is geographically heterogeneous (Rim et al., 2003; Sayasone et al., 2011). In all the provinces infections with *O. viverrini* are present with the highest infection rates in the central and southern parts exceeding 80% in adults (Figure 1.4) and 50% in children (Chai et al., 2007; Rim et al., 2003; Sayasone et al., 2007; Forrer et al., 2011). Rim and colleagues reported on a nationwide stool survey among primary schoolchildren with the prevalence of *O. viverrini* infection about 10.9% (Rim et al., 2003). These numbers underestimate the true infection rates in the population as they have been conducted on a single stool examination which has only a low sensitivity. A recent epidemiological survey on helminths in three districts on Khong Island and Mounlapamok districts revealed the prevalence of *O. viverrini* infection ranging from 64.0-92.0%, respectively (Sayasone et al., 2011). In addition, the infection intensity was found to increase with age and decease in high land areas. Hence, it emphasizes that the parasitic infections are accumulating over time by repeated infection and geographical areas.

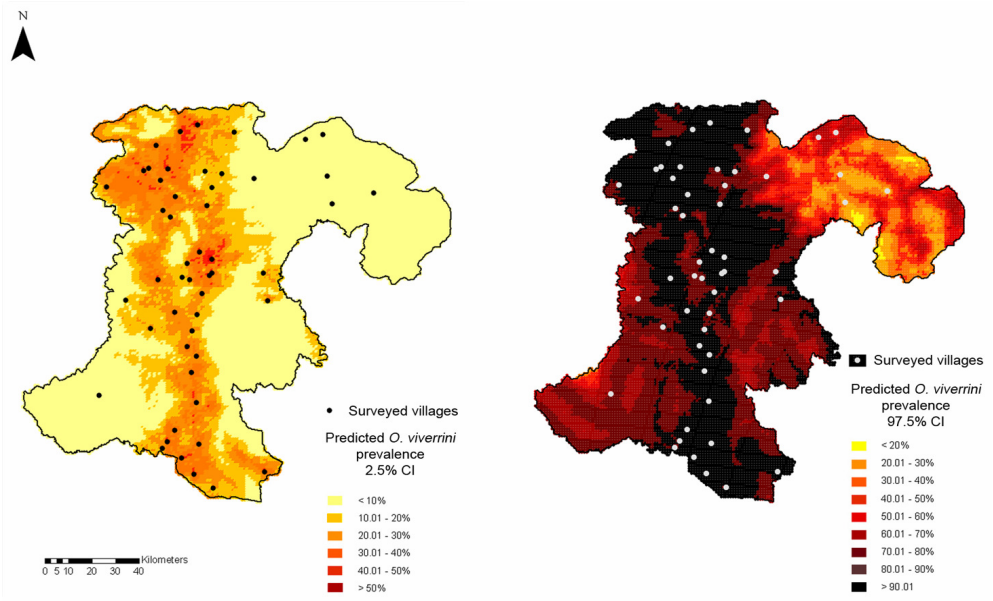

Figure 1.4 Predicted prevalence of *O. viverrini* in Champasack province, Southern Lao PDR (Forrer et al., 2011).

1.3.2 *Schistosoma mekongi* (*S. mekongi*)

Schistosomes are the blood-dwelling flukes embedding in urinary bladder and intestinal venules of humans (Gryseels et al., 2006). There are five main species affecting humans, of which the most common are *Schistosoma mansoni* (Africa, South America and the Arabic peninsula), *S. haematobium* (Africa and the Middle East), *S. japonicum* (China, the Philippines, and Indonesia), *S. intercalatum* (west and central Africa) and *S. mekongi* (the Mekong Basin in Lao PDR and Cambodia) (Gryseels et al., 2006; Muth et al., 2010). In addition, *S. intercalatum and S. haematobium* primarily infect the urinary tract and may cause lesions in the reproductive system, whereas *S. mansoni*, *S. mekongi* and *S. japonicum* provoke the intestinal, hepatic schistosomiasis (Gryseels et al., 2006).

An estimated 779 million people are at risk of schistosomiasis. An estimated 207 million in 76 countries are infected with this parsite, 120 million are symptomatic, 20 million suffer severe illness and 280,000 death per year (Chitsulo et al., 2000; Steinmann et al., 2006; WHO, 2008). Schistosomiasis is the most important human helminth infection in terms of morbidity and mortality with a global burden of disease of about 1.7- 4.5 million DALYs (Utzinger and Keiser, 2004; Utzinger et al., 2009; WHO, 2008) (Figure 1.5). However, the global burden of schistosomiasis has been seriously underestimated (Bergquist et al., 2008; Doenhoff et al., 2008; King et al., 2005).

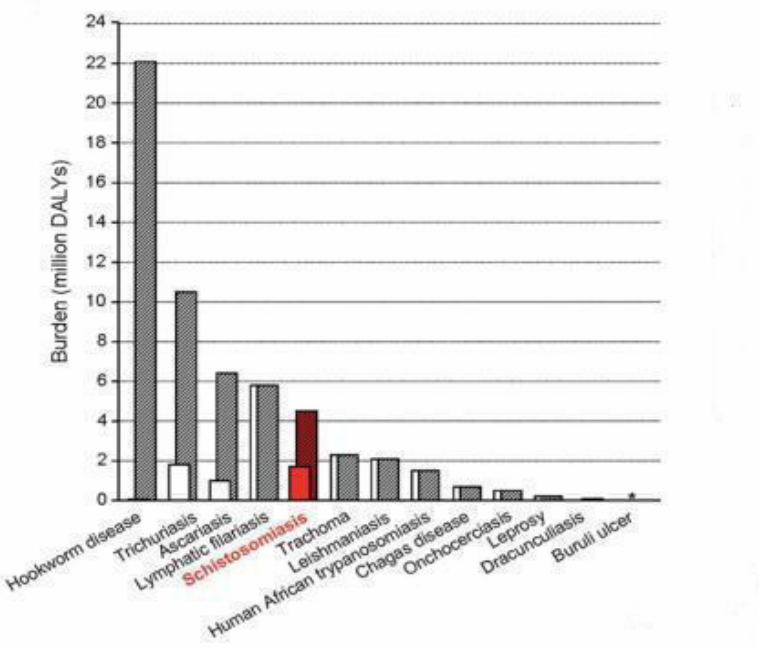

Figure 1.5 Global burden of schistosomiasis (Utzinger et al., 2009)

S. mekongi is restricted to the lower Mekong Basin and mainly endemic in Lao PDR and Cambodia with approximately 140,000 individuals at risk of infection, of which 60,000 in Lao PDR (Muth et al., 2010). Recent helminth surveys on Don Long, Khong Island showed prevalence of *S. mekongi* among school aged children were 87.8% (Lovis, pers. comm.) and adult accounted for 68 % (Sayasone et al., 2011).

1.3.3 Hookworm

STHs constitute a major public health problem worldwide. However, the bulk of the burden due to hookworms is concentrated in rural areas and deprived urban settings of low-income countries where clean water and good sanitation are lacking and access to health services and treatment is inadequate (Utzinger and Keiser, 2004). It is currently estimated that globally more than 2 billion individuals are infected with at least one species of STH and more than 135,000 die annually (de Silva et al., 2003). Thus, the burden of disease is comparable, in disability-adjusted life years (DALY), to malaria and tuberculosis.

It is estimated that globally more than half a billion individuals are infected with hookworms (*Ancylostoma duodenale* and *Necator americanus*), the majority of whom are children with prevalence ranging from 9.3-11.4 % globally (de Silva et al., 2003; Bethony et al., 2006). As many as 65,000 individuals might die each year due to the long-term complications of chronic hookworm disease, the majority of whom is school-aged children (Bethony et al., 2006; de Silva et al., 2003; Hotez et al., 2004).

The global burden of disease study has attributed 3,000 deaths due to hookworm (Brooker et al., 2004; WHO, 2002a). Scale-up administration programme resulted dramatically in overt reduction of hookworm prevalence in SEA (Figure 1.6) (Loukas et al., 2006). In Lao PDR, STHs are of a major public health importance, in particularly school-aged children, with an estimated overall prevalence of hookworm infection above 50 % and 19.1% (3.0-45.1% by provinces) in schoolchildren, respectively (Figure 1.6) (Rim et al., 2003). High hookworm prevalence rates have been reported from rural parts of Lao PDR. However, the highest prevalences were found in the Northern provinces due to limited access to the health facilities, poor hygiene and infrastructures and high exposure to animal faeces (Rim et al., 2003; Sayasone et al., 2007).

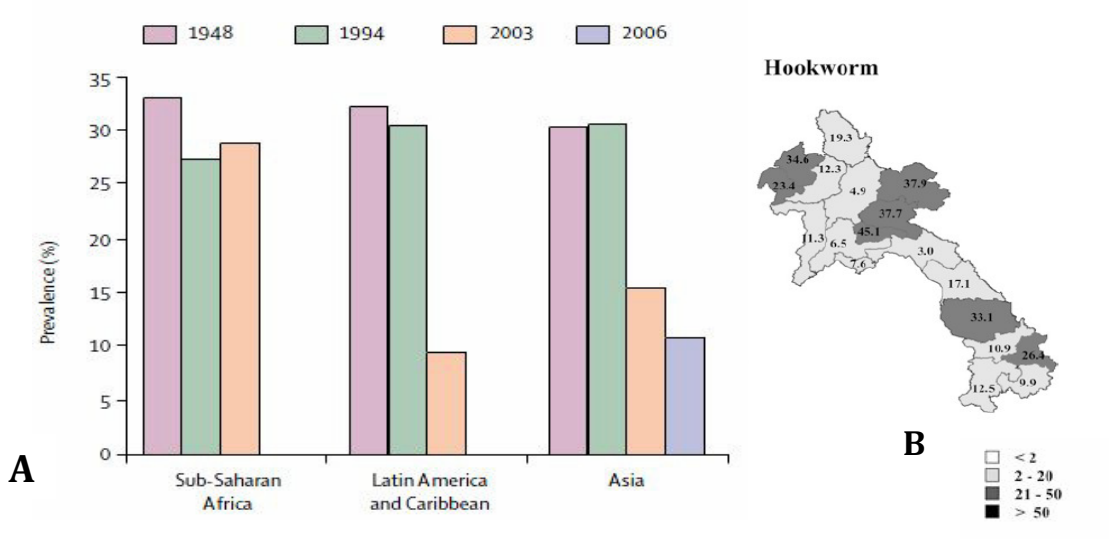

Figure 1.6 (A) Prevalence of hookworm infection by region overtime (Loukas et al., 2006; Rim et al., 2003)

1.3.4 *Capillaria philippinensis* (*C. philippinensis*)

Approximately 2,000 cases with almost 200 deaths have been documented worldwide, mostly from Southeast Asia. Subsequently sporadic cases have been reported from Europe and Africa (Odermatt et al., 2010). Outbreak of Capillariasis was mainly found in the Philippines and Thailand (Cross, 1992; Saichua et al., 2008).

1.4 Clinical manifestations and consequences

1.4.1 *Opisthorchis viverrini* (*O. viverrini*)

Opisthorchiasis is considered as one of the neglected tropical disease (Hotez et al., 2007; Keiser and Utzinger, 2009) and poses a public health threat particularly in Southeast Asia (Sripa, 2008; Sripa et al., 2010b). *O. viverrini* is the most important of the foodborne trematodes due to its link to advanced pathogen-specific disease or hepatobiliary disease including cholangiocarcinoma (Honjo et al., 2005; Sripa et al., 2007; Sripa et al., 2003). Clinical manifestations show transient or unspecific signs and symptoms of abdominal disease except for heavy infection. The unspecific signs may include upper right quadrant pain or intestinal irritations, to severe manifestation (Mairiang and Mairiang, 2003). The insidious consequences of opisthorchiasis remain unnoticed by infected individuals for a long period and the most dangerous parasite in terms of mortality (Bouvard et al., 2009; Shin et al 2010). In addition, jaundice is caused

by mechanical obstruction due to a large number of flukes in the bile duct obstruction caused by cholangitis or cholangiocarcinoma as a late complication of long-term chronic infection (Mairiang and Mairiang, 2003; Sithithaworn et al., 2009). CCA is a common cancer in Southeast Asia, with the world highest prevalence in Northeast Thailand with the respective aged-standardized incidence rate of 33.4 and 12.3 per 100, 000 in men and women (Sripa and Pairojkul, 2008). Most importantly, studies revealed that the incidence of CCA is strongly associated with the prevalence of *O. viverrini* infection. It is well established that *O. viverrini* infection is triggering the CCA development. It is estimated that 60% of the CCA cases is due to *O. viverrini* infection (Haswell-Elkins et al., 1992; Honjo et al., 2005; Mairiang et al 2006; Mairiang et al., 1992; Parkin et al., 1991; Sithithaworn et al., 1994; Sriamphorn et al., 2004; Sripa et al., 2007).

Cholangiocarcinoma (CCA) is defined as an adenocarcinoma arising from epithelial of the intra and extra hepatic bile ducts. CCA is classified into intra and extra hepatic CCA. The respective intra hepatic (IH-CAA) and extra hepatic cholangiocarcinoma (EH-CAA) account for 40 % and 60% of all cases CCA (Uttaravichien et al., 1999). IH-CAA originates in the cholangiole or small peripheral bile duct. EH-CAA is tumours which are located at the large bile ducts, at the portal hepatic, common hepatic duct, cystic duct, common bile duct and peri-ampullary region (Uttaravichien et al., 1999). However, most of the CCA cases are IH-CCA in Thailand (Sripa et al., 2008). The disease does not cause specific symptoms until very late (Sithithaworn et al., 2009). It is usually diagnosed at advanced stages, when effective treatment for cure or prolongation of life no longer exists. Primary and secondary prevention are therefore the primary strategies to reduce disease burden and CCA mortality (Kaewpitoon et al., 2008; Sripa et al., 2007; Sripa and Pairojkul, 2008).

A number of definite precancerous conditions (risk factors) are strongly associated with increased CCA risk, including primary sclerosing cholangitis (PSC), hepatolithiasis, biliary malformations (choledochal cyst, Caroli's disease), ulcerative colitis, thorotrast, liver fluke infection *(O. viverrini)* and secondary sclerosing cholangitis (SSC) related to mechanical obstruction. Other risk factors are possibly associated CCA factors namely, abestose, isniazid, methyldopa, oral contraception and polychlorinated biphenyls (Ben-Menachem, 2007; Shin et al., 2010; Yeo et al., 1990). Additional hypothesized CCA risk factors lending themselves as potential targets for prevention include dietary intake of nitrate and nitrosamines, aflatoxin B1, smoking, excessive alcohol consumption, obesity,

chronic hepatitis C (HCV) and hepatitis B (HBV) infection, cirrhosis, toxins (dioxin, polyvinyl chloride), alcohol and biliary-enteric drainage procedures (Ben-Menachem, 2007; Honjo et al., 2005; Shin et al., 2010). To date, the association of these precancerous conditions with chronic *O. viverrini* infections is unknown. The evidence for the etiological role of these additional factors as well as for their interaction with chronic *O. viverrini* infections is still limited and inconsistent (Sripa et al., 2010; Sripa and Pairojkul, 2008).

There is a paucity of data on the incidence rate of cholangiocarcinoma (CCA) in Lao PDR due to the absence of diagnostic and treatment possibilities in health services, a population-based cancer registry as well as population-based surveys on CCA. Most suspected cases admitted at the hospitals, in particularly from provincial hospitals are referred for further investigations at the central hospitals in Vientiane – for instance specific tumor-marker test, CT scan and recently ERCP has been available only at central hospitals in Vientiane, Lao PDR. At present, suspected CCA case numbers presented at the admission are accompanied by advanced signs and symptoms, including jaundice and right upper quadrant pain. Some of them were seen by annually check-up at the hospital. All suspected cases had been firstly examined by target abdominal ultrasound, then CT or ERCP. Therefore, today the incidence of CCA is unknown. Nonetheless, the incidence of CCA must be very high and similar to Thailand, given the comparable endemicity level of *Opisthorchis* infection in northeast Thailand, which has the highest incidence of CCA worldwide (Sripa and Pairojkul, 2008; Vatanasapt et al., 1990).

1.4.2 *Schistosoma mekongi* (*S. mekongi*)

Katayama fever or acute schistosomiasis is the most common acute symptoms occurring in *Schistosoma japonicum* and in *S mansoni* infections and rarely with *S. haematobium* (Ross et al., 2002). Most of infected individual with *S. mekongi* remain asymptomatic. There is no evidence that *S. mekongi* cause any acute symptoms previously (Gryseels et al., 2006). The onset of symptoms starts 4 to 8 weeks after the first exposure to infested water may result in anaemia, fatigue, abdominal pain, and intermittent diarrhoea or dysentery in the early evaluation of infection. *S. mekongi* egg deposit in the peri-intestinal tract and liver and release the antigens, evoking the inflammatory or granulomatous reactions at the site of intestine and liver, especially periportal vein. Ectopic organs can be found but rare (Carmody et al., 2008; Houston et

al., 2004). *S. mekongi* secretion causes microulceration in the intestinal tract, resulting in diarrhoea and also bloody stool. Later complication occurs several years after exposure and is associated with sustained heavy infection and several exposures to infected water bodies (Gryseels et al., 2006). Chronic disease are characterized by hepatomegaly, splenomegaly, liver fibrosis, and eventually lead to the progressive obstruction of blood flow, portal hypertension and ultimately varices or rupture of oesophageal varices, which is the most important cause of death due to this blood fluke (Biays et al., 1999; Gryseels et al., 2006).

1.4.3 Hookworm

Acute infection can occur in areas where the transmission is high. Hookworm inactive phase (L3) infect human through skin penetration. It is characterized by a cutaneous syndrome or ground itch or the so-called puritic erythematous papulovesicular rash appearing most frequently on the feet and hands, where the larval (L3) migration are entered to body (Brooker et al., 2004). Parasites migrate to the lungs and penetrate the alveolar walls, ascend through the trachea and ultimately are swollen down into the small intestine (Haddad et al., 2008) Visceral larval migration to the lung may cause pathologically in form of transient pneumonitis (Loeffler's like syndrome; Sarinas and Chitkara, 1997) or eosinophilic pleural effusion (Yassin et al., 2007).

The major cause of hookworm infections is intestinal blood loss and hemaetophagy contributing to iron deficiency anaemia (Bethony et al., 2006). Anaemia is a major public health concern particularly in at-risk population notably, children, women child-bearing age and pregnant women living in the least developed countries (Hotez et al., 2004; Hotez et al., 2007). Chronic infection causes impaired physical and mental growth, reduced school performance, educational attainment in pre and school aged children and reduced productivity in adulthood. Anemia attributable to hookworm infections can lead to low birth weight, premature birth in pregnant women and women with child bearing age. Moreover, death may occur in individuals concurrently infected with hookworm and immuno-competent human, for in stance diabetes, HIV/AID tuberculosis (Bethony et al., 2006; Hotez et al., 2007; Lammie et al., 2006).

1.4.4 *Capillaria philippinensis* (*C. philippinensis*)

Clinical findings of *C. philippinensis* include acute-on-chronic diarrhea with malabsorption. Capillariasis patients suffer with gastrointestinal symptoms, for instance abdominal pain, borborygmus, anorexia, nausea and episodic vomiting. Other symptoms, such as oedema, pitting oedema of the lower limbs, muscle wasting, severe weight loss were reported (Odermatt et al., 2010; Saichua et al., 2008). Patient may lead to fluid loss and electrolyte depletion (hypokalaemia), which may cause death due to heart failure and hypovolemic shock (Cross et al., 1992). *Capillaria* eggs has received little attention and may be frequently overlooked or confused with *T. trichuria* infection (Figure 1.2) in chronic diarrhoea and immunosuppressant disease such as Crohn disease and HIV/AIDS (Odermatt et al., 2010).

1.5 Diagnosis

The diverse diagnostic methods, namely direct parasitological examination, immunological- and molecular diagnostic tests, and imaging are essential for the diagnosis helminth infection and assess helminth-related morbidity (Keiser et al., 2010). Diagnostic capability is crucial to determine distribution, prevalence and severity of helminth diseases, which allow estimating the burden of disease (Bergquist et al., 2009; Johansen et al., 2010). Infection intensity is a crucial determinant to assess indirectly helminthiases related-morbidity (WHO, 2002b). Nonetheless, the true burden can not be determined without clinical examination and imaging techniques (Bergquist et al., 2009; Keiser et al., 2010; Lun et al., 2005).

1.5.1 Parasitological diagnosis

A single diagnosis test does not enable to identify all species helminthic eggs because the helminthic diseases necessitate various diagnostic tools (Keiser et al., 2010). Kato-Katz thick stool smear is widely used for parasitological diagnostic approach due to relatively simple and inexpensive tool, except for *S. stercoralis*. However, use of a single Kato-Katz thick stool smear lacks of sensitivity in detecting specific parasite of trematode eggs compared to ether-based concentration techniques (Keiser et al., 2010; Sayasone et al., 2009b). Multiple Kato-Katz slides and multiple stool sample analysis increase the sensitivity for helminth infection (Lovis et al., 2009). Other parasitological

techniques including immunological assays (i.e., ELISA or the enzyme-linked immunosorbent assay or specific parasitic antibodies), molecular assays (i.e., polymerase chain reaction [PCR]) and metabolic profiling (biological samples i.e., blood, stool and urine) and the recently developed FLOTAC apparatus possess higher sensitivity in detecting helminth eggs than the routine stool examination than Kato-Katz and also ether-based concentration techniques (Keiser et al., 2010; Knopp et al., 2009; Utzinger et al., 2008). However, these techniques have properties which make them challenging for field applications: more sophisticated technique, requiring equipments and well-and extensive-trained laboratory personnel (e.g. Speich et al., 2010).

1.5.2 Ultrasound (US) examination

Abdominal US is usually used as an initial imaging diagnostic tool for assessing trematodiasis related morbidity. It is non-invasive, relatively simple to perform and well-accepted by patients in hospital settings and in the communities (Hatz et al., 1992; Mairiang et al., 2006; Mairiang and Mairiang, 2003). A standardized method of US was developed by WHO experts and researchers for assessing periductal fibrosis related to schistosome pathogenesis (Niamey Working Group., 2000). US image findings document pathologies of a mass, a bile duct dilatation due to mechanical obstruction; chronic cholecystitis: gall bladder wall thickening with or without gall stone or hydrop gall bladder due to inflammation (Mairiang et al., 1992) increased periportal echo-prominence of echoes along the portal triad (Hatz et al., 1992). US of the liver and biliary tree are used as the initial examination in patient with suspected obstructive jaundice (Saini, 1997; Sharma and Ahuja, 1999). In opisthorchiasis patients, US demonstrates hepatobiliary pathologic changes. Additionally, ultrasound can identify obstruction and ductal dilatation with providing a direct image of pathologic changes and in some cases may be sufficient to diagnose CCA (Bloom et al., 1999).

1.5.2 Computed topographies

US examination is useful as an initial screening assessment in demonstrating early stages of morbidity, e.g. dilatation of all or part of the biliary tree in patient with obstructed jaundice (Mairiang and Mairiang, 2003). Abdominal computed topographies scanning (CT scan) are examinations for in-depth investigations of potential tumours, regional node involvement, particularly in suspected CCA patients. Furthermore,

percutaneous transhepatic cholangiography (PTC), endoscopic retrograde chanlangiopancreati-cography (ERCP), magnetic resonance imaging (MRI) and magnetic resonance cholangipancreatography (MRCP) are additional most suffisticated tools (Aljiffry et al., 1999). For suspected CCA patients, the extent of ductal involvement is best demonstrated MRI or MRCP providing a more accurate diagnosis than the invasive ERCP or PTC (Manfredi et al., 2001). Today, early diagnosis and resection are the only reliable therapies for CCA. If the tumour mass can be removed surgically, the prognosis remains poor. The non–jaundice CCA patients were shown to possess a significantly longer survival time than CCA patients with a jaundice (Aljiffry et al., 2009; Uttaravichien et al., 1999).

1.6 Current control, treatment and potential drug candidates

Today, there is no vaccine for the prevention of helminths infection (Hotez et al., 2007, McManus et al., 2009). Control efforts for *O. viverrini*, *S. mekongi* and STH infections rely on preventive chemotherapy accompanied with information education campaigns (IEC), improved sanitation and hygiene, access to clean water (de Silva et al., 2003; Lammie et al., 2006; Utzinger and Keiser, 2004). Molluscicide for control of *S. mekongi* was abandoned as it was not effective against the snail *Neotricula* in the huge masses of water of the Mekong (Muth et al., 2010).

Benzimidazole, pyrantel pamoate and levamisole are recommended drugs by World Health Organization (WHO) for STH (i.e. *A. lumbricoides*, *T. trichiura* and hookworm infection) and trematode infections (i.e. schistosomiasis and opisthorchiasis) (Keiser et al., 2010; WHO, 2002b). Albendazole (ABZ) and mebendazole (MBZ) belong to the benzimidazoles carbamates, which are the most widely used anthelminthics in large-scale control programmes (Utzinger and Keiser, 2004; WHO, 2002b). These drugs have an excellent safety profile with low frequency and generally mild adverse events. They can be administered by non-medical personnel, such as school teachers in school-based mass treatment campaigns (Keiser and Utzinger, 2010). Recent studies show that ABZ shows high efficacies against *A. lumbricoides*. However lower and often unsatisfactory efficacy against hookworms and *T. trichiura*. Furthermore, ABZ has a low-to-moderate efficacy against *S. stercoralis* (Keiser and Utzinger, 2008; Olsen et al., 2009; Steinmann et al., 2008b). Resistance to helminthic drugs has been widely reported in veterinary

medicine (Prichard, 1990; Wolstenholme et al., 2004). There is a danger that the wide effort of expanded mass drug administration programme against STH may lead to the emergence of drug resistance (Geerts and Gryseels, 2000).

For many trematodiasis praziquantel (PZQ) is only drug available for the treatment (Keiser and Utzinger, 2010). Today, there is little incentives to develop new drugs. It is time-consuming and has financial limitations. The dependence on a single drug for treatment and control spur a concern about treatment resistance (Geerts and Gryseels, 2000). Today there is no evidence on PZQ resistance against trematode infections in humans. Nonetheless, since the number of available dugs has been limited for the treatments of trematode infection, there is a pressing need for concerted effort to discover and develop new drugs against trematodiasis, notably opisthorchiasis, schistosomiasis and soil-transmitted helminthiasis (Keiser and Utzinger, 2010).

Recent advances are being made with promising broad spectrum Chinese anthelminthic drug (tribendimidine) for the treatment of trematodes and other helminth infection in hamster models (Keiser et al., 2010). Tribendimidine is a derivative of amidantel, synthesized and developed by the National Institute of Parasitic Disease in Shanghai, PR China. It has been approved for human use in China. Tribendimidine possess a broaden spectrum against nematodes (i.e. *A. lumbricoides, Enterobius vermicularis* and hookworms) (Xiao et al., 2005). Furthermore in vitro and vivo studies in animal modesl indicated that mefloquine, artesunate, artemether and tribendimidine possess activities against *O. viverrini* infections (Keiser et al., 2009; Keiser et al., 2006; Keiser et al., 2008).

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2. Aim and Objectives

2.1. Aim

The aim of this thesis is to deepen our understanding on the importance of helminthic parasitic infections in Lao PDR and challenges of their treatments by documenting severe morbidity due to parasitic infections in Lao PDR and assessing the effects of available and new treatments on infection and morbidity status of common helminth infections.

2.2. Objectives

The PhD thesis pursued the following 3 main objectives:

1. To document of severe morbidity of parasitic infection such as *C. philippinensis* and *S. mekongi* in Lao.

2. To assess morbidity of trematode infections and resolution of morbidity after treatment.

    2.1 To document hepato-biliary and -splenic morbidity in areas where *S. mekongi* and *O. viverrini* are coendemic

    2.2 To assess dynamics of infection and morbidity resolution after praziquantel treatment in opisthorchiasis and schistosomiasis patients

    2.3 To assess *O. viverrini*-induced hepatobiliary morbidity including precursors lesions of cholangiocarcinoma in Lao PDR

3. To assess efficacy of present anhtelmintic drugs and potential candidates.

    3.1 To assess the efficacy of a single oral dose of albendazole (400 mg) and mebendazole (500 mg) against hookworm infection among school-aged children in Lao PDR.

    3.2 To assess the efficacy and safety of mefloquine, artesunate, mefloquine–artesunate, and tribendimidine compared with that of praziquantel in patients with parasitologically confirmed *O. viverrini* infectio

3. Approach and Methodology

Figure 3.1 Field activities

(A&C) A community-based survey on *O. viverrini*-induced hepatobiliary morbidity by use of abdominal ultrasonography in Saravane province, Lao PDR

(B&D) A Randomized controlled trial on efficacy and safety of mefloquine, artesunate, mefloquine-artesunate, tribendimidine, praziquantel against *O. viverrini,* Lao PDR

3.1 An overview of approach and methdology

In this chapter and in Figure 3.1 an overview of the research approach and methodologies used in the thesis resaerch work will be provided. It will give information on the study area, study subjects, and approach and methods used. There are separately in the three parts of the Ph.D research activities: (i) severe morbidity of parasitic infections (*C. philippinensis* and *S. mekongi*), (ii) assessment of parasitic infections and morbidity resolution dynamics after treatment, and (iii) assessment of efficacy of present anthelmintic drugs and potential drug candidates. A final section provides information on ethical aspects.

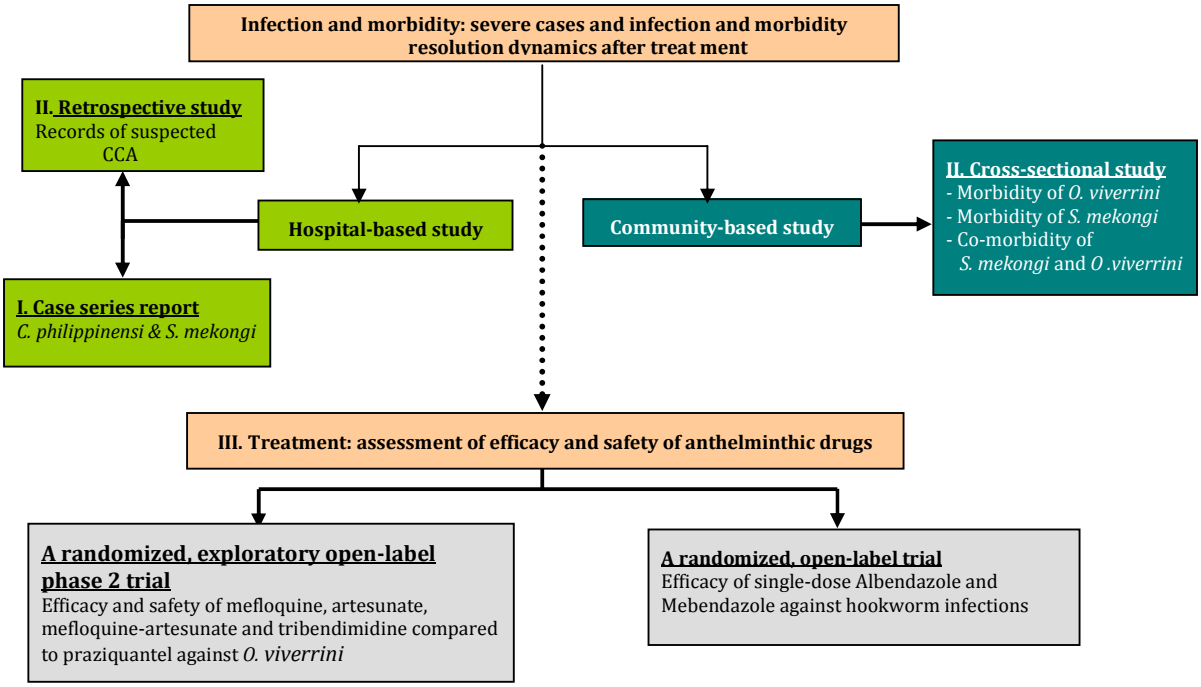

Figure 3.2 Overview research approach of present PhD

3.2 Severe morbidity of parasitic infections in Lao PDR

Severe *C. philippinensis* cases: In two referral hospitals in Vientiane and Savannakhet in Lao PDR we identified three cases from patients' admitted. A first case was reported from Savannakhet provincial hospital in 2006 (Figure 3.3). Savannakhet is located in the southern part of Laos (600 km to the south from Vientiane capital), on the shore of the Mekong River opposite of Mukdahan, Thailand. Savannakhet is the largest province

from Lao PDR with a population of 824,662 inhabitants (NSC, 2005) living in 15 districts. Two additional *C. philippinensis* cases were found in Sethathirath and Mahosot hospitals in Vientiane in 2007. Both hospitals are acting as referral and university hospitals. Vientiane Capital is the capital of Lao PDR, situated on the Mekong River with the population of 695,473 and the second largest province (NSC, 2005).

Severe *Schistosoma mekongi* (*S. mekongi*) cases and follow-up: The severe cases of *S. mekongi* were identified during community-based survey in 2007 and subsequently annually re-visited until 2010. They were seen on Khong Island, southern Champasack province (Khong district, estimated population: 80,000) (Mouth., et al 2010, Sayasone S et al., 2011). Khong Island district is located in the southern part of the province (~120 km from Pakse city), and borders Northeaster Cambodia (Figure 3.4). All patients were examined and followed up on a yearly basis for three to four years (2006-2010). The examination consisted of physical examination, abdominal ultrasound, and various blood tests, including hepatitis test as well as liver, and renal function tests. These examinations allowed evaluating the clinical and treatment response of the patients. We informed patients about any abnormalities and provided treatment in accordance to Lao standard case management, Ministry of Health, Lao PDR (Ministry of Health, 2004). Unfortunately, some cases were lost to follow-up due to temporary work task.

3.3 Assessment of infection and morbidity resolution dynamics after treatment of *S. mekongi* and *O. viverrini* patients

Assessment of infection and morbidity caused by *S. mekongi* and co-morbidity caused by *O. viverrini* after treatment in endemic areas, Lao PDR: We conducted a cohort study from 2006 to 2008 in three villages on Khong district, Champasack province. *S. mekongi* and *O. viverrini* and co-infection are endemic. It allowed us to investigate spleno- and hepatobiliary morbidity due to the two infections and document the infection and morbidity resolution dynamics. We used standard laboratory, Kato-Katz thick smear techniques (3 stool samples each examined with single Kato-Katz smear, Katz et al., 1972), and formalin ether concentration technique (FECT, Marti et al., 1990). A detailed description of the procedure of stool collection and examination is provided by Sayasone and his colleagues (Sayasone et al., 2009). A target abdominal ultrasound (US) examination focusing on hepato-biliar morbidity was carried out based

on the WHO guideline (Niamey working group, 2000) for assessing schistosomiasis-related morbidity. Organometry (liver and spleen) was performed using Hackett score (Hackett score, 1944). All organ measurements determined by US were hight adjusted (Li et al., 2004). Yearly follow-up investigations (2008 and 2009) included the assessment of infection status and hepato-biliar morbidity in which infection and morbidity resolution dynamics after a single oral dose of praziquantel treatment (40 mg/kg BW) was documented.

Assessment of severe morbidity caused by *O. viverrini*: the study focused on documenting severe morbidity due to *O. viverrini* infection; in particular (suspected) cases of cholangiocarcinoma (CCA) and other hepato-biliar morbidity. Two sub-studies were conducted: In a first retrospective hospital-based study suspected cases of CCA were identified in patient records of all referral hospitals in Lao PDR. The study was limited to records between 2006 and 2010. The second community-based study, suspected CCA cases and other hepato-biliar morbidity were diagnosed by US in a cross-sectional study in *O. viverrini* infected adult patients in endemic district of Saravane, Saravane province, Lao PDR (Figure 3.5), from February to March 2011. No internationally validated standard US guideline for assessing *O. viverrini*-related morbidity is available today (Mairiang et al 2011). The assessment of the liver parenchyma fibrosis was done by using a modified version of the Niamey protocol (Niamey working group, 2000) with information from community-based ultrasonographic studies from Khon Kean, Thailand (Mairiang et al., 2011; Sripa et al., 2010). Eligible for this study were adult individuals with parasitologically confirmed *O. viverrini* infection, detected by stool examination using Kato-Katz thick smear technique and FECT (Katz et al., 1972; Marti & Escher, 1990). An *O. viverrini*-positive patient was defined as the presence of at least one *O. viverrini* egg in one of the quadruplicate Kato-Katz fecal thick smears examined. Additional data on risk factors were obtained from each study participant.

3.4 Assessment of efficacy of currently used anthelmintic drugs and potential new drug candidates

We employed Kato-Katz thick smear (Katz et al., 1972) to detect helminth eggs, and FECT (Marti & Escher, 1990) was used to discriminating *O. viverrini* eggs from eggs of

other trematode. Standard procedure of stool examination is described elsewhere (Sayasone et al., 2010). Drug efficacy was determined by cure and egg reduction rates in both intention-to-treat (ITT) and per-protocol analysis (PPA). Adverse events were assessed at 3, 24 and 120 hours post-treatment.

Efficacy of single-dose ABZ and MBZ against hookworm: The first study investigated the efficacy of single-dose ABZ and MBZ against hookworm using a randomized open-label trial. The trial was conducted in two primary schools in Batieng district, Champasack province, southern Lao PDR from February to March 2009 (Figure 3.4). Bachieng is located on the Bolavan plateau and a district of Champasack Province (~10 km far from the Pakse city). We compared the efficacy of single dose of 400 mg albendazole and 500 mg mebendazole against the hookworm infection. Other helminths endemic in the settings were also included for the efficacy evaluation such as *O. viverrini*, *A. lumbricoides*, and *T. Trichiura*.

Efficacy and safety of mefloquine, artesunate, mefloquine-artesunate, tribendimidine, praziquantel against *O. viverrini*: With a randomized, exploratory open-label trial we assessed the efficacy and safety of mefloquine, artesunate, mefloquine-artesunate and tribendimidine against *O. viverrini* infection among schoolchildren, compared to the standard praziquantel treatment regimen. The trial was carried out in a secondary school in Attapeu Province, Lao PDR in 2010. Attapeu Province has a total area of 10,320 km^2 and is the most south-easterly province of Lao PDR bordering Vietnam to the east and Cambodia to the south. Attapeu is one of the four poorest provinces of Lao PDR (Figure 3.3). Mass drug administration programme against *O. viverrini* has not started yet in Attapeu province, although the prevalence of liver fluke is known to be high (Rim et al., 2003).

3.5 Ethical considerations

All study protocols were submitted to the Lao and international ethics committees prior to implementation, notably, the Lao National Ethics Committee for Health Research (NECHR), Ministry of Health, Vientiane, Lao PDR, the internal reviewing board (IRB) of the Swiss TPH, the ethics Committee of Basel, Switzerland (EKBB), and the ethical committee board of the World health Organization Pacific Region (WPRO), Manila, the Philippines. In addition, permission for field work was obtained from the Lao Ministry

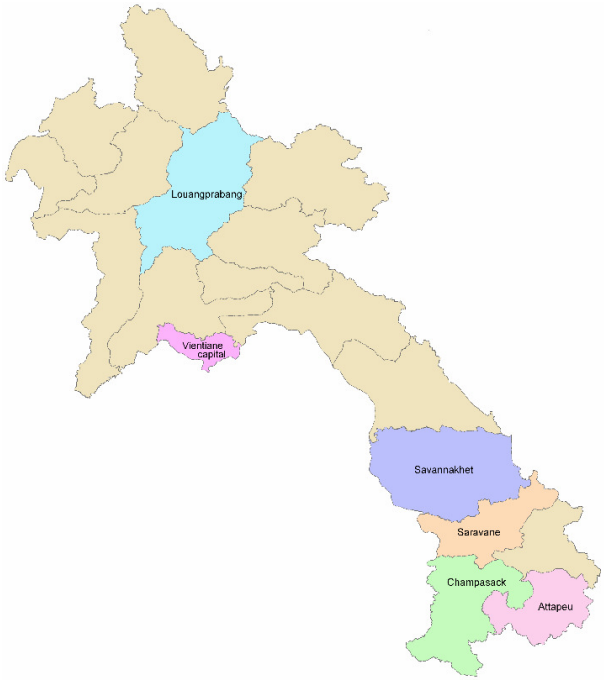

Figure 3.3 map of Lao PDR and stdy sites

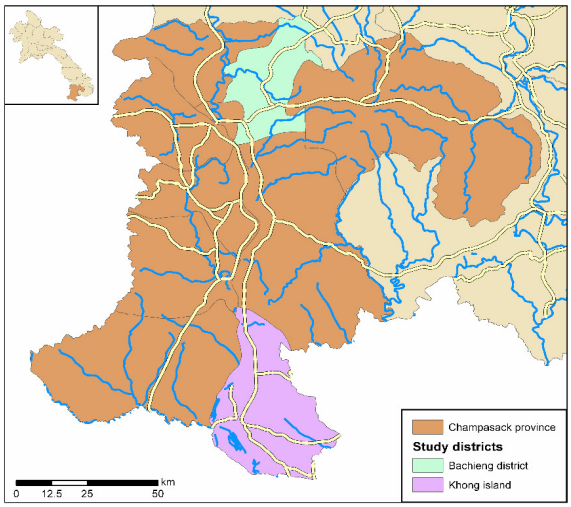

Figure 3.4 map of Champasack province

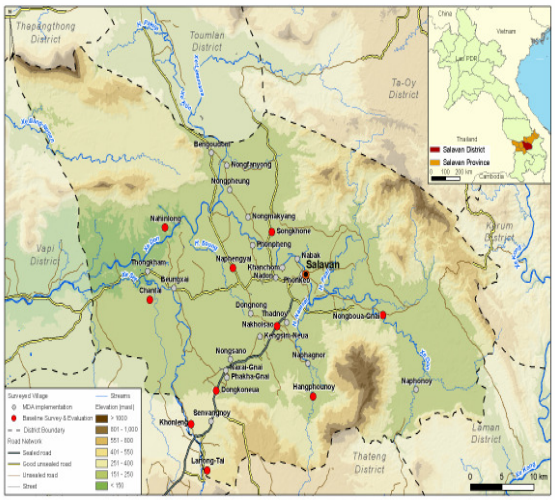

Figure 3.5 map of Sarvane distirct, Saravane province

of Health, the provincial health departments and additionally the provincial education offices and other authorities concerned (chief of villages, school authorities, youth union and so on).

Our study involved experimental trials on human subjects. At the onset of the study, participants were given detailed information on the study processes, and the possible

risks and benefits were clearly explained to all study participants in Lao language. In case participants were illiterate or belonged to ethnic minorities, we had the chiefs of the villages, relatives or school teacher acting as a translator and witnesses. In particular, we made sure that each participant understood that he/she could receive a potentially unfavourable diagnosis and/or might experience drug-induced adverse events. If patients agreed to participate in the study, they voluntarily signed a consent form. In case of children under 18 years old, we obtained their assent and written consent of their patents.

At the end of the study, helminth egg-positive individuals who were enrolled in our study were treated with with the adequate drugs, i.e., praziquantel (40 mg/kg) and/or a single oral of ABZ (400mg) according to the national scheme for mass drug administration in Laos (Ministry of Health, 2004).

All participants with any precursor CCA and liver lesions were given additional counselling. They were informed about the diagnosis, its implication and the next possible diagnostic and treatment steps were explained. Additonally, informed consent form was required before any intervention took place.

All cases that had adverse events were offered an adequate treatment at the provincial hospital or health centre depending on the severity of their condition. Further details of informed consent are provided in each chapter.

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4. Three Cases of Intestinal Capillariasis in Lao People's Democratic Republic

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This article has been published in
The American Journal of Tropical Medicine and Hygiene. 2008 Nov;79(5):735-8

4.1 Abstract

Capillaria philippinensis is a rare zoonotic intestinal parasite that emerged in the 1960s. The outcome of intestinal capillariasis may be fatal if untreated in due time. We report three cases of intestinal capillariasis in Lao People's Democratic Republic (Lao PDR). The three patients were unrelated previously healthy young men (24, 26, and 27 years of age) with no underlying disease or immune depression. They had chronic diarrhea, abdominal pain, edema, and severe weight loss. Two of them acquired the infection in Thailand; the other patient had no travel history outside Lao PDR. All patients were seen several times in different hospitals before the diagnosis was made. All had concurrent parasite infections: *Giardia lamblia, Entamoeba histolytica*, *Strongyloides stercoralis*, *Opisthorchis viverrini*, and hookworm. The patients frequently consumed uncooked fish. After treatment with albendazole (400 mg/day for 21–30 days) all patients recovered. In Lao PDR, consumption of raw small freshwater fish is common. Therefore, the possibility of a capillariasis outbreak should be considered.

4.2 Introduction

Intestinal capillariasis, which is caused by *Capillaria philippinensis*, is a rare food-borne nematodiasis that was first described in The Philippines in 1962 and emerged during the 1970s. It has received little attention and may be frequently overlooked or confused with *Trichiuris trichuria* infection. However, *C. philippinensis* infection is remarkable because of its potential severity and fatal outcome in cases of delayed diagnosis or inappropriate treatment. Thus, approximately 2,000 cases with almost 200 deaths have been documented worldwide, mostly from Asia.[1] We report three cases of intestinal capillariasis in Lao Peoples' Democratic Republic (Lao PDR).

4.3 Case description

The patients were three unrelated previously healthy young men (24, 26, and 27 years of age). They showed no underlying disease or immune depression. All patients had non-febrile, chronic diarrhoea associated with abdominal pain, oedema, and severe weight loss. Two of them had low levels of serum protein, albumin, and cholesterol, but a normal level of haemoglobin. All patients were negative for human immunodeficiency virus, and one was positive for hepatitis B surface antigen. Detailed information on the three patients is shown in Table 4.1.

4.3.1 Case 1

A 24-year-old man from Vientiane. In May 2007, he reported an eight-month history of recurrent diarrhoea associated with colicky pain that started after returning from southern Thailand where he was a seasonal migrant worker. Infections with G*iardia lamblia*, *Trichomonas* spp., and *Trichuris trichiura* were diagnosed and he was treated with standard regimens. Two months later (10 months after symptom onset), the diarrhea persisted and he had a weight loss of 12 kg. He was then admitted to Setthathirath Hospital in Vientiane. Clinical examination showed subcutaneous fat and muscle wasting, moderate dehydration, pallor, a soft non-tender, non-distended abdomen, and a marked pitting edema of the lower limbs. The liver and spleen were not enlarged. Stool examinations showed numerous eggs (1–2 eggs/10 × 40 microscopic field) that were elongated and peanut-shaped with flattened bipolar plugs, striated shells (46.1 × 22.1 μm), and Charcot-Leyden crystals. Eggs were identified as those of *C.*

philippinensis. Adult or larval *C. philippinensis* was not found in stool samples of all patients.

Levels of serum protein and albumin were low (1.7 and 0.9 g/dL, respectively), and proteinuria was not detected. A chest radiograph and abdominal ultrasound did not disclose any specific abnormalities. Gastroscopy showed a non-specific duodenitis with superficial erosions. A biopsy specimen of the duodenum did not show any parasites or specific abnormalities.

The patient reported eating small raw or insufficiently cooked fresh water fish and prawns in Lao PDR and Thailand. All other family members were healthy without any diarrhea. A routine stool examination performed for all family members showed negative results (no *C. philippinensis* eggs detected). The patient was given a one-month course of albendazole (400 mg/day) and he made uneventful and stable recovery.

4.3.2 Case 2

A 26-year old man from Vientiane. In December 2007, he came to the emergency department at Mahosot Hospital in Vientiane with a one-month history of profuse watery diarrhea. He was extremely emaciated and had severe prolonged dehydration. He was admitted for further investigations and treatment. During the preceding month, the patient had been repeatedly seen in district and military hospitals in Vientiane for the same symptoms. No diagnosis was made, including after stool examination, and multiple and empirical treatment regimens were given that did not relieve the symptoms.

He had approximately 10 bowel movements per day of non-dysenteric, watery stools associated with abdominal pain, borborygmus, anorexia, and episodic vomiting. The patient reported a weight loss of 13 kg within the past 4 weeks. He admitted eating raw fish. Other family members remained free of symptoms. Two months before admission, he had returned from central Thailand where he had been a migrant worker for approximately one year.

On physical examination, he appeared emaciated, pale, and in poor general health. He had subcutaneous fat and muscle wasting, marked pitting edema on the lower limbs, a temperature of 36°C, low blood pressure (80/50), a heart rate of 119 beats/minute, and a respiratory rate of 25 breaths/minute. The abdomen was distended. Results of chest

and heart examinations were normal. The patient received intravenous fluids to correct dehydration and electrolyte imbalance.

Table 4.1 Laboratory investigations and treatments in three case-patients infected with *C. philippinensis*

Characteristic*	Case 1		Case 2		Case 3	
	First admission May 2007	Second admission July 2007	Follow-up October 2007	Admission December 2007	Follow- up January 2008	Admission September 2005
Hemoglobin (10.5-15 g/dl)	15	13.5	14.8	16.1	16	11.3
WBCs (4000-9000/ µL)	8,800	10,800	5,200	5,400	6000	8700
Neutrophils (50-65%)	65	68	60	50	55	50
Lymphocytes (20-40%)	30	35	39.3	48.5	44	17
Eosinophils (1-4%)	2	1	0	6	0	12
Serum creatinin (0.6-1.3 mg/dl)	1.2	1.4	1.1	1.3	1.2	ND
Glycaemia (75-115 mg/dL)	88	98	85.2	122	120	ND
Serum Na (135-445 mmol/L)	122	132	122	115	133.8	ND
Serum K (3.5-4.1 mmol/L)						
Serum Cl (95-105 mmol/L)						
TSP (6.0-8.5 g/dL)	1.7	1.7	7.2	1.5	6.5	ND
Serum albumin (3.5-5.0 g/dL)	0.9	2.3	3.7	2.1	3.4	ND
Cholesterol (119.7-220.1g/l)	104.2	173.2	169.2	112	159	ND
ALT (0-40 U/L)	18	18	20	22	25	22.4
Urine analysis	Normal	Normal	Normal	Normal	Normal	Normal
Stool examination result	Watery, WBC 100-150 / 40 power field, no RBC, mucus=1+ Stool culture negative	Watery, Absence of Stool culture negative		Watery, Stool culture negative		Watery
Parasites detected	*G. lamblia (cyst) Trichomonas sp. E.histolytica (cyst) T. trichiura*	*C. philippinensis*	Negative	*C. philippinensis, O. viverrini, hookworm*	Negative	*C. philippinensis O. viverrini S. stercoralis (larvae), hookworm*
Treatment	Antibiotics (metronidazole, ofloxacin), IV fluids and albumine, ABZ 400mg / day / 3 days	Albendazole400 mg p.o. for 30 days	ND	supportive care with IV fluid Albendazole 400 mg p.o. 30 days, PZQ 40 mg/ kg	ND	Albendazole 400 mg 21 days + PZQ 40 mg/ kg and supportive care

* Values in parentheses are normal ranges. ALT = alanine aminotransferase; TSP = Total serum protein; ND = not done

Stool examinations showed *C. philippinensis* eggs and parasitic co-infections (Table 4.1). A chest radiograph and an abdominal ultrasound were normal. Stool examinations were carried out for all family members; none showed parasitic infections. After a full month course of albendazole (400 mg/day), the patient fully recovered. At a one-month follow-up visit, weight gain (8 kg), negative stool examination results, and normal levels of serum albumin were noted.

4.3.3 Case 3

A 27-year-old man (farmer) from southern Lao PDR (Phine District, Savannakhet Province). He was seen in September 2005 at Savannakhet Provincial Hospital with chronic diarrhoea of six months duration. On admission, he had at least six daily bowel movements, persistent abdominal pain, and episodic vomiting. He was wasted and emaciated, moderately dehydrated, and reported a weight loss of 10 kg during the past 3 months.

The patient had no history of travel outside his province. After the start of illness, he visited district hospitals and a neighbouring hospital in Vietnam where no diagnosis was made. Treatment with several anti-infective drugs, including ampicillin, sulfamethoxazole, and metronidazole, did not improve his condition. Results of stool examinations by the Kato-Katz (KK) thick smear technique and a formalin-ether concentration technique (FECT) were positive for *C. philippinensis* (1,696 eggs/gram [of feces] [epg] by KK and 346 epg by FECT). Moreover, *Opisthorchis viverrini* (336 epg by KK and 58 epg by FECT; 9 adult worms isolated after treatment with praziquantel and purgation), *Strongyloides stercoralis* (9 larvae), and hookworm (408 epg by KK and 14 epg by FECT) were also present. The patient received albendazole, 400 mg/day for 3 weeks, and showed gradual and significant improvement. He was lost to further follow-up.

4.4 Discussion

The first case of capillariasis was diagnosed in 1962 in Luzon, The Philippines. Soon afterwards, several outbreaks were described in this country[2] and in Thailand.[3] Other Asian countries (Taiwan,[4] South Korea,[5] India,[6] and Indonesia[7]) reported sporadic cases, followed by Middle East countries (Iran,[8] United Arab Emirates,[9] and Egypt[10]).

These results suggested that *Capillaria* spp are present in more areas than initially thought. In addition, *C. philippinensis* infections were imported into Europe.[7–12]

The genus *Capillaria* is a nematode member of the superfamily Trichinelloidea, which includes *Trichuris* spp. and *Trichinella* spp.[13] Among some 250 different *Capillaria* species, only three are human pathogens (*C. hepatica* and *C. aerophila*, which cause liver and a tracheo-bronchial disease, respectively, and *C. philippinensis*, which affects the intestinal tract). *Capillaria philippinensis* is the only species that causes severe disease. It may be endemic but also can cause epidemics.

The life cycle of *C. philippinensis* involves a small fresh water or brackish water fish harbouring the infectious stages in their viscera. Natural definitive hosts are fish-eating birds. Human become infected when they consume raw or insufficiently cooked fish or, less often, ichthyophagic birds or fish eating birds. Handling fish under poor sanitary conditions may contaminate other foods and favor indirect transmission.[14] In humans, the parasite is not opportunistic (disseminated) but is usually restricted to the small intestine where it develops, reproduces, and where the female lays eggs that mature into larvae. A notable feature is that these larvae may engage in a shorten auto-infection circle, which may produce high parasitic loads.

The pathophysiology of this nematode is largely unknown, especially with regard to detail mechanisms of malabsorption. Parasitic products may interfere with ionic exchanges and carbohydrate and protein absorption of the intestinal epithelium, which produces a protein-losing enteropathy that is an outstanding feature of capillariasis.[15,16]

Intestinal capillariasis has been known since 1973 to occur in Lao-neighbouring Thailand.[3,17] It has been previously reported in travellers to Thailand.[14] Two of our cases had stayed for several months in this country. This finding indicated that that they were infected in Thailand. However, one case from rural southern Lao PDR had no travel history out of this country. He must have been infected in Lao PDR, which documents that transmission of *C. philippinensis* infection also occurs in this country. Lao PDR and Thailand share many environmental, epidemiologic, and cultural features. In particular, the widespread habit of eating raw or insufficiently cooked fish, the main risk factor for infection with *C. philippinensis*, is common in both countries.

To our knowledge, the cases reported here are the first to be documented in Lao PDR. The patients were active healthy men with no underlying disease or immune deficiency.

Clinical and laboratory features were comparable to those initially described in The Philippines [2] and elsewhere, i.e. chronic watery diarrhoea, abdominal pain, and notably pronounced weigh loss and pedal oedema, the latter as part of a malabsorption syndrome. The diagnosis was easily achieved by finding ova of characteristic size and shape in stool specimens. However, *C. philippinensis* eggs may be sparse or inconstantly shed in the stools. They also have a similar appearance to those of *T. trichiura* in size and peanut shape with two polar opercula, and are often mistaken for those of the latter species.

The clinical presentation of capillariasis may be confused with those of other protozoans (*Entamoeba histolytica*), nematodes (*S. stercoralis*), trematodes (*Fasciolopsis buski*), or opportunistic parasites associated with acquired immunodeficiency syndrome (*Microsporidium* spp.). In addition, capillariasis may be confused with other chronic diarrhoeas of unclear origin such as tropical sprue, collagen colitis, adult celiac, or Crohn disease. Associated hypovolemic cardiac failure has also been described.[18] It is important to note that intestinal capillariasis even when properly recognized and treated recurs frequently because of auto-infestation or reinfection.

All three patients experienced clinical and biologic recovery (negative stool results for parasites, serum albumin levels returning to normal values) after completing the 3–4-week regimen of albendazole. To avoid frequent relapses, high dosages and prolonged (3–4 weeks) or sequential treatments have been advocated.[19] Several anti-helminthic drugs have been recommended for treatment of intestinal capillariasis. All compounds of the azole family of drugs are effective. However, several studies[7,13,19-22] have consistently recommended albendazole as the drug of choice.

Unawareness and delayed diagnosis may cause serious medical problems. This issue has been documented in South Korea, Egypt, and India, and may even be fatal (fatality rate up to 30% in an outbreak in The Philippines), which is an exceedingly uncommon outcome for other nematode infections.[5,10,16,23,24]

Finally, food-borne parasites are an emerging public health problem, particularly in Southeast Asia.[25] Intensified production of fish farming and the persisting habit of eating raw or undercooked fresh water fish contribute to increased transmission rates, including *Capillaria* spp.[1,4,6,26] As an example, in Lao PDR, despite increased development, as many as 75% of the population still eat traditional raw fish dishes[27]

such as Koy Kung (prawn salad), Koy pa or lap pa (minced fish salad with spices), and Koy pa siew (raw *Rasbora borapetenis*). Therefore, *C. philippinensis* deserves particular attention in this and neighbouring countries that have similar environments and traditional behaviour patterns. Because this parasite is endemic in this region, the occurrence of outbreaks cannot be excluded.

4.5 Acknowledgments

We thank the patients, and doctors, laboratory staff, and infectious ward staff at Setthathirath Hospital, Mahosot Hospital, Savannakhet Provincial Hospital, and the Department of Parasitology, University of Health Sciences, Vientiane, Lao PDR, for their participation in the study.

4.6 Financial support

This study was supported by the Swiss National Science Foundation and Swiss Agency for Development and Cooperation (project no. NF3270B0-110020), the Canton of Basel-Stadt, Switzerland, and the Institut de la Francophonie pour la Médecine Tropicale, Vientiane, Lao PDR.

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5. Severe Schistosomiasis Mekongi in Southern Lao People's Democratic Republic

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This article has been prepared for submission to
PLos Neglected Tropical Disease

5.1 Abstract

***Background*.** In 2007, within the context of a community-based survey on helminth infections in three villages we identified severe cases of schistosomiasis mekongi in Southern Lao People's Democratic Republic.

***Methodology*.** We identified and treated nine patients with praziquantel and followed them for three years performing annually a clinical and parasitological examination, and ultradonographical assessment. Our report includes photo documents of the severe *S. mekongi* patients.

***Results*.** Mean age of patients was 36 years (range: 5 - 66 years); 7 patients were male. The leading clinical features were cachexia, hepatosplenomegaly, ascites, splenic varices and rupture of oesophageal varices. Patients were co-infected with hookworm (n=7), *Opisthorchis viverrini* (n=6), and *Strongyloides stercoralis* (n=1). Three years follow-up and treatment showed improvements in three of them (case 5, 6, 9), the status of one adult patient remained unchanged (case ?) and one worsened (case ?). Two patients (case 4, 7) died due to oesophageal bleeding.

***Conclusions*.** Liver pathology improved after treatment in young patients. Young children affected with severe *S. mekongi* infections document that transmission is ongoing. Current measures employed in Laos' endemic areas are insufficient to stop transmission and eliminate schistosomiasis. A long-term control intervention including access to treatment and case management, health education, sanitation and infrastructure is warranted.

***Keywords*.** *Schistosoma mekongi*, case-report, Lao PDR, hepatosplenomegaly

5.2 Author summary

The willow-leaflike, blood fluke, *Schistosoma mekongi* is a chronic parasitic diseases living inside the mesenteric vein of human. *S. mekongi* is focally limited epidemiology in the Lao PDR and Cambodia. It remains exclusively a major public health in this region. Since the largest effort of schistosomiasis control, using praziquantel, the prevalence of its infection was dramatically reduced and considered as under control among villagers in Khong Island and along the outlying Mekong. We aimed to report on nine severe *S. mekongi*, determined during a community-based survey on helminth infections from 2007 to 2010 on three Khong islands in the district of Kong, southern Champasack province. Among nine patients, male was predominant sex. Mean age was 36 years. All patients had dwelled in Khong Island since birth. Patients had previously been treated with several praziquantel rounds. Most of patients developed hepato-splenic disease. Three patients improved after treatment, two adult patients remained unchanged or the status worsened. Two patients died due to digestive bleeding. Liver condition improved after treatment in particular in young aged patients. This case report raises awareness of the re-emerging of *S. mekongi* in this region. Hence schistosomiasis-morbidity control requires urgently attention.

5.3 Introduction

Schistosomiasis is a most common parasitic infection in human caused by blood flukes of the *Schistosoma* genus. Schistosomiasis-related morbidity poses a significant public health problem in endemic settings [1, 2]. More than 800 million people are at risk of infections, 200 million people are infected and 10% of whom develop severe hepato-splenic morbidity [3]. *Schistosoma mekongi* has been identified first in the late 1960ties. It is exclusively confined in communities along the Mekong and its tributaries in several districts in the northern provinces of Kratié and Stung Treng in Cambodia and southern province of Champasack in Lao People's Democratic Republic (Laos, Lao PDR). An estimated 140,000 people are at immediate risk for *S. mekongi* infection [4]. Chronic inflammation due to *S. mekongi* leads to a fibrotic liver disease, which is associated with fatal consequences of portal hypertension and digestive bleeding by the eruption of oesophageal bleeding. It was frequently observed in the endemic communities. In the 1990ties substantial control efforts were undertaken which dramatically reduced infection and morbidity. Since 2005 schistosomiasis mekongi is considered under control [5]. Here we report on nine severe *S. mekongi* patients from southern Lao PDR diagnosed, treated and followed from 2007 to 2010.

5.4 Methods

5.4.1 Study area

Khong district is the most southern district of Champasack province in Laos, and bordering Cambodia. It has 131 villages most of which are located on islands in the Mekong river. The district has a total population of 55,000 inhabitants. The majority belongs to the Lao Loum ethnic group. They are rice farmers and engaged in fishering, and tobacco planting. A recent study on helminthic infection in Khong Island showed *Opisthorchis viverrini* and *S. mekongi* infection's prevalences were 92 and 68%, respectively [6].

5.4.2 Ethical consideration

The study was approved by the Ethics Committee of Basel (no. 255/06) and National Ethics Committee, Ministry of Health (MOH) in Vientiane (no. 027/NECHR). Permission

for field work was obtained from the MOH, and Provincial and the District Health Offices. Prior to enrolment, all patients signed an informed consent form. Parents or legal guardians signed for children (< 16 years).

5.4.3 Identification and follow-up of patients

Patients were identified during a community-based survey on helminth infections in 2007-2009 on three Khong islands (Don Long, Don Kaden and Loppadi) in Khong district [6]. Each year the patients were traced for a follow-up visit. At each visit a detailed clinical and ultrasonographical examination was performed including an interview. Stool samples of two consecutive days were examined on the presence and intensity of helminth infection using the Kato-Katz method [7]. The infection intensity of *S. mekongi* were classified as negative (0), mild (1-100 eggs per gram stool [EPG]), moderate (101-400 EPG), heavy (501-999 EPG) and very heavy (> 1000 EPG) [8]. *O. viverrini* infections were grouped in negative (0), light (1-500 EPG), moderate (501-999 EPG), heavy (1000-1999 EPG) and very heavy (≥ 2000 EPG) infection intensity [9].

Information on anaemia, liver function and co-infection with hepatitis B (HBV) and C (HCV) was obtained from a serum sample examination. All patients were interviewed on their history of bloody stool, onset of disease, family history of deaths due to schistosomiasis and the treatment with praziquantel (PZQ), and their water contract habits the Mekong river.

Liver pathology was assessed about four hours after meal in supine position using an ALOKA SSD-900 (3.5 MHz convex probe) portable ultrasonography machine. Liver image pattern (IP) and periportal fibrosis (PPF) was scored and graded according to the Niamey protocol [10]. Patients were grouped in liver patterns A-F, depending on the extent of visible PPF around the portal bifurcation and in liver parenchyma. Grades A and B were considered normal patterns, whereas grades C, D, E, and F were manifestations of liver disease with increasing severity. The size of the left liver lobe (SLL) was measured with a longitudinal liver scan. The measurements were taken in the left parasternal line from the upper to the caudal margin. The portal vein diameter (PVD) was measured in the right oblique view along the axis of the vessel with measurement of the internal diameter of the portal vein at the entry point into the liver.

The size of spleen was measured in the left intercostals oblique view with the maximum length by measuring through the splenic helus. All organ measurements were height-adjusted [11]. The organs were considered abnormal if the height-adjusted value exceeded two standard deviations (2SD) of the reference population (e.g., 0-2SD normal; 2-4SD enlarged and > 4SD much enlarged). Periportal hypertension (PH) score was calculated from the sum of three indicators [11]: (i) height-adjusted value of PVD (0 normal, 4 enlarged and 6 marked enlarged), (ii) presence of collateral veins (0 absence, 4presence), and (iii) presence of ascites (0 absence, 3 presence). The sum of these scores was categorized into four groups: 0 normal, 4 light hypertension, 6–8 moderate hypertension and 10–13 severe hypertension.

5.5 Case description

In Table 5.1 details of the examinations of the nine patients is displayed. They had a mean age of 36 years (range 5 – 66 years). Seven patients were male. Generally, at the first visit patients showed markedly deprived general and nutritional status with signs of portal hypertension (collateral circulation on abdomen), moderate to advanced splenomegaly and several with ascites. Two patients reported an episode of rupture of oesophageal varices. Ultrasound assessment revealed marked splenic varices, advanced ascites, present thickening of portal vein walls, and pronounced hepato-splenomegalies. In all patients except one an infection with *S. mekongi* could be confirmed by stool examination. Co-infections with hookworm (n=7), *O. viverrini* (n=6) and *Strongyloides stercoralis* (n=1) were diagnosed but no HBV and HCV infection was found.

5.5.1 Case 1

In March 2007 a 66-year old farmer (Figure 5.1) vomited a large amount of blood and emitted blood in stool. He was admitted to Khong district hospital. On admission, he was in shock, unconscious (temperature 35.5°C; pulse rate 108 pm; blood pressure 90/60 mmHg) and highly anaemic (Hb 5 mg/dl). No cervical lymphadenopathy was noted. Abdominal palpation revealed an ascites, and a hepatosplenomegaly. In February 2008, physical examination showed poor condition, distended abdomen with a further developed ascites, and venous collateral veins. He was anorexic and had lost weight.

Abdomen had no palpable abnormality. Six months later he deceased in an episode of severe hematemesis.

Figure 5.1: Case 1 found in March 2007

5.5.2 Case 2

A 46-year old farmer presented in 2007 with an ascites (Figure 5.2a). He reported that it developed over several years. He had no history of gastrointestinal bleeding. His younger sister suffered from the similar condition (case 5.4). He had no other illnesses or complaints. He reported to consume alcohol occasionally. He consulted two hospitals in Vientiane where a bypass operation was proposed, which he refused. On physical examination (2007) he showed pale conjunctivae, no jaundice, circulation collateral, ascites, splenomegaly, no other abnormalities were found. In 2009, the physical examination was unaltered (Figure 5.2b). In 2010 his condition had critically worsened with enlargement of abdomen and occurred liquid flowing from the umbilicus (Figure 5.2c,d). The patient and surrogates refused recommended treatments and proposed interventions. The patient's resistance was due to his younger sister (case 5.4), who underwent surgical intervention and died shortly afterwards.

5.5.3 Case 3

In March 2007 this 45-year old farmer (Figure 5.3) reported to suffer from a swollen abdomen since the age 9 years. He reported that on his island at that time several of his fellow children had the same symptoms. During childhood he was treated twice with anti-parasitic drugs (twice: 2 tablets x 2 days), thereafter he felt better.

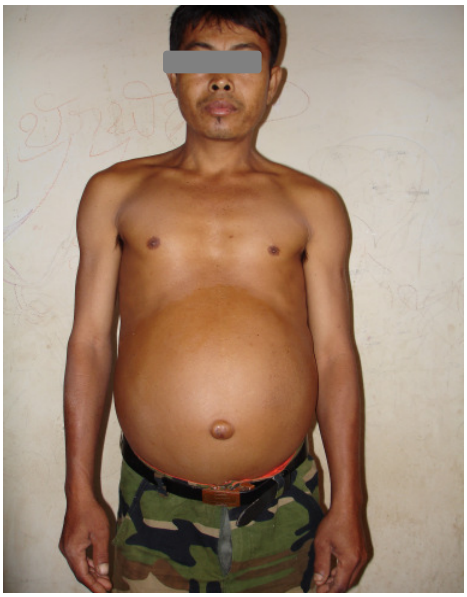

Figure 5.2a: case in March 2007

Figure 5.2b: case in March 2009

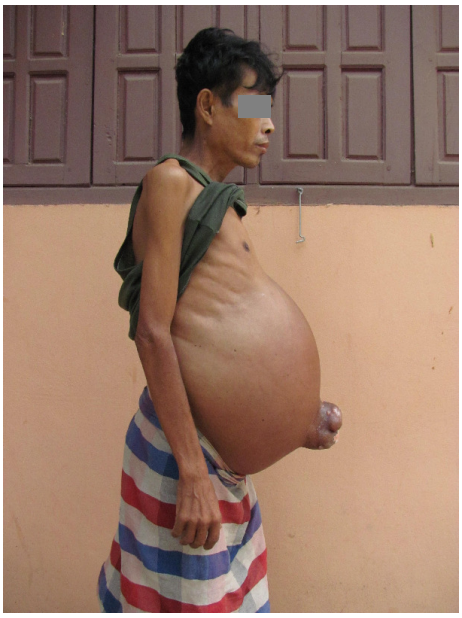

Figure 5.2c: case in March 2010

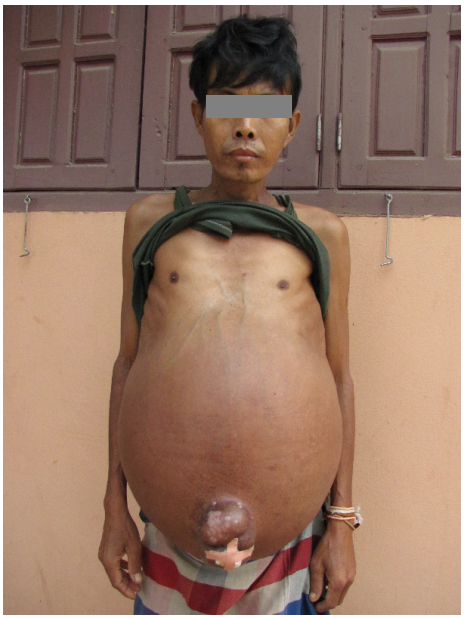

Figure 5.2d: case in March 2010

Ten years ago venous collateral veins started to appear on his belly. He repeated anthelmintic treatments including PZQ two more times since then. On examination he was in good general condition but with marked spider angiomas (Figure 5.3), no ascites, but nodular liver (4 cm) was detected below costal margin and spleen extended (4 cm)

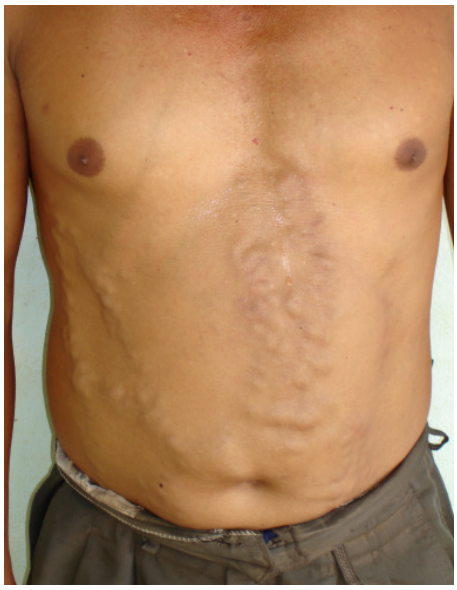

Figure 5.3: case 3 in March 2007

beyond the costal rim. The US of the abdomen revealed liver parenchyma with highly echogenic ruff around portal bifurcation and main stem (pattern D), splenomegaly and splenic varices present. The patient was lost to follow-up.

5.5.4 Case 4

In 2007, the 36-year old sister of case 2 (Figure 5.4) presented with an ascites. Clinical examination revealed general weakness, pallor with a severe anaemia (Hb: 7 mg/dL), markedly enlarged and distended abdomen with long surgery scars. The liver was not palpable. She underwent a bypass surgery at a central hospital in Vientiane in 2004. She reported no improvement after the intervention but an increase her ascites. In early 2007, she suffered from a severe episode of haematemesis and melaena. She was admitted to the provincial hospital of Paksé and after one week referred to Mahosot hospital, Vientiane, where she received a blood transfusion (totally 7 units). In Mars 2007 she died during an episode of haematemesis.

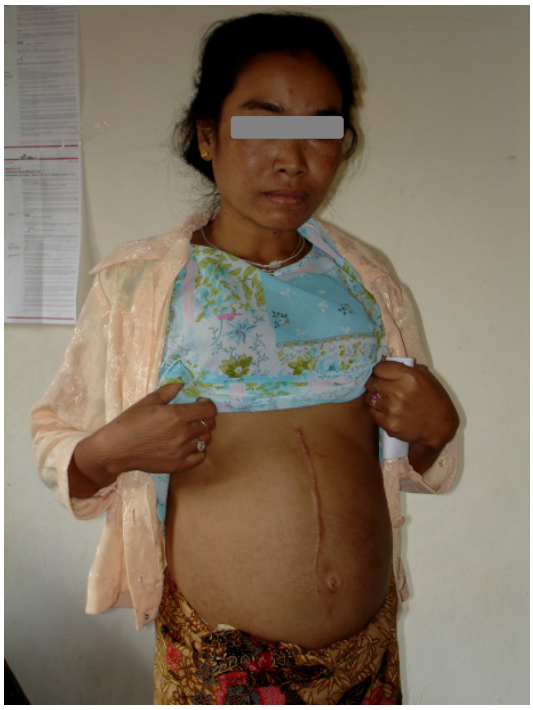

Figure 5.4: case 4 in March 2007 (a sibling of case 2)

5.5.5 Case 5

The 27 years old son of case1 had no history of bloody diarrhoea, abdominal pain or jaundice of gastrointestinal bleeding, and did not receive anti-*Schistosoma* treatment before. In February 2008, he presented to the village health staff with complaints about abdominal swelling. He reported right upper quadrant pain (temperature was 37°C, pulse rate 66 pm; respiration rate 22 pm; blood pressure 100/60 mmHg). On examination he had anaemic conjunctivae, collateral circulations, an ascites, and hepatosplenomegaly. He was treated (PZQ 40 mg / kg BW; albendazole (ABZ) 400 mg single dose). In April 2009 his condition improved. US showed a lower liver fibrosis pattern (D, earlier F), and absence of ascites and hepatosplenomegaly. Stool examination revealed an infection with *O. viverrini* and hookworms. He was again treated with anti-helminthic drugs. In April 2010 his general condition was good, and better than a year before.

5.5.6 Case 6

This 12-year old boy (Figure 5.5a) is a resident on the island since birth. In February 2007 he presented with an ascites. The illness started at age of 5 years with repeated episodes of blood in stool. Soon after, his ascites gradually developed. He reported to be treated with (1.5 tablets of praziquantel) by health workers but his condition did not improve. On physical examination poor general nutrition status, pale conjunctivae, protruding belly button, collateral circulation, abdominal distend and painful was noted. Abdominal sonography disclosed hepato-splenomegaly, liver parenchyma with highly echogenic patches extending from the main portal vein and branches to the periphery (pattern E), plenty of free fluid in abdomen, splenomegaly and varices. In 2008 the patient was well and much better with diminished abdominal distension (Figure 5.5b). Ultrasound showed splenomegaly, liver parenchyma with highly echogenic ring echoes or pipe stems (pattern C), absent ascites, varices present. In 2010, stool examination showed a re-infection with *O. viverrini* and hookworms. US examination of the liver did not show constantly liver parenchyma and splenomegaly but varices at the splenic hilum were rather improved than the year recently. No ascites was detected (Figure 5.5c-d).

5.5.7 Case 7

A 13-years old boy (Figure 5.6a-b) reported to swim twice a day in the Mekong River. He was diagnosed during a follow-up visit in 2009. As a one-year old boy, his mother noticed that his abdomen had swollen. He had no history of serious gastrointestinal pain. He received a treatment (unknown) in a nearby Cambodian health centre which did not improve his condition. Physical exam revealed that he looked weak and malnourished with being underweight (BMI, 14). Spleen extended beyond the costal rim (3 cm) and hepatomegaly was palpable. The boy was severely anaemic (Hb: 3.4 mg/dl). US examination detected a markedly ascites, hepato- and splenomegaly with dilated splenic varices. Stool examination revealed infections with *S. mekongi*, *O. viverrini* and hookworms. The boy was treated with PZQ (40 mg/kg BW) and ABZ (400mg/day/3 days). His condition improved significantly (Figure 5.6c-d). In 2010, on physical examination a splenomegaly remained. A re-infection with *O. viverrini* and soil transmitted helminths was retreated.

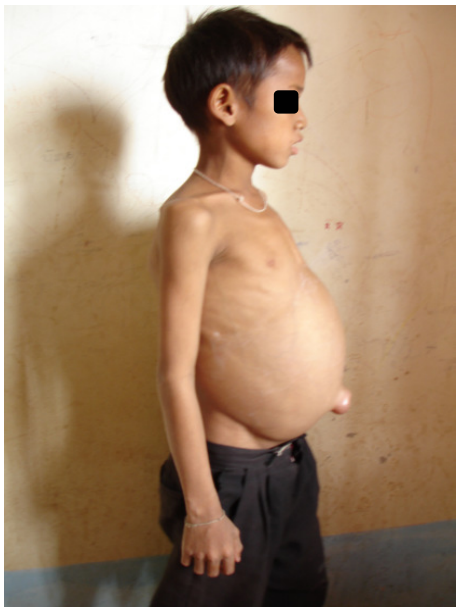

Figure 5.5a: Case 6 in March 2007

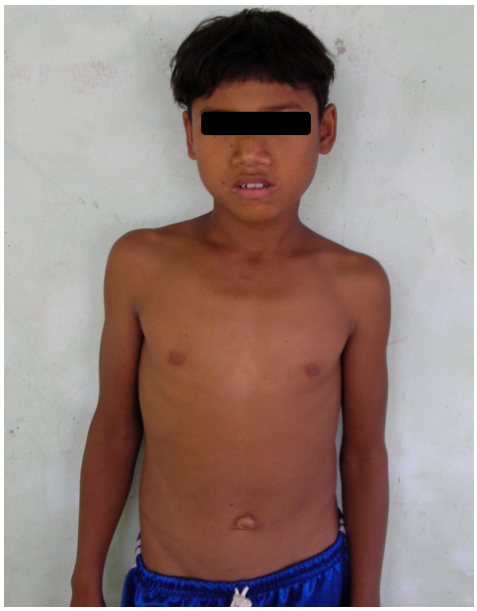

Figure 5.5b: case 6 in March 2008

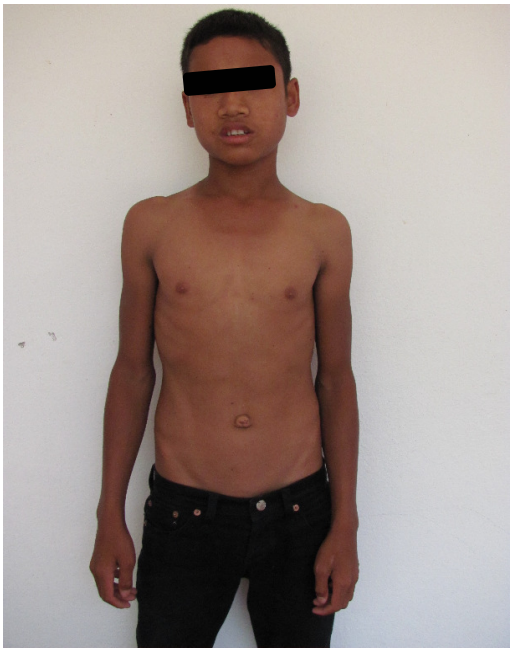

Figure 5.5c: case 6 (Face) in March 2010

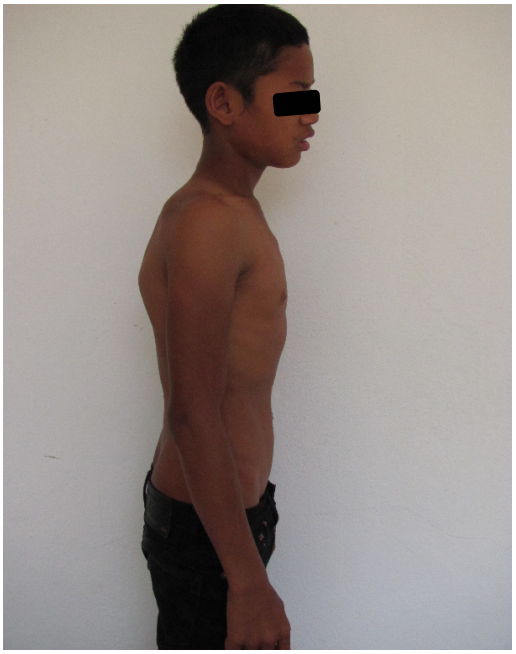

Figure 5.5d: case 6 (profile) in March 2010

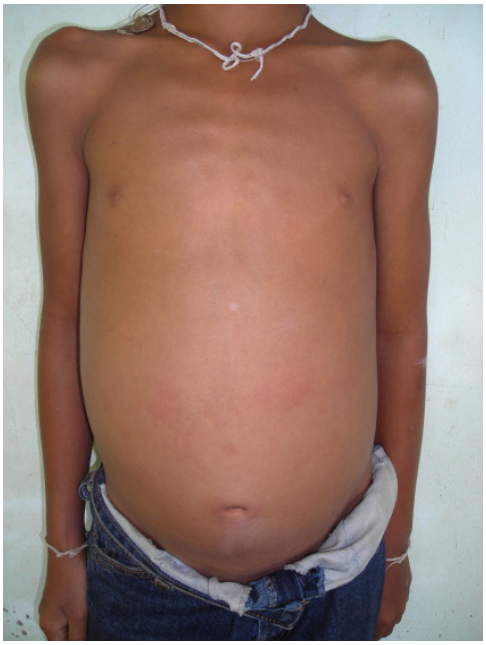

Figure 5.6 a: case 7 in 2009

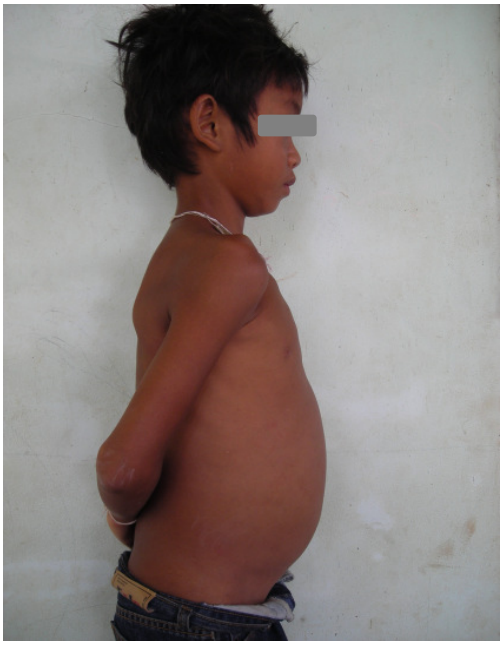

Figure 5.6 b case 7 in 2009

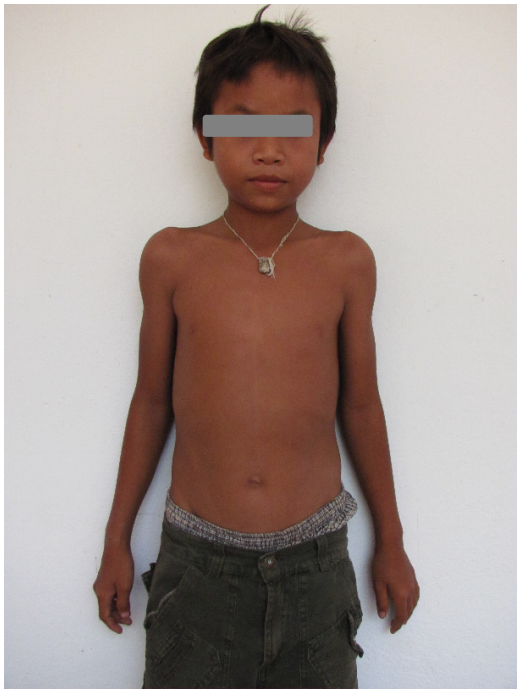

Figure 5.6c: case (face) 7 in 2010

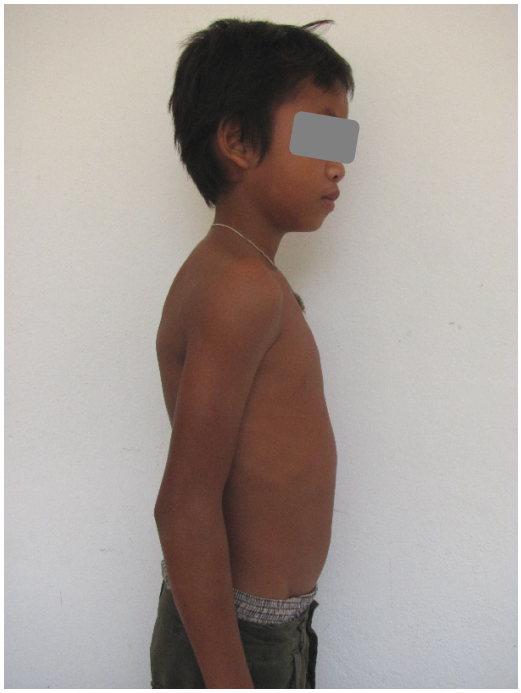

Photo 5.6d: case 7 (profile) in 2010

5.5.8 Case 8

A 6-year old girl (Figure 5.7 a-b) reported frequent baths in the Mekong since early childhood. She reported frequent episodes of blood in stool, diarrhoea and abdominal pain. As a one year old, she developed an abdominal swelling. Clinical examination showed no abnormalities. Ultrasound examination revealed an ascites, a splenomegaly and splenic varices. In 2010, she was again infected with *O. viverrini* and hookworms. US examination showed a marked improvement since a year before (Figure 5.7 c-d).

5.5.9 Case 9

A 5-year old boy (Figure 5.8a-b) also reported frequent baths in the Mekong since early childhood. She reported frequent episodes of blood in stool, diarrhoea and abdominal pain. In early childhood she developed an abdominal swelling. Clinical examination showed no abnormality. US examination revealed an ascites, a splenomegaly and splenic varices. He could not be found in the later visits between 2008 and 2010.

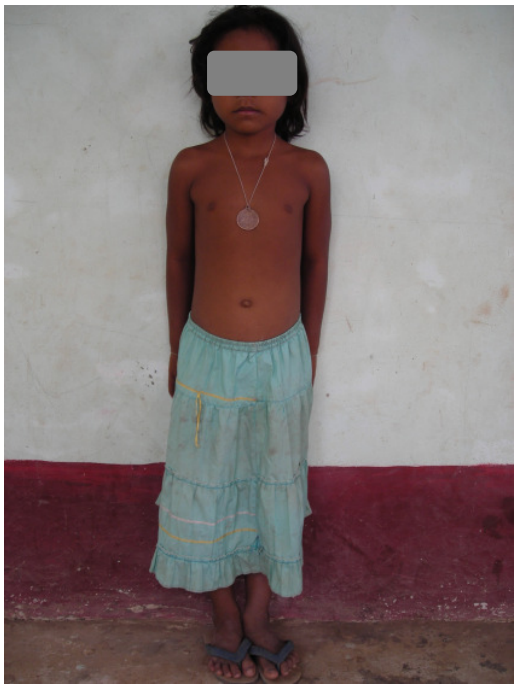

Figure 5.7 a: Case 8 (face) in 2009

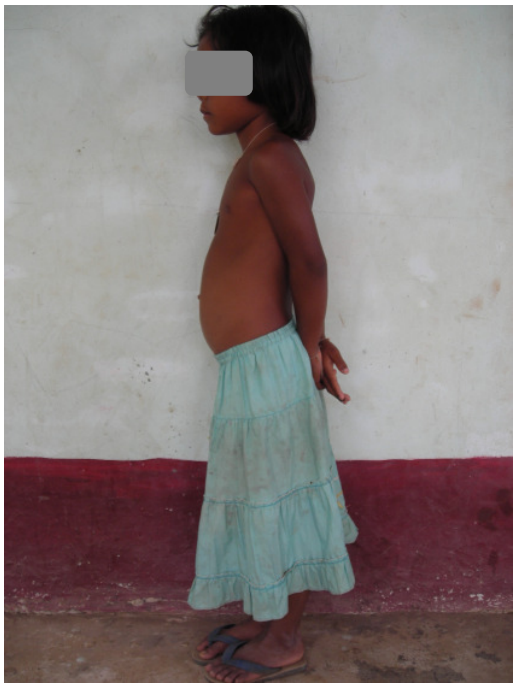

Figure 5.7 b: Case 8 (profile) in 2009

Figure 5.7 c: Case 8 (profile) in 2010

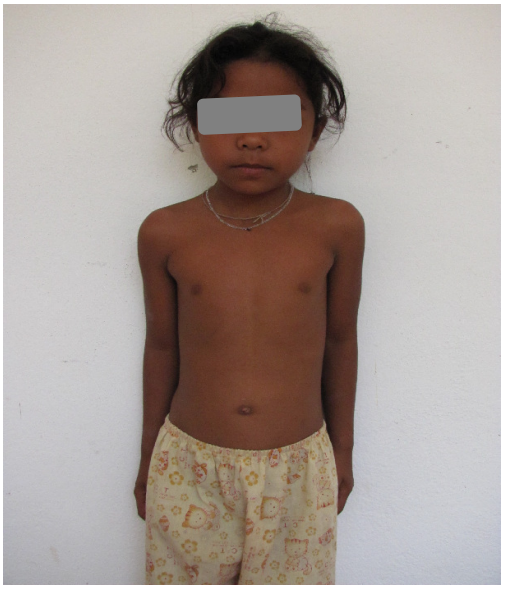

Figure 5.7 d: Case 8 (face) in 2010

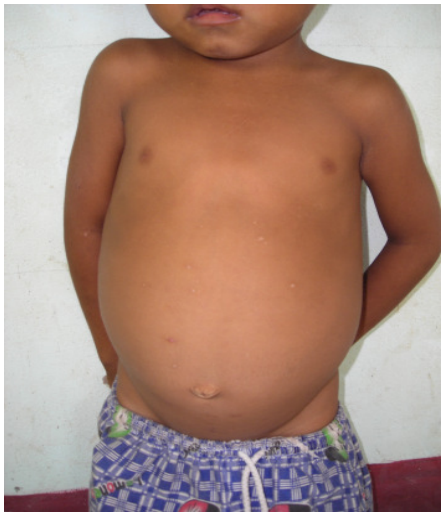

Figure 5.8 a: case 9 (face) in

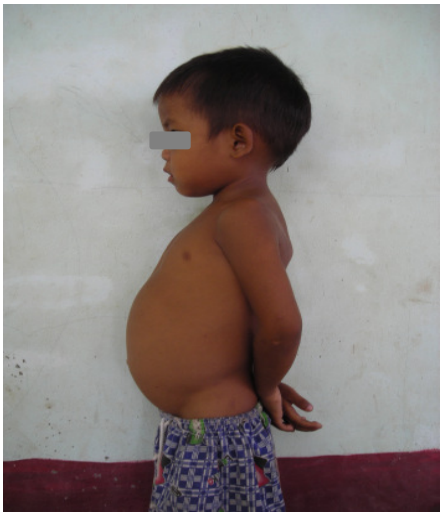

Figure 5.8 b: case 9 (profile) in 2009

TABLE 5.1: Laboratory, stool examination and ultrasonography results and treatment of nine severe *S. mekongi* patients, Khong District, 2007-2010

	Case 1 (male, 66y)	Case 2 (male, 46y)			Case 3 (male, 45y)	Case 4 (female, 36y)	Case 5 (male, 27y)		
Year	2007 (died in 2008)	2007	April 2009	April 2010	2007	2007 (died 2008)	2008	2009	Follow-up 2010
Hb (10.5-15 g/dL)	5	-	9.3		12.8	7.0	-	13.5	-
WBCs (4000-9000l)	-	-	16.1		-	-	-	18.800	-
Neutrophils (50-65%)	-	-	60		-	-	-	65	-
Lymphocytes (20-40%)	-	-	45		-	-	-	32	-
Eosinophils (1-4%)	-	-	3		-	-	-	4	-
r- GT (0-40 U/L)	-	-	20		-	-	-	32	-
T- BILI (0-1.2 mg/dl)	-	-	1.03		-	-	-	0.98	-
AST (0-40 U/L)	-	-	58		-	-	-	44	-
ALT (0-40 U/L)	-	-	32		-	-	-	8.31	-
AKP (40-150 U/L)	-	-	35		-	-	-	88.32	-
HBV	-	-	Neg		-	-	-	Neg	-
HCV	-	-	Neg		-	-	-	Neg	-
Stool Exam	*S. mekongi* (666 epg), *O. viverrini* (371 epg) Hookworm (165 epg)	ND	hookworm (83 epg)	ND	*S. mekongi* (577 epg)	ND	*S. mekongi* (774 epg), *O. viverrini* (340 epg) hookworm (6,510 epg)	*O. viverrini* (456epg) Hookworm (8,240 epg)	*O. viverrini* (672 epg) hookworm (4,176 epg)
Ultrasound examination	Pattern E, portal vein 16 mm, enlarged spleen, splenic varices	Pattern F, Ascites absent, splenomegaly marked splenic varices	Pattern F Ascites, hepato- and splenomegaly absent, marked splenic varices	Pattern F Markedly ascites, hepato- and splenomegaly absent, marked splenic varices	Pattern D (Dc), fatty liver, splenomegaly, varices shunt	Pattern F Ascites present, enlarged spleen, splenic varices present	Pattern F, Ascites present, enlarged spleen, marked splenic varices	Pattern D, ascites absent, enlarged spleen, splenic varices	Pattern merely C, ascites absent, enlarged spleen, splenic varices
Evaluation	_	--	constant	Worse	-	-		Improved	Improved
Treatment	PZQ (40 mg/ kg BW), ABZ (400mg / day / 3 days) in 2006 supportive care with IV fluid and transfusion	Lasix 25 mg daily	ABZ (400mg / day / 3 day) Lasix 25 mg daily	ND	PZQ (75 mg/ kg BW)	Not given	PZQ (75 mg/ kg BW), ABZ (400mg / day / 3 days)	PZQ (75 mg/ kg BW), ABZ (400mg / day / 3 days)	PZQ (75 mg/ kg BW), ABZ (400mg / day / 3 days)

ND Not Done, PZQ praziquantel, ABZ albendazole, BW body weight; IP score: Pattern A Normal texture; Pattern B Starry sky; Patter C highly echogenic "rings and pipe stems", Pattern D highly echogenic "patches" extending from the main portal vein and branches to the periphery; E highly echogenic bands and streaks, extending from the main portal vein and branches to the periphery; Pattern F highly echogenic bands and streaks, extending from the main portal vein and bifurcation to the liver surface, retracting the surface "bide claw" pattern.

TABLE 5.1: Continued

	Case 6 (male, 12y)			Case 7 (male, 13y)		Case 8 (female, 6y)		Case 9 (male, 5y)
Year	2007	April (2009)	2010	2009	2010	2009	2010	2007
Hb (10.5-15 g/dl)	-	12.5	-	3.4	-	8.0	-	7.0
WBCs (4000-9000l)	-	10.000	-	7.000	-	9.500	-	-
Neutrophils (50-65%)	-	65	-	60	-	68	-	-
Lymphocytes (20-40%)	-	34	-	40	-	35	-	-
Eosinophils (1-4%)	-	2	-	6	-	4	-	-
r- GT (0-40 U/L)	-	31	-	25	-	28	-	-
T- BILI (0-1.2 mg/dl)	-	0.88	-	1.11	-	0.8	-	-
AST (0-40 U/L)	-	38	-	58	-	40	-	-
ALT (0-40 U/L)	-	33	-	32	-	33	-	-
AKP (40-150 U/L)	-	89	-	31	-	38	-	-
HBV	-	Neg	-	Neg	-	Neg	-	-
HCV	-	Neg	-	Neg	-	Neg	-	-
Stool Exam	*S. mekongi* (477 epg), *O. viverrini* (334 epg) hookworm (367 epg)	*O. viverrini* (4,120 epg), hookworm (289 epg)	*O. viverrini* (264 epg), hookworm (12,000epg)	*S. mekongi* (99 epg), *O. viverrini* (462 epg) hookworm (15,0810 epg)	*O. viverrini* (224 epg) hookworm (16,200epg) *Ascaris lumbricoides* (2,200 epg)	*S. mekongi* (88 epg), *O. viverrini* (660 epg), hookworm (6,600 epg)	*O. viverrini* (14,800epg) hookworm (14,640epg)	*S. mekongi* (2,400 epg) *O. viverrini* (231 epg) *S. stercoralis (larvae),* hookworm (231 epg)
Ultrasound examination	Pattern E Ascites present, hepato-splenomegaly Marked splenic varices	Pattern C hepato-splenomegaly, moderate splenic varices Ascites absent	Pattern E Ascites present, hepato-splenomegaly Marked splenic varices	Pattern E Ascites present, enlarged spleen, marked splenic varices	Pattern E, Gallbladder sludge Ascites absent, hepato-splenomegaly, No varices	Pattern E Ascites present, enlarged spleen, splenic varices	Pattern C No ascites, hepato-splenomegaly Marked splenic varices	ND
Evaluation	-	Better	Improved	-	Improved	-	Improved	-
Treatment	PZQ (20 mg/ kg BW x 2 does, spaced 8 hrs interval), ABZ (400mg / day / 3 days)	PZQ (75 mg/ kg BW), ABZ (400mg / day / 3 days)	PZQ (75 mg/ kg BW), ABZ (400mg / day / 3 days)	PZQ (75 mg/ kg BW), ABZ (400mg / day / 3 days)	PZQ (75 mg/ kg BW), ABZ (400mg / day / 3 days)	PZQ (75 mg/ kg BW), ABZ (400mg single dose)	PZQ (75 mg/ kg BW), ABZ (400mg / day / 3 days)	PZQ (75 mg/ kg BW), ABZ (400mg / day / 3 days)

ND Not Done, PZQ praziquantel, ABZ albendazole, BW body weight; IP score: Pattern A Normal texture; Pattern B Starry sky; Patter C highly echogenic "rings and pipe stems", Pattern D highly echogenic "patches" extending from the main portal vein and branches to the periphery; E highly echogenic bands and streaks, extending from the main portal vein and branches to the periphery; Pattern F highly echogenic bands and streaks, extending from the main portal vein and bifurcation to the liver surface, retracting the surface "bide claw" pattern.

5.6 Discussion

To our knowledge, we reported on nine severe cases of *S. mekongi* from Southern Laos. These severe cases of *S. mekongi* were considered as the latest case report on *S. mekongi* from Khong district, Lao PDR, since the first *S. mekongi* case was discovered by Vic-Dupont and colleagues in 1957, France. Few severe *S. mekongi* cases have been documented from the Khong island although they have been seen. We diagnosed our patients during the community survey on parasitic infections. All patients were examined and follow-up was performed to document their improvements after treatment from 2007 to 2010.

Older patient pronounced demonstrating sequelae after treatment compared to younger aged patients. Most of younger patients tent to improve clinical and sub-clinical morbidity dramatically; however, liver, spleen and other lesions remained sequelae or a little subtly changed to unchanged especially disproportionately enlarged liver and its surface in severe cases were irregular and bosselated surface with slightly improved periportal fibrosis particularly cirrhosis like. The size and lesion of spleen were irreversible after treatment. Ascites were completely reversible similar to cases report from Cambodia [12, 13]. Some of our cases particularly in older patients may development to severe life-threatening disease and even death, others maintained sequelae with worsening. Children with severe disease (clinical and subclinical features) were documented ongoing transmission and its distribution within on island. The infection may widespread than we thought. Clinical management focusing on ultrasonography follow-up, stool examination and biological tests after first round of treatment with PZQ are required accordingly. Health services needs to improve preventive measures and clinical diagnosis and management.

Portal hypertension attributable to chronic schistosmiasis mekongi resulting in rupture of gastro-oesophageal varices is common in endemic settings. Generally, there are three main aetiologies of portal hypertension, including upper the liver, within the liver and lower liver which is mainly due to the mechanism of venous blood flow [1, 2]. Clinically hypertension is unable to detect since pressures are increased [2]. In areas where schistosmiasis is endemic, most of the severe schistosmiasis cases were assumed due to the complication of schistosmiasis [1-3, 5]. Other further evidence for association cause

of liver chronic infection due to virus (mainly hepatitis B and C) and alcoholism are not clearly elucidated [14]. However, evolution to a more severe form is a co-infection with hepatitis and alcoholism which were not observe in our patients.

S. mekongi has a complex life cycle. Eggs are released in the faeces and infect intermediate host snails, *Neotricula aperta,* possessing three strains (alpha, beta and gamma) [15]. *N. aperta* gamma-strain is the main intermediate host and distributed along the Mekong and its tributaries in southern Lao PDR. The molluscs shed cercariae which penetrate human skin. Buffalo, dogs and other mammals are suspected being reservoir animals but their contribution to the transmission is uncertain [15-17]. The peak of the transmission appears during the dry season during low–water period, especially from April to June [5, 18]. Human acquires infection by exposure to infected water bodies.

The first case of *S. mekongi* was discovered in a Lao immigrant to France in 1957 [19]. Subsequently, cases were diagnosed in Thailand and Cambodia. A foci of *S. mekongi* was discovered in 1968 in the province of Kratie, northern Cambodia, and described in 1995 [5]. An estimated 140,000 people are at immediate risk of *S. mekongi* infection in Cambodia and Lao PDR. The number of people at risk of *S. mekongi* in Khong Island and outlying villages was estimated to be as high as 60,000 people [5]. Clinical observations documented in the 1970s and extensive epidemiology surveys investigated in the 1980s [5] revealed that Lao people residing in Khong Island, Champasak province, were at high risk for *S. mekongi* infection. Subsequently a community-based intervention, carried out by WHO and the MOH of Lao PDR was carried out in the 1980s. Several mass drug administrations with PZQ (40 mg/kg BW) were executed in the target villages where the infection prevalence was beyond 25-50% [17]. This program was effectively reducing infection prevalence in villages along the Mekong in Khong district. However, the mass drug administration could not interrupted transmission [12]. Recent reports on *S. mekongi* infection revealed the prevalence was exceeded 68% in some villages in Khong Island [6, 12].

The control of schistosmiasis mekongi is similar to those in other schistosome species (*Schistosoma japonicum, S. haematobium and S. mansoni*). Preventive chemotherapy is

usedwith single treatment of PZQ. In addition, information, education and communication campaigns aim to reduce water contact and contamination by changing human behaviour. Improving sanitation and access to safe water supply is performed whenever economic conditions permit [5, 20].,. The elimination of schistosmiasis mekongi may be feasible in light of restricted geographical distribution. However, intersectorial approaches are required to achieve this goal [18, 20].

Our case series diagnosed in the last few years document that *S. mekongi* transmission is still ongoing, particularly on the Khong Islands where most of our cases reside. Of particular epidemiological importance are our observations on the youngest cases. They had no travel history and therefore had acquired the parasite in their residential villages, documenting that the transmission is on-going. We have diagnosed and followed patients with severe disease. Patients were predominantly males. Their age ranged from 5 to 66 years. The patients presented with symptoms associated with hepatic pathology. Dominant signs were ascites and other symptoms and signs associated with portal hypertension such as collateral veins, and splenic varices. Two patients (aged male, 66 and f, 36 years) died in the course of our follow-up due to rupture of oesophageal varices.

Our cases were similarly to those described in the period when the parasite was discovered. Of nine cases, four patients were children [19]. All of them were originating from the Mekong Islands. Six cases (case 2&6, case 1&5, case 7&8) were from the same family. The majority cases (5 of 9) were diagnosed with an ascites. It is often being the most obvious sequelae resulting in chronic and severe *S. mekongi* infection. The consequence of *S. mekongi* liver disease is thrombocytopenia with the risk of bleeding. Two of our cases died (case 1 & 4) due to the complication of the rupture of oesophageal varices. Case 4 had a splenectomy which did not improve her condition but other patient it did [21]. However, it has been shown to be less effective and having limited benefit to this patient [5, 19].

Out of nine cases, seven (case 1, 2, 3, 5, 6, 7, 9) had marked liver parenchymal changes and typical network pattern E-F with periportal fibrosis, thickness of portal vein walls. The other significant findings were ascites, hepatosplenomegaly, and the development

of varices was markedly noted. Only one case (case 3) had a pattern D and absence of the additional complications. Liver parenchymal changes due to *S. mekongi* infection were found to be similar to morbidity patterns induced by *S. japonicum* [22, 23].

Ultrasonography is a non-invasive, rapid and relatively inexpensive tool that can be performed both at the hospital and in the community [24]. To date, it has become a important tool to diagnose various parasite-induced liver pathology, in particular used to diagnose liver fibrosis induced by trematode infections. Today, extensive experience with the US is available in schistosomiasis control. It shows that a major challenge is the standardisation of diagnosis and reporting, and comparability of the results. An international working group was established which developed a validated standard procedures for ultrasonographical diagnosis for *S. haematobium* and *S. mansoni* and later also for *S. japonicum*. *S. mekongi* is assessed using the *S. japonicum* approach.

Praziquantel is the drug of choice for *S. mekongi* treatment [5]. In mass-drug administrations (MDA) a single dose of 40 mg / kg BW of PZQ is used [5, 25]. Adverse effects are generally quite frequent but are mild and transient [26]. PZQ treatment of our cases revealed not only improvements.

Our case description documented substantial morbidity of chronic infection with *S. mekongi* including mortality. It shows that severe disease is still present in this district. Cases of children with severe manifestations document ongoing transmission in this area. A control program based on community-based MDA of praziquantel, combined health education and improved infrastructures has recently been started. In addition to an improved sanitation health services should also benefit for clinical training to manage current and future severe cases of this disease. Curative and preventive health services of *S. mekongi* endemic area need to be further strengthened to successfully combat this neglected tropical disease.

5.7 Acknowledgements

Authors are thankful to the patients, laboratory staff at CMPE in Vientiane, Malaria stations and provincial hospital in Champasak, and health and technical staff at Khong district hospital, the village chiefs and villagers in three islands for their kind support.

Finally the authors respectfully thank Dr. Souban Khanhyavong, Director of District Health Office and staff, Khong Hospital, Khong Island Lao PDR, for his kind support.

5.8 Funding

The study was funded the Swiss National Science Foundation and the Swiss Agency for Development and Cooperation (project no. 3270B0-110020). PAS is supported by the City of Basel, Switzerland. YV is supported by the Forlen Foundation, Basel, Switzerland. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

5.9 Potential conflicts of interest.

All authors: None declared

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6. Resolution of Hepatobiliar and Intestinal Morbidity Induced by *Schistosoma mekongi* and Co-infection with *Opisthorchis viverrini* after Praziquantel in Lao People's Democratic Republic

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Working paper

6.1 Abstract

Background. Food and waterborne trematodiasis caused by *Schistosoma mekongi* and *Opisthorchis viverrini* are medically flukes importance in Southeast Asian, particularly afflicting the poorest in the poor-stricken settings. Chronic infections due to these flukes can lead to serious hepatobiliary and -splenic morbidity and mortality, from portal hypertension, hepato-splenomegaly and risk of rupture from oesophageal varices (*S. mekongi*) and cholangiocarcinoma (*O. viverrini*) if untreated.

Methods/principle findings. A cohort study was carried out from 2006 to 2008 in three villages in Khong district, Champasack province, Lao PDR. In total 306 persons were screened for *S. mekongi* infections using Kato-Katz thick smear, 151 (49.3%) individuals were *S. mekongi* infected and underwent ultrasound examination. All individuals were treated with a single-dose of 40 mg/kg praziquantel and albendazole (500mg) at the baseline (2006). Repeated stool and ultrasonographic examinations were performed at 11 months (mid-point) 24 months (end point) after treatment. At baseline co-infection with *O. viverrini* was diagnosed in 88.7%. The individuals did not demonstrate any severe sequelae. Overall, liver pathology was observed in a high prevalence of 37.1%. At midpoint follow up, two cases had developed ascites and one died due to rupture of oesophageal varices. There were significantly improvements such as reversals of periportal fibrosis among mild and moderate group ($p<0.001$) and a decreasing size of the left liver lobe, spleen and splenic veins ($p<0.001$). Re-infection with *S. mekongi* (54.3%) and *O. viverrini* (74.2%) were observed at the endpoint. Liver pathology at endpoint remained unchanged compared to midpoint.

Conclusion/significance. Liver morbidity due to *S. mekongi* and co-infection with *O. viverrini* are substantial on Khong district. Ultrasonographic liver findings showed a significant reduction of morbidity after praziquantel treatment. However, severe morbidity and mortality due to *S. mekongi* were observed as well. Regular deworming programmes are needed to reduce the re-infection. Integrated and sustainable control of these two trematode infections is urgently needed on Khong district.

6.2 Introduction

Trematode parasites, schistosomiasis and opisthorchiasis are an emerging but largely unrecognized public health and economic importance in the Mekong River basin concern (Andrews et al., 2008; Keiser and Utzinger, 2005; Muth et al., 2010). About 40 million people are infected and 750 million people at risk of infection (Keiser and Utzinger, 2009). The infection caused by these flukes can lead to liver fibrosis and calcifications of portal venous system (schistosomiasis), which includes increased morbidity and mortality from portal hypertension, hepato-splenomegaly, and risk of rupture from oesophageal varices (Gryseels et al., 2006) and cholangiocarcinoma (Sripa et al., 2007).

Schistosoma mekongi and *Opisthorchis viverrini* belong to the family plathyhelminth, which are cause *s*chistosmiasis and opisthorchiasis. Both parasites cause hepatosplenic morbidity and mortality in the affected communities (Muth et al., 2010). They result in different liver parenchymal changes seen in ultrasound images (Hatz, 2001; Hirose et al., 2007). Asian liver fluke, *O. viverrini* is prevalent in the Mekong River, namely Thailand, Lao PDR, Vietnam and Cambodia (Sithithaworn et al., 2011; Sripa et al., 2010b). Foci of blood dwelling fluke, *S. mekongi* was restricted to Lower Mekong Basin. The geographic overlap of *S. mekongi* and *O. viverrini* is a unique situation and represent an high socio-economic burden in Lao PDR and Cambodia (Andrews et al., 2008; Keiser and Utzinger, 2005; Muth et al., 2010). Preventive treatment with praziquantel (PZQ) is the mainstay of control today. However, it is not clear in how far liver morbidity induced by *S. mekongi* and *O. viverrini* is resolved after treatment.

The objective of this study is to document the resolution dynamics of *S. mekongi* induced liver morbidity in a setting where co-infection with *O. viverrini* is prevalent. We carried out a cohort study for years following *S. mekongi* infected patients after an initial treatment with PZQ (40 mg / kg BW).

6.3 Patients and Methods

6.3.1 Study area and population

A cohort study was conducted between June 2006 to June 2008 in three villages consisting of Long Khang, Long Song, Hang Long (on Don Long island) in Khong district, Champasak province, Lao PDR. 15-20 households (100 individuals) in each village were

randomly selected and screened for *S. mekongi* infection. The selection of household was based on the village family register. All members of selected household age ≥ 6 months were invited to participate in the study.

Three villages are known to be *S. mekongi* and *O. viverrini* endemic area. Khong district is located in Champasack Province (~200 km), Southern Lao PDR and borders to Cambodia. In Khong district an estimated 80,000 people dwell adjacent to the Mekong River.

6.3.2 Laboratory procedures

For each individual, 3 stool samples were collected over 6 consecutive days. The stool samples were collected in the early morning between 7h00-9h00am and provided the empty container for the next day. All stool samples were shipped to district laboratory in the main island, Khong district. One Kato-Katz thick smear (41.7 mg) was prepared from each specimen (Katz et al., 1972) and allowed to clear for 30-40 minutes 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) (Marti & Escher, 1990) and then analyzed by use of a formalin-ether concentration technique at the parasitological department of the Faculty of Medicine, University of Health Sciences (Vientiane, Lao PDR). Approximately 10% of the Kato-Katz thick smears were randomly re-examined for quality control, performed by laboratory staff from the Swiss Tropical and Public Health Institute (Basel, Switzerland). Helminth eggs were counted and recorded for each species separately. Finger pick for hemoglobin was performed for each individual using a hemoglobin meter (HemoCueB-Hemoglobin, Microvettes®)

6.3.3 Abdominal ultrasonography examination

Ultrasonography was performed, using a portable ultrasonographic machine (SSD-500, Aloka, Tokyo, Japan) with 3.5 MHz convex abdominal transducers. The US examination was conducted by an experienced radiologist of Mahosot hospital (a director of radiological department), Ministry of Health, Vientiane, Lao PDR and a physician who was trained in performing the ultrasound protocol, using the recent WHO guideline (Niamey Working Group, 2000). Both examiners were blinded with regards to

parasitological results. The image was recorded on a DVD disc, using a Sony DVD recorder (RDR-HX780).

Liver image pattern (IP) was taken in supine position. IP was scored and graded according to Niamey document (Niamey Working Group., 2000). IP was designated as A to F: A as normal pattern; B as liver with defuse echogenic foci 'starry sky'; C as liver with highly echogenic 'rings and pipe stems'; D as liver with a highly echogenic 'ruff' around the portal bifurcation and main stem; E as liver with highly echogenic 'patches' extending from the main portal vein and branches to the periphery; and F as liver with highly echogenic bands and streaks, extending from the main portal vein and its bifurcation to the liver surface, where they retract the organ surface 'bird's claw' pattern. The size of the left liver lobe (SLL) was measured by longitudinal liver scan. The measurements were taken in the left parasternal line from the upper to the caudal margin. This view is similar to the one used to demonstrate paraumbilical and coronary vein collaterals. The portal vein diameter (PVD) was measured in the right oblique view along the axis of the vessel with measurement of the internal diameter of the portal vein at the entry point into the liver. The size of spleen was measured in the left intercostals oblique view with the maximum length by measuring through the splenic helus. All organ measurements were high-adjusted. The cut-off numbers were based on the healthy uninfected Chinese population (Li et al., 2004). The organs were considered as abnormality if the height-adjusted value exceeded two standardized Deviation (2SD) of the referenced population (e.g., 0-2SD is normal; 2-4SD is enlarged and > 4SD is much enlarged). Finally, periportal hypertension (PH score) was calculated from the sum of the three indicators such as the height-adjusted value of portal vein diameter (PVD) (normal = 0, enlarged = 4 and marked enlarged = 6), the presence of collateral veins (absence = 0 and presence = 4) and the presence of ascites (absence = 0 and presence = 3). The sum of these scores was categorized into four groups: 0 = normal, 4= light hypertension, 6–8 moderate hypertension and 10–13= severe hypertension and adjusted for each individual. Each study individuals was asked to fast at least 4 hours before examination. The examination was only performed in the study individuals with body height ≥ 80cm.

6.3.4 Questionnaire and physical examination

Socio-economic characteristics of the study family, demographic (e.g., age, sex, education, and profession), and behavioral data (e.g., food consumption habits) were obtained with a questionnaire (Sayasone et al., 2011). Self-reported morbidity recalled within past two weeks was obtained from each individual. Each study individual underwent a physical examination by a general physician. The physical examination was subjected to a general condition of study individuals, skin abnormality observed, abdominal, liver and spleen palpation. The clinical classification of liver and spleen were based on the criteria modified by Hackett (Hackett, 1944). Moreover, an anthropometric assessment (body weight, body height and upper arm circumference) was done for each study individual. An electronic bathroom scale was used to measure the body weight of study participants. Body height was measured using a standard meter in standing position. Mid upper arm circumference was collected from each study individual using a measuring tape.

6.3.5 Data management

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). Only volunteers who completed abdominal US and a full set of 3 Kato Katz thick smears with parasitologically confirmed *S. mekongi* infections at the start of the cohort were evaluated in the final analysis. Age was categorized into seven groups: (i) < 6 years, (ii) 6–15 years, (iii) 16–30 years, (iv) 31-55, (v) > 55 years. Infections intensity with *S. mekongi* and soil-transmitted helminths (STHs) was classified in categories put forth by WHO (WHO, 1995): *S. mekongi*: 0; 1 – 100; 101 – 400; > 400 egg per gram [EPG] hookworm, (1-1999 (EPG), 2000-3999 EPG, and ≥4000 EPG), *A. lumbricoides*, 1-4999 EPG, 5000-49,999 EPG. *O. viverrini* were grouped into negative, light infection, moderate infection and severe infection (0; 1 – 999; 1.000 – 9.999; ≥ 10.000 epg) cut-offs put forward by Maleewong (Maleewong et al., 1997).

Descriptive analyses are presented as counts, percentages, means and standard deviations, as appropriate. Pearson's x^2 and Fisher exact test were applied to compare the baseline binary characteristics between three villages as appropriated. Negative

binomial regression was applied to calculate egg reduction rate ratio (ERRR) between the numbers of helminth eggs recovered in stool examination between three year follow-up. Random effects multinomial logistic regression model was fitted and employed to investigate the association between morbidity and parasitic infection at individual over the period of time.

6.3.6 Ethical considerations

The study was approved by the Ethics Committee of the Kanton of Basel (EKBB; reference no. 255/06) and the National Ethics Committee, Ministry of Health in Vientiane (reference no. 027/NECHR). Written informed consent was obtained from the heads of participating household and an individual prior to enrollment. An informed consent form was written and translated in Lao language, detailed on potential risks, benefits, procedures. An anti-spasmodic treatment and oral dehydration was provided in case of adverse effects due to drug administration. Once our study participants completed all the evaluations, including clinical, US, parasitological examination had been performed, they were treated with anthelminthic drugs in a corresponding to their current helminth infection. If any anormalities were observed during the examination, we informed and suggested for further investigation according to Lao Ministry of Health guideline. Additionally, at the first year and the end of the study, all individual in the three villages were administered a single dose of 400 mg albendazole and a single dose of 40 mg/kg praziquantel in respective to Lao national scheme for mass drug administration (Ministry of Health, 2004).

6.4 Results

6.4.1 Baseline characteristics.

Of 409 participants randomly selected in three villages, we excluded 89 (21.8%) individuals since they provided only single stool sample and absence during the survey. In total 306 (100.0%) individuals were enrolled at baseline and examined by 3 Kato-Katz slides. While 81 (26.5%) individuals were excluded due to missing both stool samples and US examination, only 225 (73.5%) individuals were included with parasitologically confirmed *S. mekongi* in mid point follow-up (2007). 74 (24.2%) individuals could not be traced on the last follow-up. Overall, 151 individuals completed

the all data records (3x3 Kato-Katz thick smears, 3x US and 3 physical exams) at the final evaluation (Figure 6.1).

Overall, demographic characteristics of study participants did not differ between the villages (Table 6.1). There were slightly more women (52.3%) than men (47.7%). Mean age (SD) was 23.2 years(18.5) ranging from 6 months to 87 years. Of these, children aged < 6 and elderly > 55 years accounted for 13.9 % and 6.6 % of the cohort, respectively. Most participants had a primary school education level (63.6%) while some were illiterate (26.5%) or reached a secondary school level (9.9%). Agriculture or/and fisherman were the main professional activity in three villages, while there were only few without any profession or housewifes and government employees.

Table 6.2 summarizes signs and symptoms related to *S. mekongi* and *O. viverrini* infection. Study participants felt tiredness (14.6%), RUQ pain (23.2%), and repored blood in stool (6.6%), was significantly different with three year evaluation (2006 to 2008) ($P< 0.03$). On physical exam the most frequent sign was a hepatomegaly (82.7%) and splenomegaly (48.7%) with statistical significance with three year evaluation (2006 to 2008) ($P<0.001$).

Table 6.1: Baseline characteristics

Village		Long Kang (n= 52)	Long Song (n=50)	Hang Long (n=49)	*P*-value
Sex	Male	42.3	52.0	36.7	
	Female	57.7	48.0	63.3	0.11
Age	Mean (year)	21.3	23.5	24.8	
Age groups	≤ 5	21.1	10.0	10.2	
(year)	6 – 15	34.6	42.0	36.7	
	16 – 30	7.7	12.0	14.3	
	31 – 55	32.7	30.0	28.6	
	>55	3.9	6.0	10.2	0.12
Weight	Mean (SD) kg	33.7	34.0	32.9	
Height	Mean (SD) cm	134.4	133.6	135.9	
Education	Illiterate	26.9	32.0	20.4	
	Primary school	55.8	62.0	73.5	
	Secondary	17.3	6.0	6.1	0.17
Occupation	No occupation	26.9	22.0	28.6	
	Farmer /Fisher	71.1	78.0	71.4	0.76

Data are no; (%) of subject, otherwise indicated; MAUC, mid upper

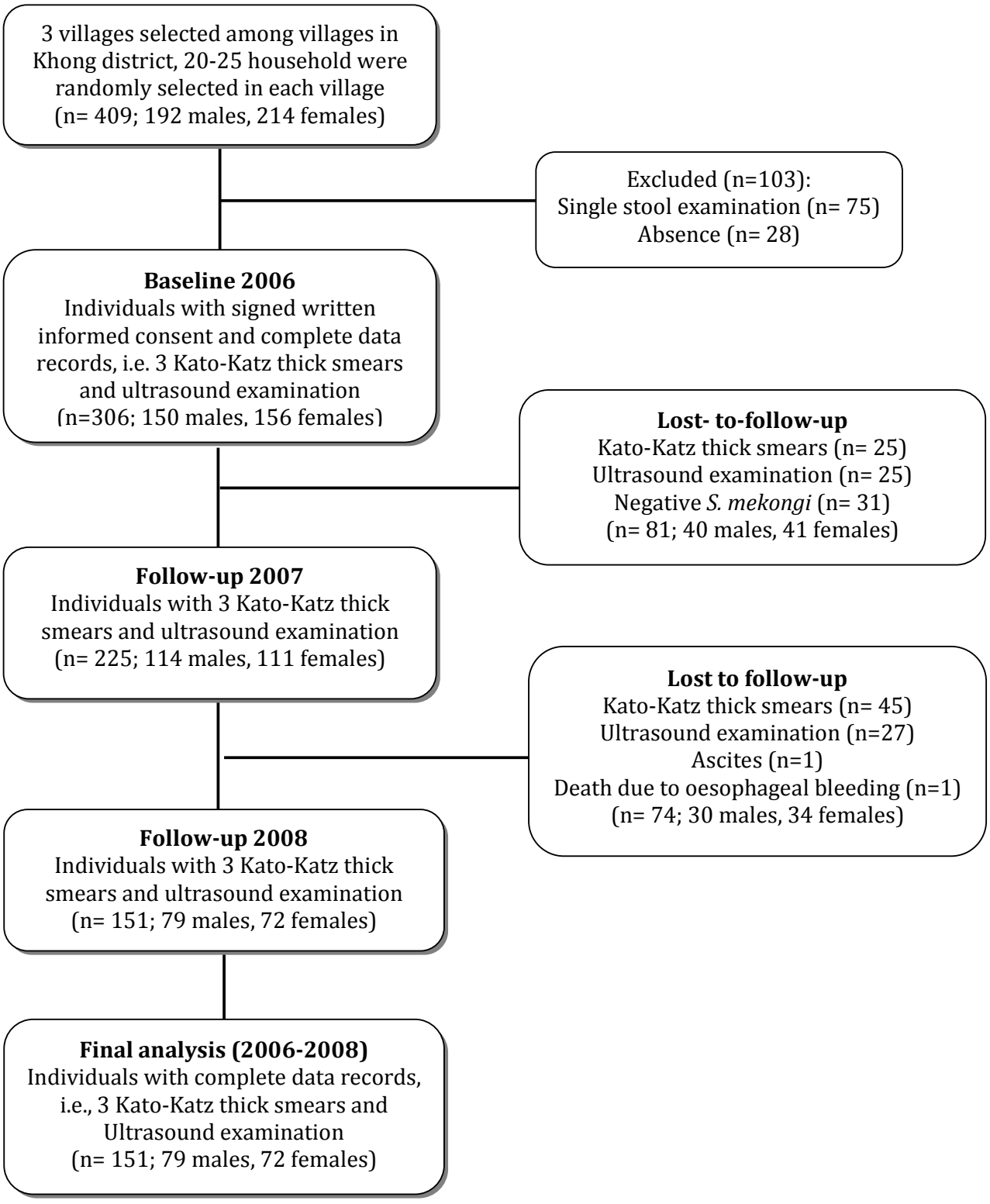

Figure 6.1 Study participation and compliance. Flow diagram of cohort study on *S. mekongi* and co-infection with *O. viverrini* in three villages, Don long, Khong District, Lao PDR between 2006 and 2008.

6.4.2 Morbidity resolution dynamics

Among our patients we observed the following hepatobiliary pathology: portal hypertension, enlargement of the left liver lobe, portal vein dilatation and splenomegaly. Table 6.3 summarizes the development of morbidity in the 2 years cohort. Overall liver morbidity decreased initially (2006-2007) but increased between 2007 and 2008 (P< 0.001).

Table 6.2: Medical history and physical among 151 residents in 3 villages of Don Long Island, Khong province, Champasak Province

	2006 (n=151)	2007 (n=151)	2008 (n=151)	χ2-trend
Medical History				
Morbidity reported (past 2 weeks)				
Lost weight	4.2	18.9	1.3	<0.001
Tiredness	11.5	26.0	6.1	0.02
RUQ pain	4.2	18.2	16.4	0.01
Blood in stool	0.0	10.0	10.2	0.03
Physical examination				
Good condition	96.2	94.0	100.0	0.32
Jaundice	0	1.9	0.0	Na
Pale conjunctiva	13.4	16.0	10.2	0.72
Big belly	36.0	18.0	28.6	0.08
Coronary collateral vein	1.8	0.5	0.9	0.89
Hemoglobin, mean (SD) mg/dl	11.5	11.6	12.3	0.78
Hepatomegaly				
1	31.3	71.5	91.7	
2	52.5	21.7	4.7	
3	15.0	5.8	2.4	
4	1.3	0	1.2	<0.001
Splenomegaly (Hackett score)				
1	17.2	75.5	94.7	
2	66.9	23.2	4.6	
3	15.9	3.3	0.7	0.001

Data are no; (%) of subject, otherwise indicated; RUQ- right upper quadrant pain at palpation; Hackett score (1944) 1-normal, 2-mild, 3- moderate; 4. large, coronary collateral vein-vessel on abdominal observation;

6.4.3 Infection resolution dynamics

At the baseline, among 151 infected with *S. mekongi*, 88.7% were infected with *O. viverrini*. Overall prevalence of hookworm, *A. lumbricoides*, *T. trichiura* were 56.0%, 4.0% and 10.6%, respectively. Other helminthic infection such as *Taenia* spp., *Strongyloides stercoralis* and *Enterobius vermicularis* were 10.6%, 27.3% and 0.7%,

respectively. The *S. mekongi* and *O. viverrini* geometric mean egg counts obtained by Kato-Katz and FECT at baseline ranged from 879.2 to 565.4 and 2296.2-3970.7 EPG, respectively. At the end of the study, 54.3% were positive with *S. mekongi* and following by 63.6% were found egg positive for *O. viverrini*. There were statically significances observed between GM and over two years evaluation (Table 6.4).

Table 6.3 Ultrasonographic findings in villages infected by co-infection of *O. viverrini* and/or *S. mekongi* following treatment by praziquantel

		2006 (n=151)	2007 (n=151)	2008 (n=151)	χ^2 – trend
Image pattern	A	39.7	4.7	5.3	
	B	23.2	35.1	35.8	
	C	19.2	43.7	41.7	
	D	11.3	6.6	9.9	
	E	3.9	6.6	4.0	
	F	2.7	3.3	3.3	< 0.001
Portal hypertension					
	Normal	82.1	69.5	78.8	
	Light	0	27.2	0	
	Moderate	17.9	3.3	21.2	< 0.001
Left liver lobe					
	Normal	10.6	17.9	21.9	
	Enlarged	54.3	38.4	48.3	
	Much enlarged	35.1	43.7	29.8	0.0002
Portal vein diameter					
	Normal	88.7	63.6	64.9	
	Dilated	8.6	8.6	25.2	
	Marked dilated	2.7	27.8	9.9	< 0.001
Spleen					
	Normal	88.7	66.9	86.1	
	Enlarged	7.3	6.6	11.3	
	Much enlarged	4.0	26.5	2.6	<0.001
Other findings					
	GBST	1.3	3.1	1.3	
	GBWT	2.7	2.6	1.8	
	GBL (Mean)	5.3	5.6	6.0	
	GBWI	3.6	2.0	0.9	
	GBSL	0.4	1.6	1.8	
	Ascites	0	1.0	0.5	

Data are no; (%) of subject, otherwise indicated; GBST-gallbladder stone, GBWT-gallbladder wall thickening, GBL- gall bladder length (cm), GBWI-gallbladder wall irregular or with a halo, IHBDL: intra-hepatic bile duct dilated, GBSL-gallbladder sludge

Table 6.4 Prevalence of infection, diagnosed by Kato-Katz and formalin-ethyl-acetate concentration in three villages, Khong district, Champasack province

	2006 (n = 151)	2007 (n = 151)	2008 (n = 151)	*P-value*
Schistosoma mekongi [a]				
GM egg per gram of stool (range)	322.3 (79.2-565.4)	119.0 (38.8-199.3)	486.1 (234.4-737.9)	0.09*
Negative	0	66.9	45.7	
Light (1-999 EPG)	88.1	32.4	42.4	
Moderate (1000- 9999 EPG)	8.6	0.7	7.3	
Heavy (≥10,000 EPG)	3.3	0	4.6	
Opisthorchis viverrini [b]				
GM egg per gram of stool (range)	3133.4 (2296.2-3970.7)	2501.9 (1361.3-3642.6)	2443.0 (1177.9-3708.1)	< 0.001*
Negative	11.3	35.8	36.4	
Light (1-999 EPG)	41.7	45.7	41.7	
Moderate (1000- 9999 EPG)	38.4	13.9	18.6	
Heavy (≥10,000 EPG)	8.6	4.6	3.3	
Hookworm [c]				
GM egg per gram of stool (range)	476.6 (358.3-594.9)	534.8 (343.6-726.0)	275.6 (164.4-350.8)	0.94*
Negative	44 (29.1)	91 (60.2)	60.3	
Light (1-1999 EPG)	103 (68.2)	56 (37.1)	40.0	
Moderate (2000- 3999 EPG)	4 (2.7)	4 (2.7)	39.7	
Ascaris lumbricoides [d]				
GM egg per gram of stool (range)	7268 (5968.4-20504.4)	1772.0 (955.4-4499.4)	552 (381.9-1485.9)	0.04*
Negative	9.0	96.0	96.0	
Light (1-4999 EPG)	2.7	3.3	2.7	
Moderate (5000- 49,999 EPG)	1.3	0.7	1.3	
Trichuris trichiura [e]				
GM egg per gram of stool (range)	139.5 (52.9-331.9)	140.0 (72.5-207.5)	228.9 (24.6-482.5)	0.001*
Negative	89.4	92.1	91.4	
Light (1-999 EPG)	10.6	7.9	8.6	
Taenia spp.				
Negative	89.4	99.1	100	
Positive	10.6	92.0	0	
S. stercoralis				
Negative	72.7	84.8	100.0	
Positive	27.3	15.2	0	
E. vermicularis				
Negative	99.3	99.3	98.7	
Positive	0.7	0.7	1.3	

[a] According to guidelines put forth by WHO [xx], based on Kato-Katz thick smear examinations
[b] According to Maleewong and colleagues [xx], based on Kato-Katz thick smear examinations
Data are no; (%) of subject, otherwise indicated (95% confidence interval); EPG, eggs per gram of stool; GM, geometric mean; * ERRR, egg reduction rate ratio

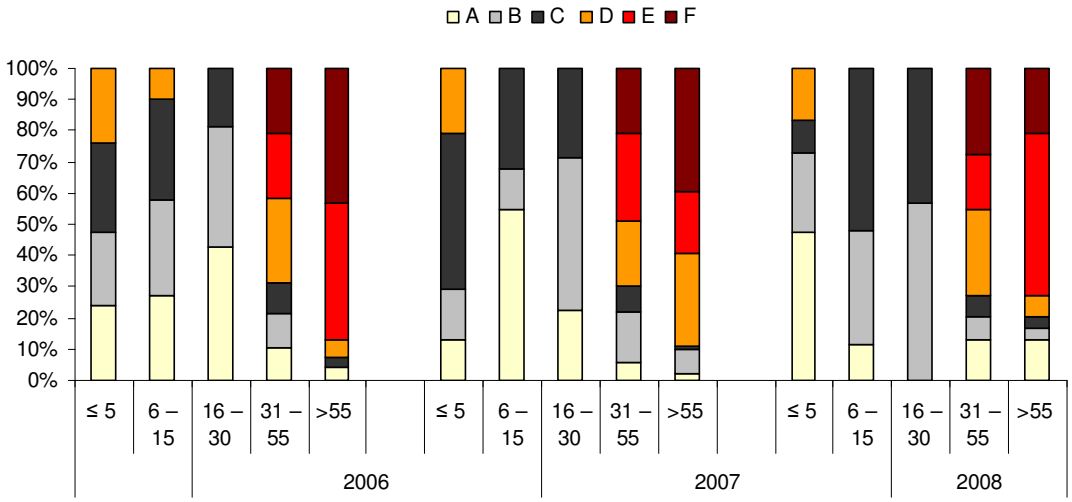

Figure 6.2 Prevalence of periductal fibrosis (A - normal, B - 'starry sky', C - highly echogenic 'rings and pipe stems', D - highly echogenic 'ruff' around the portal bifurcation and main stem, E - highly echogenic 'patches' extending from the main portal vein and branches to the periphery, F - highly echogenic bands and streaks, extending from the main portal vein and its bifurcation to the liver surface moderate 151 individuals infected with *S. mekongi*, underwent ultrasound examination by aged groups from 2006 to 2008.

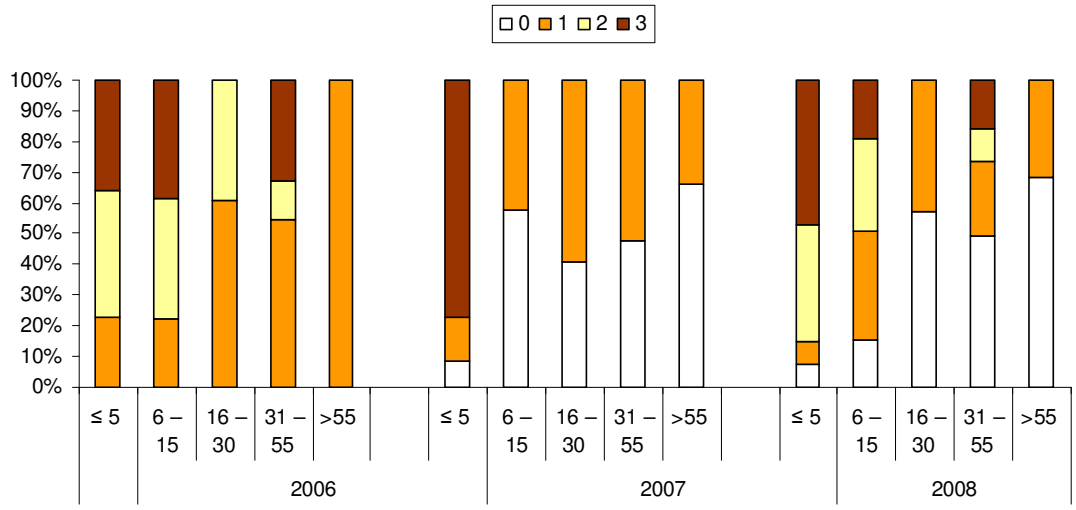

Figure 6.3 Infection intensity (0- negative, 1- mild 2- moderate 3-heavy) among 151 individuals infected with *S. mekongi* by aged groups from 2006 to 2008.

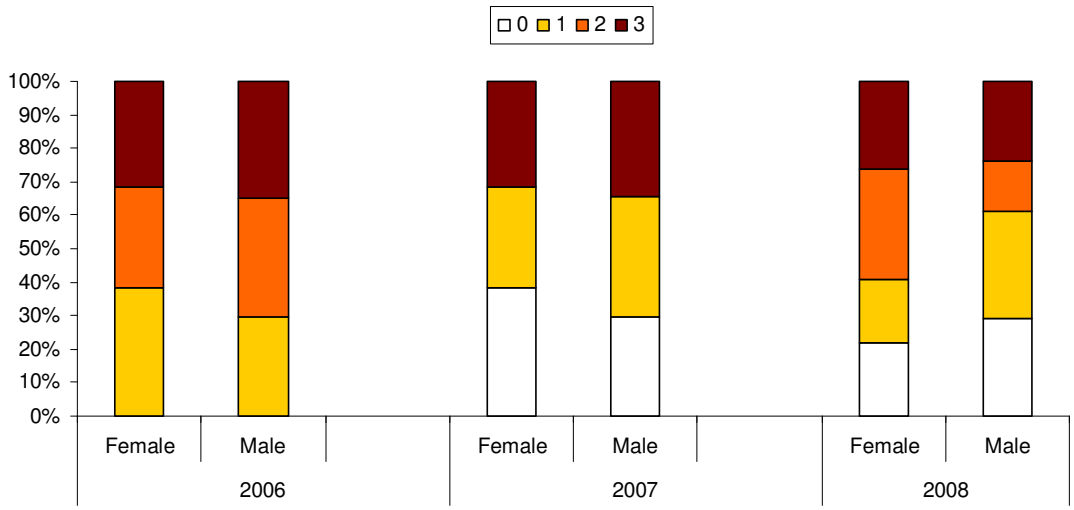

Figure 6.5 Infection intensity (0- negative, 1- mild 2- moderate 3-heavy) among 151 individuals infected with *S. mekongi* by gender from 2006 to2008

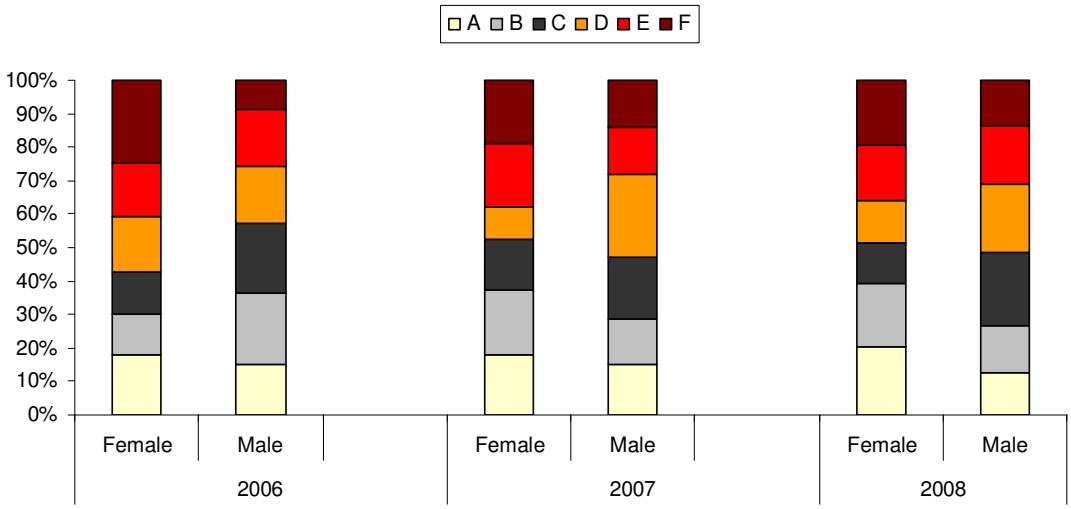

Figure 6.4 Prevalence of periductal fibrosis (A-normal, B- a liver with defuse echogenic foci 'starry sky', C -highly echogenic 'rings and pipe stems', D -highly echogenic 'ruff' around the portal bifurcation and main stem, E -highly echogenic 'patches' extending from the main portal vein and branches to the periphery, F-highly echogenic bands and streaks, extending from the main portal vein and its bifurcation to the liver surface moderate 151 individuals infected with *S. mekongi*, underwent ultrasound examination by gender from 2006 to 2008.

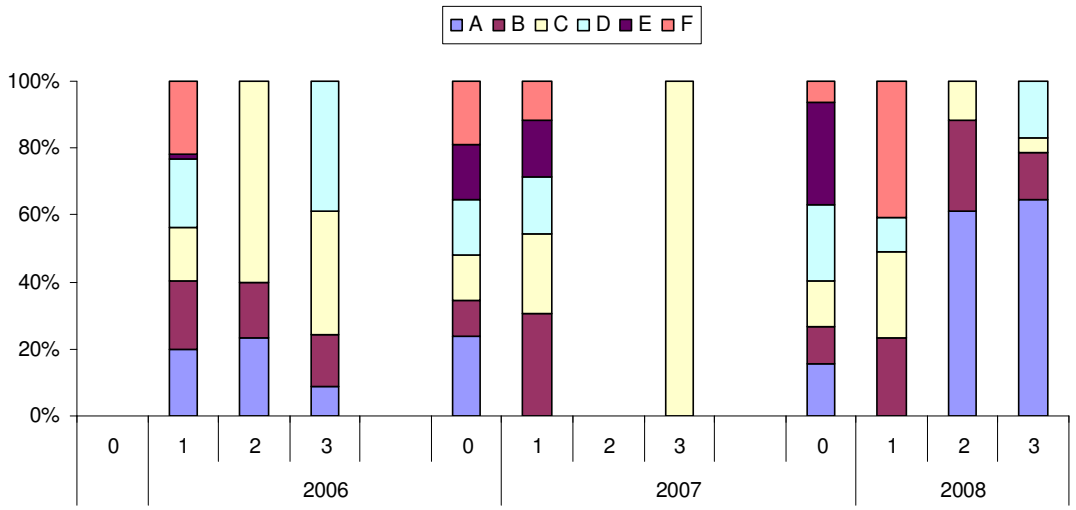

Figure 6.6 Infection intensity (0- negative, 1- mild 2- moderate 3-heavy) among 151 individuals infected with *S. mekongi* by classification of periportal fibrosis from 2006 to2008

6.5 Discussion

This is the first community based study investigating dynamics of *S. mekongi* infection and related morbidity after praziquantel treatment. This cohort study was carried out in a setting where co-infection with other parasites are highly prevalent. In particular is also *O. viverrini* present which also produces significant hepato-biliar morbidity. We found that *S. mekongi* infection prevalence was reduced but increased again in the second year. On the morbidity side similar observations were made.

Chronic infection with *S. mekongi* associated significantly with hepatosplenomegaly, granulomatous of liver or periportal fibrosis, portal hypertension, leading to rupture of oesophageal bleeding, which is a main cause of death due to this parasite (Biays et al., 1999; Chiavalori et al., 2008). While *O. viverrini* contribute to a wide range of hepatobiliary disesses, including hepatomegaly, obstructive jaundice, gallbladder stones, cholecystitis, cholangitis and, most severely, a fatal bile duct cancer (cholangiocarcinoma) (Andrews et al., 2008; Sripa et al., 2010b). Little is known about the association and the underlying mechanism between *S. mekongi* and *O. viverrini.* Nevertheless, co-infections of these flukes might aggravate the host-organ pathology (Sayasone et al., 2011).

Abdominal ultrasound (US) is a safe, simple, rapid diagnosis tool in hospital and communities studies (Hatz et al., 1992a; Hatz et al., 2001; Mairiang and Mairiang, 2003).

A standard used of ultrasound, developed by WHO expert and researchers for a decade in detecting *Schistosomiasis*-related morbidity (Niamey Working Group, 2000). As morbidity due to *S. mekongi* was similar to findings in *S. mansoni* and *S. japonicum* infections (Hatz et al., 1992a; Hatz et al., 1992b). Today no standardized protocol is available for assessing morbidity due to *S. mekongi* and co-infecting *O. viverrini*. Researchers of the Southeast Asia region have adapted the Niamey protocol and added periductal fibrosis indicators specific for *O. viverrini* infection (Mairiang et al., 2011). Niamey protocol was developed for standardized protocols for the assessment ultrasonographical morbidity due to *Schistosoma haematobium* and *Schistosoma mansoni* (Niamey Working Group, 2000; Richter, 2000).

Our study confirmed that the impact of praziquantel treatment reduces hepatosplenic diseases by decreasing significantly liver and spleen sizes. US findings demonstrated that enlargement of left liver lobe, spleen, splenic vein; sign of portal hypertension were improved. Other parameters such as periportal fibrosis and portal vein diameter started to regress. Similar achievement of pathology resolution were observed in Cambodia after several rounds of praziquantel treatment (Keang et al., 2007; Stich et al., 1999). In our study population liver fibrosis remained unchanged.

Prevalence of *S. mekongi* and *O. viverrini* were found to be highly prevalent before treatment, other soil transmitted helminths were also endemic. Rapid re-infection obviously occurred after praziquantel and albendazole treatment within a year. For example, prevalence of *S. mekongi* and *O. viverrini* reached pre-treatment level within subsequent years. Our finding was in line with a recent study, conducted in the school, which was the same study site as our study revealed the respective prevalence of *S. mekongi; O. viverrini* and hookworm were highly prevalent by 87.8%, 98.9% and 97.6%, respectively (Lovis et al., 2011, submitted to PLoS NTD).

Our study confirmed the previous studies on eating raw or insufficient cooked fish (i.e., “Lap-pa” and “Koy-pa”) and having contact with infected water are the main key factor for re-infection (Grundy-Warr et al., 2011; Sayasone et al., 2011; Sayasone et al., 2007; Sayasone et al., 2010b). Additional to behavior characteristics and water contact, socioeconomic, and environmental characteristics such as infrastructures and sanitation are key determinants for prevalence and intensity of several parasitic infections (Sayasone et al., 2011; Steimann et al., 2011). For example in 2007 in our study survey of

four villages (Long Kang, Long Song, Hang Long and Hauo Long), on Khong district, only 14.5% of the households possessed latrines and 76.0% reported daily use of the Mekong River for bathing (K. Phongluxa, pers. communication). Given the fact that snail population control by application of molluscicide and limited mammal reservoirs (i.e., dog, cat and beaver) might be not feasible to curb Schistosome and Opisthorchis-induced morbidities (Muth et al., 2010; Thamkeo and Pholsena, 2003). Nonetheless, without elimination with snail, the control of *S. mekongi* could be possible (Ohmae et al., 2004). In our current work, improved Infrastructure and sanitation are being carried out on four villages, Don Long Island this intervention would considerably help to cease the transmission of these parasites (S. Sayasone, pers. communication).

In Lao PDR, a community-based intervention against schistosomiasis mekongi has been performed in the late 1980s. The program was led by Lao Ministry of health and World Health Organisation using a single dose of 40 mg/kg praziquantel for treatment of schistosomiasis. Since then, the public health problem had only been resolved partially. Today, there is a raising concern on re-emerging of *S. mekongi* transmission in Lao PDR, since nine sever cases of *S. mekongi* with signs and symptoms of schistosomiasis mekongi have recently determined during the epidemiological helminth surveys on Khong District, Champasack Province (Soukhathammavong., in preparation for PLos NTD). Our study further documents that re-infection and re-emergence of morbidity is rapid and occurs already 1 year after treatment. Therefore, an effective community intervention must operate at an annual basis, i.e. annual preventive treatment.

A single dose of 40 mg/kg praziquantel and 500 mg albendazole are well-tolerated and recommended by WHO (WHO, 2002) as a drug of choice for the use of mass drug administration against mainly food-and waterborne trematodiasis and soil transmitted helminth in endemicity, particularly emphasis was placed in population at risk (Muth et al., 2010; Utzinger and Keiser, 2004). In our population study showed periductal fibrosis were regressed and significantly improved after treatment, notably in mild (pattern A) and moderate (pattern B to C) periportal fibrosis groups. On contrary, among advanced periductal fibrosis group were remained the same as pretreatment but ascites were not detected after treatment.

We have encountered limitations, for example alcohol consumption is commonly observed in Laos and is known to be a potential risk factor for cirrhosis (Gatto et al.,

2010). Frequency and onset had been difficult to estimate because villagers had started at early ages. Additionally, viral infections caused by HVB and HVC could not be ruled out in our population, which are mainly prevalent in Southeast Asia and considered as a risk factor leading to liver cancer (Gatto et al., 2010; Shin et al., 1996), however, no data have been documented in Laos. The images resulting from these risk factors may be confused with a liver US imaging attributable to *S. mekongi* (Kardorff et al., 1999) and may aggregate liver pathogenesis for co-infection with *O. viverrini*. Lost-to-follow-up or participant compliance was an issue found in our study, since people had to leave their home for work.

In conclusion, our findings underscore that morbidity and remerging of *S. mekongi* and co-infection of *O. viverrini* are of particular concern on Khong district. Co-infection of these trematodes might deteriorate the pathogenesis of host organs. Follow-up and monitoring of schistosmiasis and opisthorchis induced morbidity in endemic areas are warranted. Multi-component integrated and sustainable control disease control programme on prevention strategies are fundamental (Utzinger et al., 2010; Utzinger et al., 2003), for instance promoting health education campaigns, improved sanitation and hygiene, better access to clean water are needed to reinforce.

6.6 Author Contributions

PO, SS, TK, JK, JU and MT conceived and designed the study; PAS, TK, KP, SS, YV collected data; KA had the overall responsibility of data collection; TK entered the data; PAS, PO and PV analyzed data and interpreted results together with CH; PAS and PO wrote the working paper; PAS and PO are guarantors of the paper.

6.7 Acknowledgements

We are grateful to villagers, communities and chief of village for their kind collaboration, commitment and participation in our study. We would like to thank Dr. Souban Khanhyavong, Director of District Health Office and staff, Khong Hospital, Khong Island Lao PDR, for his kind support. We thank Dr. Samlane Phompida and staff at Center for Malaria and Parasitology and Entomology, Vientiane, and also staff at Champasack malaria station for their kind support.

6.8 Funding

The study was funded the Swiss National Science Foundation and the Swiss Agency for Development and Cooperation (project no. 3270B0-110020). PAS is supported by the City of Basel, Switzerland. YV was supported by the Forlen Foundation, Basel, Switzerland. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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7. Subtle to Severe Hepatobiliar Morbidity Associated with Opisthorchis viverrini Infection in Southern Laos

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This article has been submitted to
PLos Neglected Tropical Disease

7.1 Abstract

Background. Hepatobiliar morbidity including cholangiocarcinoma (CCA) are outcomes of chronic *Opisthorchis viverrini* liver fluke infection. Detailed information on hepatobiliar morbidity associated with *O. viverrini* infection including CCA is scare in Laos although *O. viverrini* infection is highly prevalent. We assessed *O. viverrini*-related hepatobiliar morbidity using abdominal ultrasonography (US) in confirmed *O. viverrini* infected adult patients in Saravan province, southern Laos.

Principle findings. A random sample of 431 *O. viverrini* patients from 10 villages underwent abdominal US. Mild, moderate and markedly advanced periductal fibrosis was diagnosed in 7.0%, 66.5%, and 17.0%, respectively. Normal liver parenchyma was seen only in 9.5% of patients. Presence of gall stones (13.2%), sludge (1.4%), gall wall thickening (1.2%), bile duct dilatation (1.6%), fatty liver (12.0%), and kidney stones (8.6%) and cysts (7.9%) were seen in considerable frequencies. In five patients (1.2%) hepatobiliary lesions suggesting CCA were diagnosed. The diagnosis of CCA cases could not be confirmed. Tumour markers, i.e., Interleukin-6, plasminogen activator inhibitor, and carbohydrate antigen 19-9 were within normal range.

Conclusion. High prevalence of CCA suspected liver masses and hepatobiliar diseases related to *O. viverrini* infection were seen among clinically asymptomatic adult patients in the endemic areas of Laos. Creation of awareness highlighting routes of infection and cultural culinary preference from very early ages, improved infrastructure and surveillance of population in distinct endemic settings are warranted, in order to reduce and prevent opisthorchiasis-related morbidity including a liver fatal cancer, cholangiocarcinoma.

Keywords *Opisthorchis viverrini*, hepatobiliar morbidity, cholangiocarcinoma, ultrasonography

7.2 Introduction

In Laos, information on morbidity due to *O. viverrini* infection is scare and virtually absent for cholangiocarcinoma (CCA), a bile duct cancer associated with chronic *Opisthorchis viverrini* infection. A recent study documented morbidity associated with liver flukes *O. viverrini* and *Schistosoma mekongi* infections in Southern Laos [1]. However, CCA cases and precursor lesions were not assessed. Endemicity levels of *O. viverrini* in the country [1,2] suggest that hepatobiliar morbidity and CCA incidence in Laos are at least as high as or higher than in Northeast Thailand.

CCA is a rare bile-duct cancer with a poor prognosis [2,3]. Chronic *O. viverrini* liver fluke infection is a main risk factor for CCA [4]. The highest CCA incidence worldwide is recorded in *O. viverrini* endemic areas in Northeast Thailand where on average 119 cases per 100,000 persons occur per year in adults aged 35 to 64 [5], with approximately five thousand cases are diagnosed per year [6]. Costs associated to CCA and *O. viverrini* fluke infection amount to an estimated USD 120 millions in Thailand alone [7].

Opisthorchiasis is a fish-borne trematode infection belonging to the neglected tropical diseases (NTDs) [8–10]. Almost 67 million people are at risk of infection, and an estimated 10 million people infected live in Northeast Thailand and Lao People's Democratic Republic (Laos, Lao PDR). In Laos, a third of the 5.5 million inhabitants are infected [8,11]. Infections occur in all provinces [2] but highest rates are seen in the Central and Southern parts with rates reaching up to 90% of the population [12–14]. Although burden of infection is high public health control activities are largely lacking.

We assessed hepatobiliar morbidity and found lesions suspicions of CCA in *O. viverrini* infected adults in rural communities of Southern Laos.

7.3 Methods

7.3.1 Ethical considerations

Ethical approval was granted by the Lao National Ethics Committee for Health Research (NECHR, N° 278/NECHR) and Ethical Review Group of the World Health Organization-Western Pacific Region in Manila, Philippines for investigating hepatobiliary morbidity.

The Lao National Ethics Committee for Health Research (NECHR, N° 169/NECHR) Vientiane, Lao PDR and the Ethical Committee Board, World Health Organization, Geneva, Switzerland to assess *O. viverrini* infection status.

Study aims, procedures and potential risks and benefits were explained to village authorities and all selected villagers in Lao language. Prior to enrolment informed written consent was obtained. Patients were informed on any abnormal ultrasound result and referred to treatment according to standard health care of the Lao Ministry of Health [15]. All patients with lesions suspecting CCA were given additional counseling and a free follow-up investigation was proposed. All patients were treated with praziquantel (40 mg/kg, single oral dose).

7.3.2 Study Design and Area

From January to April 2011 a cross-sectional study was carried out in adults (aged ≥ 20 years) of ten *O. viverrini* endemic villages in Saravan district, Saravan province, Southern Laos. Approximately 350,600 inhabitants live in the province (9 districts, 168 villages); 15.5% are children under five-years of age [Pers. Communication, Saravan Statistic Division, Saravan District Health Office]. Parasitological studies in the district of Saravan documented high *O. viverrini* infection rates in the general population of above 50% [3].

For this study, 840 participants from randomly selected households were screened for *O. viverrini* infection (Figure 1) of whom 85.0% had an *O. viverrini* infection. Abdominal ultrasonography examination was performed in adults (431, 51.3%).

7.3.3 Laboratory Analysis of Stool and Blood

Stool examination followed a standard procedure. In brief, each participant provided two fresh stool samples on consecutive days. Stool containers were transferred to the laboratory in the morning. From each sample, two Kato-Katz thick smears were prepared using standard 41.7 mg templates and examined under a light microscope (100 x magnification) [16]. Number of *O. viverrini* eggs per slide was recorded. Slides were read within 30-45 min after preparation. Ten percent of smears were re-examined

for quality control [17]. An *O. viverrini*-positive patient was defined as the presence of at least one *O. viverrini* egg in at least one of the four Kato-Katz fecal thick smears.

From patients with CCA suspected lesions a 30 mL venous blood sample was drawn. Tumor markers, i.e., carbohydrate antigen 19-9 (CA 19-9), Interleukin-6 (IL-6) and plasminogen activator inhibitor (PAI) were assessed in Khon Kaen University's reference laboratory [18,19].

From each patient demographic data were recorded (i.e., sex, age, place of residence, contact details and occupation) and information on abdominal symptoms and raw fish consumption was obtained.

7.3.4 Assessment of Hepatobiliary Morbidity

Abdominal US examination was performed in the study village using a portable US machine (SSD-500, Aloka, Tokyo, Japan) with a 3.5 MHz convex abdominal transducer. Patients were asked to fast 8 hours before US examination. Liver parenchyma fibrosis was assessed using an adapted examination protocol from Niamey [20] in combination with the standard protocols used in earlier in community-based studies [19]. Liver parenchyma pattern was graded as normal or no echoes (=0), starry sky (=1+), rings and pipe stems (=2+), and as highly echogenic 'patches' extending to peripheral areas (=3+). Patients were grouped to "none or mild advanced periportal fibrosis" and "advanced fibrosis" according to the US grade (equal ≤1 *versus* ≥2). Gallbladder was examined once before and 30 minutes after consumption of a fatty meal (a sterilized milk of 250 mL and two boiled eggs).

US examiners (PAS, VR) were blinded with regard to the laboratory results. Images were recorded on a DVD (Sony DVD recorder RDR-HX780) for quality control conducted by senior radiologists (OR, EM).

7.3.5 Statistical Analysis

Data were double-entered and validated in EpiData, version 3.1 (Epidata Association; Odense, Denmark). STATA software, version 10.1 (Stata Corp., College Station, TX, USA)

was employed for analysis. *O. viverrini* infections were grouped in light (1-500 eggs per gram stool [EPG]), moderate (501-999 EPG), heavy (1000-1999 EPG) and very heavy (≥ 2000 EPG) infection intensity. Descriptive statistics was used (counts, percentages, and means and standard deviations [SD]). Kruskal Wallis rank test was used to compare egg counts of *O. verrini* among patients with liver lobe enlargement and patients without this condition. ANOVA, logistic regression and multinomial logistic regression was used. A *P-value* below 5% was considered significant.

7.4 Results

7.4.1 Patients' Characteristics

Ultrasound examination was performed in 431 *O. viverrini* infected adults (Figure 7.1). Enrolled patients had mean age of 43.2 years (SD 0.5 years, range 20 to 86 years); 30.2% were between 30 and 39 years old; 31.0% older than 50 years (Table 1). There were more women (57.8%) than men (42.2%). Most patients were farmers (99.1%) and illiterate (95.8%). Patients' mean weight was 49.8 kg (SD 3 kg). Overall, *O. viverrini* geometric mean egg counts were 3961 EPG (range 24 – 69,648 EPG); 40.1%, 14.7%, 14.2% and 31.1% had a light, moderate, heavy and very heavy *O. viverrini* infection intensity, respectively.

Patients reported abdominal discomfort (75.6%), pain in the right upper quadrant (RUQ, 60.5%), and having "hot sensations" around the RQU (25.0%). Two patients had a jaundice and reporting skin itching. Most patients (99.5%) had consumed *Pa dek* (raw fermented fish sauce) within the last week; three quarters (75.8) reported regular consumption of raw *Koy-pa* and *Lap-pa* (meal based on raw fish). Twenty five percent reported to have a latrine at home but less than 5% use it regularly but defecate outside.

7.4.2 Hepatobiliary Morbidity

Only forty one patients (9.5%) had a normal liver parenchyma (Table 7.2); in 66.6% and 16.9% moderate and advanced periductal fibrosis was diagnosed (Figure 7.2). Gallbladder abnormalities were observed such as wall thickening (1.2%), gallbladder wall irregularities (0.5%), and sludge (1.4%). Gallbladder stones were observed frequently (13.2%). Other pathologies were diagnosed in considerable frequencies:

fatty liver (12.0%), kidney stones (8.5%), kidney cysts (7.8%), and cirrhosis-like condition (0.7%).

We did not identify any significant association between the O. viverrini infection intensity and the left liver lobe enlargement (P=0.27), gallbladder retraction capacity after meal (length difference pre- and post-fatty meal, P= 0.46), grade of periductal fibrosis (P=0.38), presence of gall stones (P= 0.44) and other pathogenesis (i.e., kidney stone(s) and kidney cyst(s), P>0.05).

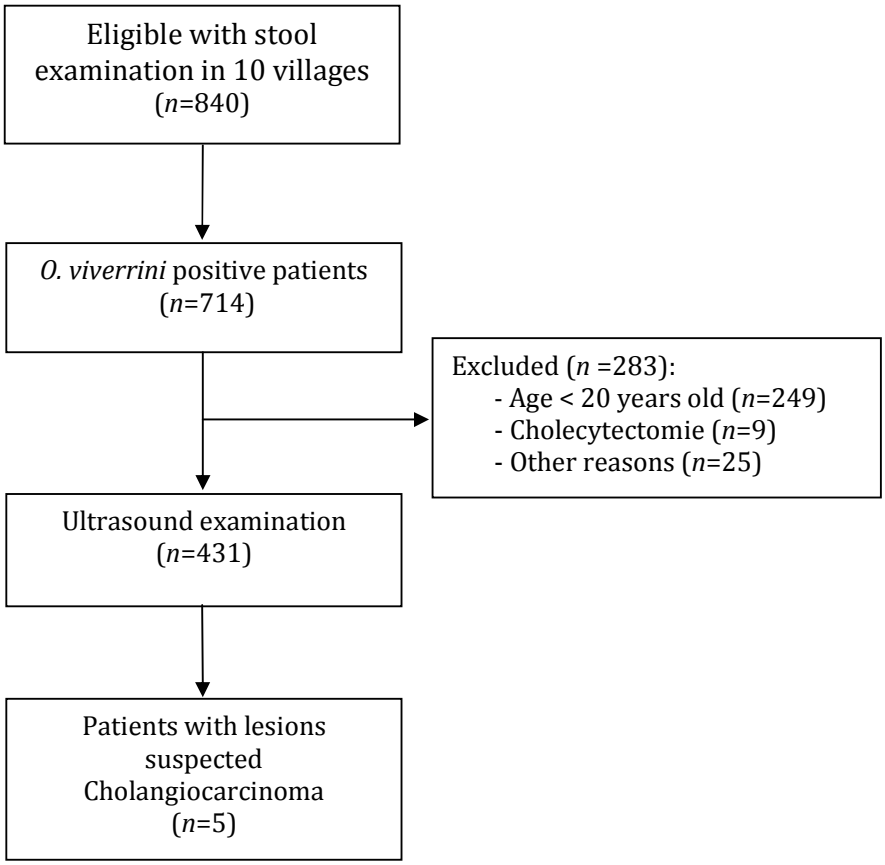

Figure 7.1 Study flowchart detailing the study participants in 10 villages, Saravane district, Saravane province, Lao PDR

Table 7.1 General characteristics of study participants (n = 431)

Characteristics		n (%)
Age (years]		
	20-29	75 (17)
	30-39	130 (30)
	40-49	92 (21)
	≥ 50+	134 (31)
Sex		
	Male	182 (42)
	Female	249 (58)
Ethnic group		
	Lao Loum	294 (68)
	Lao Theung	137 (32)
Profession		
	Farmer	427 (99)
	Other	4 (1)
Education		
	Illiterate	413 (96)
	Primary	8 (2)
	Secondary, and above	10 (2)
O. viverrini infection intensity		
	GM egg counts (EPG)	3961
	Min – Max of egg counts (EPG)	24 – 69,648
Intensity groups [a]		
	Light (≤ 500 EPG)	173 (40)
	Moderate (501-999 EPG)	63 (15)
	Heavy (1000-1999 EPG)	61 (14)
	Very heavy (≥ 2000 EPG)	134 (31)

Data are numbers and (%) of subjects; [a] according to Sripa and colleagues [18], based on Kato-Katz thick smear examinations; GM- geometric mean, EPG eggs per gram stool

7.4.3 Prevalence of Suspected CCA

Five patients had liver masses suspected for CCA diagnosed by ultrasonography, representing 1.2% of the random sample of *O. viverrini* infected patients (Table 7.2, 7.3). These patients, 2 men and 3 women, had a mean age of 52 years (range 36 – 67 years). They were referred to the regional hospital in Pakse (Champasack province) for further examinations. All had abnormal renal function tests that did not allow performing further diagnostic procedures such as e.g. endoscopic retrograde cholangiopancreatography. Hence, no further targeted diagnosis could be performed.

Two patients had a heavy and three had very heavy *O. viverrini* infection intensity (Table 7.3). All cases had normal liver function tests (AST, ALT, ALP, bilirubine), except one male and one female patient with markedly increased levels of AST, ALT, ALP (248.16 mg/dl) and bilirubine (13.3 g/l), indicating that pathology may develop to prevent the biliary circulation (Figure 7.3). In none of the cases a hepatitis B or C was diagnosed. Tumor markers of all five patients, namely IL-6, PAI and CA19-9, were in a normal range.

Table 7.2 Hepatobiliary morbidity in *Opisthorchis viverrini* infected adult patients (n=431)

Morbidity		n (%)
Height of left liver lobe (cm)	Mean (SD)	5.4 (1.0)
Normal parenchyma	Grade 0	41 (9)
Mild periductal fibrosis	Grade 1+	30 (7)
Moderate periductal fibrosis	Grade 2+	287 (67)
Advanced periductal fibrosis	Grade 3+	73 (17)
Suspected lesion of CCA		5 (1)
Cirrhosis		3 (1)
Fatty liver		52 (12.1)
Length pre-fatty meal (cm)	Mean (95% CI)	6.9 (6.8-7.1)
Length, post-fatty meal (cm)	Mean (95% CI)	5.5 (6.9-7.1)
"Pre" minus "post" fatty meal (cm)		1.4
Gallbladder pathologies		
Wall thickness		5 (1)
Wall irregularity		2 (0.5)
sludge present		6 (1)
stone present		57 (13)
Bile duct dilated		10 (2)
Intra hepatic duct stone		1 (0.2)

Data are numbers; (%) of subjects, unless otherwise indicated

Table 7.3 Follow-up examination of five suspected CCA cases

	Case 1	Case 2	Case 3	Case 4	Case 5
Age (years), sex	65, m	48, m	36, f	45, f	67, f
Liver function tests					
AST(IU/l)	127	39	49	30	23
ALT (IU/l)	47.0	35.9	45.9	37.9	16.7
Total bilirubine	13.3	8.6	9.5	8.5	10.3
Albumin (g/l)	6.8	4.9	5.3	4.3	3.3
ALP	248.16	75.2	55.4	65.34	74.8
GGT (IU/l)	147.9	10.3	6.8	9.9	8.8
AFP (IU/l)	0.8	0.3	0.2	0.04	0.1
Creatinine (mg/dl)	1109.0	173.0	1335.0	1128.0	1278.0
Tumor markers					
IL-6 (pg/ml)	<10	<10	< 10	11.5	<10
PAI (pg/ml)	5700.8	14249.6	9891.1	19840.9	16826.5
CA 19-9	25.0	20.0	12.9	12.8	14.6
Infections					
O. viverrini (EPG)	1968	2878	3734	1752	4680
(infection intensity)	(heavy)	(very heavy)	(very heavy)	(heavy)	(very heavy)
HBsAg-HBV	neg.	neg.	neg.	neg.	neg.
HCsAg-HCV	neg.	neg.	neg.	neg.	neg.
Reported ill health	Intense jaundice	Absence	Absence	Absence	Moderate jaundice, mass palpabal at RUQ
Ultrasonography findings	Liver categorized as grade 2+, liver mass in segment 7, markedly dilated bile duct, intrahepatic stones	Liver grade 2+, liver mass in segment 5, no dilated bile duct detected	Liver grade 3+, liver mass in segment 5, no dilated bile duct detected	Liver grade 2+, fatty liver mass presence in segment 5 of liver, no dilated bile duct detected, left hydronephosis	Liver grade 3+, fatty liver mass presence in segment 5 of liver , no dilated bile duct detected

Data are no; (%) of subject; EPG, egg per gram faeces, ALT alanine aminotransferase; AFP Alpha fetoprotein; ALP alkaline phosphatase; AST aspartate aminotransferase; CA 19-9 Carbohydrate antigen 19-9; f female; GGT Gamma-glutamyl transferase (GGT); HBsAg hepatitis B surface antigen; HCsAg hepatitis C surface antigen; IL-6 Interleukin 6, m male; neg. negative; PAI plasminogen activator inhibitor; RUQ right upper quadrant

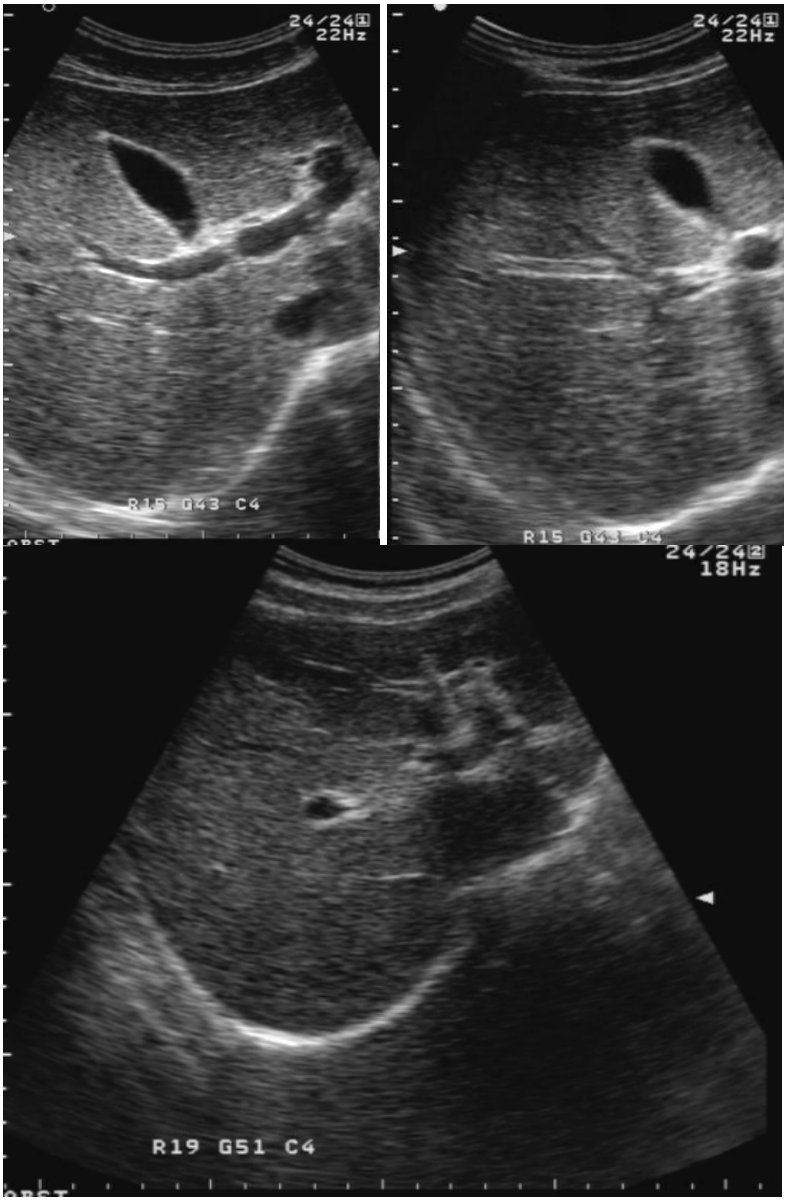

Figure 7.2: Liver parenchyma fibrosis observed: (1+) starry sky (top, left); (2+) rings and pipe stems (top, right); (3+) highly echogenic 'patches' extending to peripheral (bottom) areas

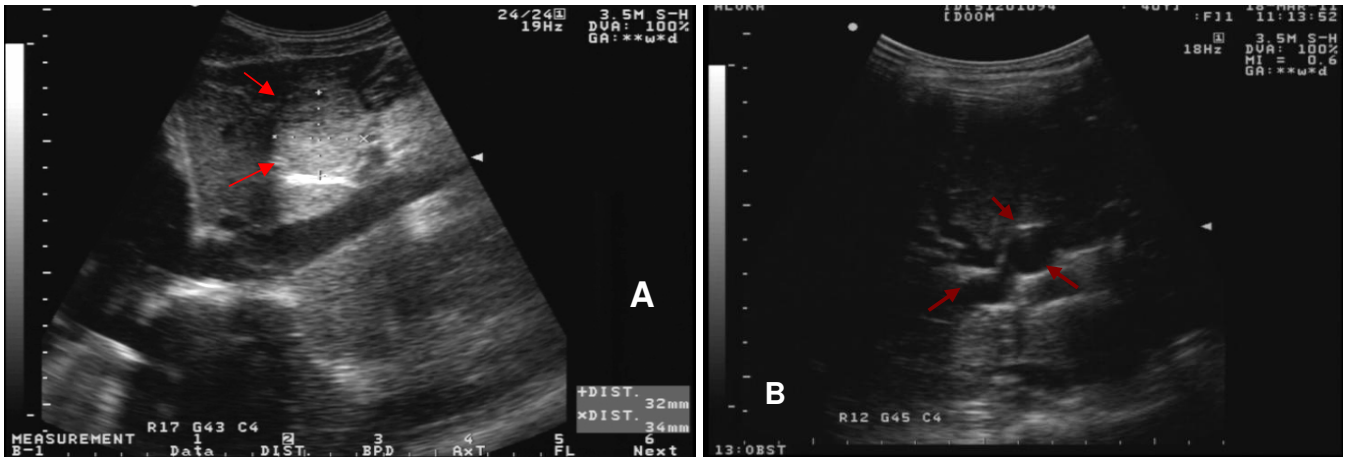

Figure 7.3: Patient (male, age 65 year): (A) with solid mass lesion with well-defined contour in right liver lobe; (B) Mechanical bile duct dilatation due to the mass

7.5 Discussion

We assessed the degree of hepatobiliary pathology including lesions suggestive of CCA in rural communities in Southern Laos where *O. viverrini* infection is highly endemic. Among a random sample of *O. viverrini* infected adult individuals of 10 villages, we identified a rampant prevalence of clinically hepatobiliar pathology, i.e., 83.5% had a moderate or advanced periportal fibrosis; only 9.5% of the examined persons had a normal liver parenchyma. Fatty liver were very frequent (12.5%). In five patients representing 1.2% of the sample such lesions were observed. This is the first study reporting on suspecting CCA lesions in communities in Laos.

O. viverrini infection leads to variety of hepatobiliar diseases [21,22] ranging from non-specific or asymptomatic cases, such as upper right quadrant pain or abdominal irritations to severe manifestations, namely cholangitis, obstructive jaundice, cholelithiasis, gall stones and liver periductal fibrosis, and the most severe outcome of a chronic infection, a fatal bile-duct cancer (cholangiocarcinoma). The clinical manifestations in our study were similar to previously reported manifestations.

However, they were diagnosed in rampant prevalence rates. In our study advanced periportal fibrosis was seen in 83.5% of participants, which is three times higher compared with reports from neighboring Thailand [18,21]. Also, gallbladder and intrahepatic stones, bile duct dilatation and fatty livers were diagnosed in high frequencies. Of note, 9 of the identified study participants could not be enrolled due to their precedent cholecystectomy which is further documenting the excess hepatobiliar pathology burden in our setting. The heavy *O. viverrini* infection intensity in our setting is the most likely explanation for the high level of morbidity. The mean infection intensity of 3961 eggs per gram of stool was observed and 45% of the patients had heavy or very heavy infection intensity.

O. viverrini infection has been classified as Group 1 carcinogen agent triggering CCA development [23]. In Northeast Thailand an estimated 60% of CCA cases are due to *O. viverrini* infection [6,24]. Direct irritations from the fluke in the bile ducts and immunopathological pathways of the parasite toxins are recognised mechanisms. Nonetheless, cholangiocarcinoma has multi-factorial origins [25]. Various additional stressors such as *N*-nitrosamide from foodstuff such as fermented fish sauce “Pa dek” [26], have been identified as important determinants. All the stressors reported in Thailand are also highly prevalent in our study setting in Southern Laos.

The world’s highest CCA prevalence and incidence is recorded in Northeast Thailand [25,26] where 71% of primary cancer is CCA [4,6]. In our study, of 431 subjects five patients had lesions suspicious of CCA. The suspected diagnosis was based on invasively growing liver masses. They were typical for those seen in confirmed CCA patients. Furthermore, all patients had a heavy or very heavy *O. viverrini* infection intensity which is a known determinant of CCA development [4,6]. In addition, all five cases reported other ill health problems such as high blood creatinine level, and liver function test. All had advanced periductal fibrosis (case 1, 5 = Grade 3+: case 2, 3, 4 = Grade 2+). These observations are typical signs in CCA patients in Thailand [21,24].

Early clinical signs and symptoms of CCA patients are related to biliary obstructions, jaundice, pale stool, dark urine, and puritus. In addition, other symptoms are increased liver mass, right upper quadrant pain, fever and weight loss [27,28]. Of five suspected

CCA cases, two of suspected CCA cases showed moderate to intense jaundice with bile duct dilatation detected by US.

Liver function tests performed on suspected CCA patients were with the normal range. In CCA patients liver function is preserved over a long period of time in illness [29]. The proposed tumor markers for CCA, i.e., IL-6, CA 19-9 and PAI were also in the normal range. Biochemical test are not specific for CCA except for CEA and CA19-9 [30, 31]. A recent case control study focused on the parasite-specific interleukin-6 (IL-6) promising marker in detecting the pathogenesis of advanced periductal fibrosis in individuals infected with chronic *O. viverrini* [18]. Given that these markers measures advanced CCA [19], their values indicate that our cases might still be in an earlier stage of development.

A final diagnosis for our suspected CCA cases could not be confirmed. High blood creatinine levels were measured in all patients which is a contraindication for imaging diagnosis, namely CT scan, and Endoscopic Retrograde Cholangiopancreatography (ERCP). These imaging techniques include the use of iodinated radiocontrast through intravenous injection, which can lead to renal failure in patient with high blood creatinine.

In the absence of these further diagnostic results we can not definitively conclude on a CCA diagnosis. Hence, these suspected CCA lesions are currently the best available information on CCA in communities in Laos. In a recent ultrasonographical hepatobiliary morbidity survey in Champasack province a similar prevalence of 1% (8 among 800 examined) suspected CCA lesions were recored (pers. communication, Dr. Bouasy Hongvanthong). These findings warrant further investigations with confirmation of CCA.

We noted *Opisthorchis*-associated nephropathy in our population study such as kidney stone(s) (8.6%), kidney cyst(s) (7.9%), and hydronephosis (1.6%) among individuals infected with *O. viverrini*. Only kidney cyst showed association with APF. This finding is consistent with recent reports on kidney pathogenesis observed in hamsters with infected *O. viverrini* [32].

In our study a relative small number of study participants were included. As *O. viverrini* infections occur in clusters, larger studies in settings with varying prevalence rates are required to assess the region specific morbidity and cancer risks, and hence to assess the Lao morbidity burden. In a recent global burden of disease initiative the burden of food-borne trematodiasis was estimated at 665,352 DALYs (479,496 - 859,051 DALYs) [33]. This is most likely crude underestimation of the burden as it did not take into account the actual burden as they were missing in high endemic areas.

However, this study documents the importance of liver fluke infection in terms of mild to severe hepatobiliary morbidity. CCA diagnosis is not possible in Laos due to the lack diagnostic (i.e., imaging techniques, liver biopsy) and adequate case management facilities. Suspected cases diagnosed in hospitals in Vientiane and elsewhere in Laos are referred to hospitals in Thailand, i.e. to Khon Kaen, Oubon Ratchathani, and Mukdahan. For those confirmed with a CCA symptomatic treatment is proposed.

Mass lesions could have resulted from other common malignant hepatobiliary, namely hepatocarcinoma or hepatitis B and C viruses which are also common in Southeast Asia and possible risk factor for CCA [34]. Furthermore, there is evidence that other predisposing factors for CCA such as primary and secondary sclerosing cholangitis, malformations (choledochal cyst, Caroli's disease), thorotrast, alcohol consumption, obesity, and smoking may play a role [35,36]. Differential diagnosis of morbidity must thus be investigated in more detail in further studies.

This study is a first attempt to quantify mild to severe morbidity including the presence of malignant lesions in rural communities in Laos where the prevalence of *O. viverrini* infection is high. The absence of a conclusive diagnosis for the severe sequelae is hampering the estimation of the CCA burden in Laos. Furthermore, the facilities of advanced and less invasive diagnostic procedures such as ERCP, MRI and MRCP for suspected CCA cases are warranted to investigate the burden of hepatobiliary morbidities in *O. viverrini*-endemic settings in Laos.

7.6 Acknowledgement

Authors thank the participants for their active participation. We thank Dr. Eimorn Mairiang from Department of Radiology, Faculty of Medicine, University of Khon Kaen, Khon Kean, Thailand for her assistance with regard to ultrasound imagines. We also thank the Saravan provincial Health Departments and District Health Office for excellent assistance and their company. We are grateful to Dr. Remigio Olveda from the Research Institute for Tropical Medicine, Philippines (RTM-DOH) and Dr. Somphou Sayasone from the National Institute of Public Health for comments and advice on an initial draft of manuscript and proposal.

7.7 Financial support

This work was supported by UNDP/WB/WHO Special Programme for Research and Training in Tropical Diseases, Joint Small Grants Programme for Operational Research in Communicable Diseases, Manila, Philippines [2010/92660-0]. PS and KP acknowledge financial support from the commission of research partnership with developing countries (KFPE). Furthermore, PAS and KP received support from the City of Basel and the Rudolf Geigy Foundation, respectively. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

7.8 Author Contributions

PAS and PO conceived and designed the study; PAS, VR, KP, PO, YV collected data; KA, OR had the overall responsibility of data collection; PAS, PO and JH analyzed data and interpreted results together with CH; PAS and PO wrote the manuscript; OR and EM performed quality control of ultrasonographical assessments; BS conducted serological analysis; KA, OR and CH assisted with manuscript revisions; all authors read and approved the final submitted manuscript; PAS and PO are guarantors of the paper.

7.9 Potential conflicts of interest.

All authors: None declared

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8. Hepatobiliary Morbidity and Suspected Cholangiocarcinoma in Lao People's Democratic Republic: a hospital-based retrospective study

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Working paper

8.1 Abstract

Cholangiocarcinoma poses a significant public health problem in Southeast Asia. Chronic infection due to liver fluke, *Opisthorchis viverrini* is believed to be a key risk factor to develop CCA. *O. viverrini* is known to be prevalent in Lao PDR, no epidemiological-based evidence of CCA has been determined in Lao PDR. A hospital-based, retrospective study was carried out in all referral hospitals in Lao PDR, including 4 representative hospitals in the center, one in the north and one in the South. All medical records of patients admitted during 2006-2010 to the hospital and diagnosed with suspected CCA were retrospectively reviewed and based on selected criteria. In total 247 were suggestive to CCA lesion detected by ultrasound and CT scan. Male gender and age of 50 years and above were predictors of suspected cases. The majority of suspected CCA case was admitted at the hospitals in the central (58.0%) and southern (29.6%) parts of Laos accompanied by abdominal pain (48.5%) and jaundice (14.2%). Of the 274 individual records, 267 (97.4%) of the patients had dilated bile duct; 40 (74.1%) had gallstone or intrahepatic stone, and 33 (12%) were parasitologically confirmed with *O. viverrini*. This study provides the first estimated numbers of suspected CCA admitted at the hospitals in Lao PDR. Definite diagnosis of CCA was not possible. Therefore, case diagnosis and good registration of suspected CCA cases are warranted as evidence for large-scale studies, and to develop preventive and curative health services.

8.2 Introduction

Cholangiocarcinoma (bile duct cancer, CCA) is a rare cancer in the world but poses a significant public health importance in Southeast (Andrews e al., 2008; Keiser and Utzinger, 2009; Sripa et al., 2010). CCA has been recognized as primary cancer of the epithelial cell of hepatic bile ducts. The main risk factor is a chronic infection with a liver fluke (*Opisthorchis viverrini*) (Keiser and Utzinger, 2009; Sripa et al 2007). other risk factors such as dietary intake of nitrate and nitrosamines, aflatoxin B1, smoking, excessive alcohol consumption, obesity as well as chronic hepatitis B and C (HBV and HBC) infection also contribute to increase a risk of CCA, however, the exact etiology is unclear (Shin et al., 2010).

Today, no cancer registration exists in Lao PDR and the incidence of CCA, precancerous lesions and precancerous conditions of CCA are unknown. However, given the high infection rates with *O. viverrini* which are similar (Chai et al., 2005; Rim et al., 2003; Sripa et al., 2007) or beyond the rates in neighboring Thailand, the incidence of CCA is believed to be comparable to north-eastern Thailand or beyond. The incidence of CCA and *O. viverrini* in Thailand from 1990-2001 account for 118.8 per 100 000 person years (Sripa et al., 2007).

Here, we aimed to investigate the estimated numbers of suspected CCA cases in hospital-based settings in Lao PDR.

8.3 Methods

8.3.1 Study site and patients

Observations were made with all medical records retrospectively reviewed of patients admitted and diagnosed with suspected CCA at the central hospitals in North, Central and South of Lao PDR. There were totally 6 hospital representatives. Of six hospitals, three hospitals are located in Vientiane capital, which are part of the university hospitals. Each suspected case of the records was filled into a questionnaire form. Demographic data (sex, age, village of residence), other pathologies and diagnosis techniques employed were filled into the questionnaire. For the sample size, we went through the patient record archives (all records) of the referral hospitals to identify all patients with ultrasound, CT scan (and other imagine device) and clinical information on suspected CCA. Records of patients who visited the hospitals between 1 January 2006 and 31 December 2010 were reviewed and included in the study.

8.3.2 Case definition to identify suspected CCA patients

The definitions of the CCA cases were based on the previous studies performed in endemic areas where the prevalence of CCA is high (Mairiang et al., 2011; Sripa et al., 2010a). (i) C*onfirmed CCA case*: the final histopathological results must be confirmed (with biopsy). This diagnosis is not performed in Lao PDR. (ii) Suspected CCA case: As indicators of morbidity the following clinical features related to CCA were used: (a) a single/multiple mass (es), (b) a dilated intrahepatic duct, or (c) a hydrop gall bladder found by U/S, CT scan, Magnetic resonance imaging (MRI), ERCP or MRCP; Biochemical test required related to CCA were serum alkaline phosphatase (ALP), gamma-glutamyltransferase (γ-DT), CEA and CA19-9 and AFP. In addition, other data were collated from the records including: the sex and age of each patients, original province of patients, hospital complaints on admission, onset of symptoms, individual and family past history, laboratory data collected during their hospitalization, location or the tumour detected by ultrasound, CT scan, MRI, ERCP, MRCP, final histopathological results, final diagnosis and prognosis, appropriated treatment. In records of ultrasound examination and CT scans of abdominal tract hepatobiliar abnormalities were reported: dilation of biliary tree, mass in liver associated +/- gall bladder anormalities (Gall bladder stone (GBS) and bile duct stone; any evidence of intrahepatic duct dilatation (IHDD), extrahepatic duct dilatation diagnosed was transcribed from records to the study questionnaire.

8.3.3 Data management and analysis

Data was double-entered and validated in Epidata 3.1 (Epidata Association; Odense, Denmark). Statistical analyses were performed with STATA statistical software, version 10.1 (Stata Corp.; College Station, TX, USA). Descriptive data were presented in percentage and numbers.

8.4 Results

Of 274 suspected CCA patients admitted at the 6 referral hospitals across the country, namely one hospital in the Northern, 3 hospitals in central and 2 hospitals in the southern part of Lao PDR. Male (74.8%) was the most predominant among the suspected cases versus female (25.3%). Median age was 57 years, with peak of age group was among those > 50 years (76.3%). The overall admission of cases was the

referral hospitals in the capital city (66.5%). The average of year of documentation archives at the hospital was 3.3 years. Half of cases were found mostly in the central part of Lao PDR (43.1%) (Table 8.1). Additionally, we found 58% of patients were originally from the central part, 29.6% from the south and 12.4% from the north part of the country made by their permanent residency (Figure 8.1).

Table 8.1 Patients' characteristics

		N° of patients (n= 274)
Age [years]		
	Median	57
	20-29	0 (0)
	30-39	8 (2.9)
	40-49	57 (20.8)
	50+	209 (76.3)
Sex		
	Male	205 (74.8)
	Female	
Hospitals		
	Luang Prabang Hospital	15 (5.47)
	Savanakhet Hospital	22 (8.3)
	Champasak Hospital	55 (20.1)
	Mahosot hospital*	77 (28.1)
	Friendship hospital*	55 (20.1)
	Sethathirath hospital*	50 (18.3)
Original province of patients		
	Oudomxay	1 (0.36)
	Xieng Khouang Province	4 (1.46)
	Luang Prabang Province	25 (9.12)
	Sayaboury Province	4 (1.46)
	Vientiane Province	29 (10.58)
	Vienitane capital Province	118 (43.07)
	Bolikhamxay Province	5 (1.82)
	Khammouane Province	7 (2.55)
	Savanakhet Province	25 (9.12)
	Champasack Province	51 (18.61)
Number of years included related to patients' record [Years]	Luang Prabang Hospital	2
	Savanakhet Hospital	5
	Champasak Hospital	4
	Mahosot hospital	2
	Friendship hospital	2
	Sethathirath hospital	4

Data are no; (%) of subjects, otherwise indicated [year], * centered hospitals in Vientiane capital

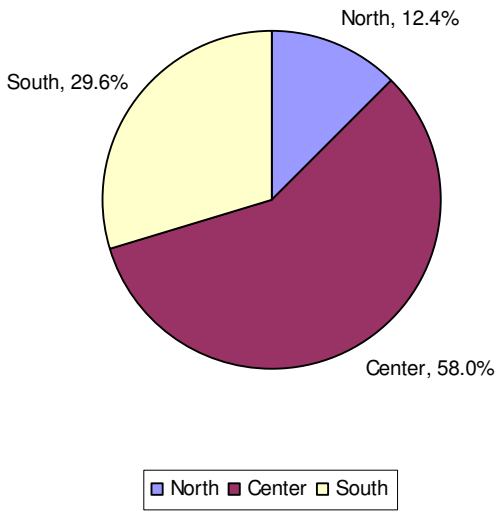

Figure 8.1 Records of suspected CCA patients diagnosed at the referral hospitals of Lao PDR from 2006-2010 by area

Of 274 suspected CCA cases, 57 (20.8%) patients were previously admitted to a hospital. Suspected CCA patients had also abdominal pain (48.5%), jaundice (20.9%) and jaundice with abdominal (14.2%) (Table 6). All CCA suspected patients (100%) underwent ultrasonography examination, however, CT scan results was found only in 55 cases (20.1%). 267 patients (97.4%) had a bile duct dilatation, 40 patients (74.1%) presented either stone in the gall bladder or intrahepatic ducts (Table 8.2).

The biochemistry of suspected CCA patients was shown in Table 8.3. Most of levels of liver function test ALT, AST, and ALP were increased among the advanced pathology cases. All patients were negative for HBsAg-HBV and HBcAg-HCV. Of 222 cases with parasitological examination, 33 (12.0%) cases were infected with *O. viverrini*. Histopathology is not performed for any suspected CCA case because it was not available in Lao PDR. Only symptomatic treatments were offered to patients. No resectable tumor and other surgical bypass namely palliative plastic or metal stents were available at the hospitals across the country.

Table 8.2 Results of patients' investigations

	Total	N° of patients (n= 274)
Chief of complaints	(n= 274)	
Jaundice or icteric skin		57 (20.9)
Abdominal pain		133 (48.5)
Fever		31 (11.3)
Jaundice and abdominal		39(14.2)
Others (abdominal mass, fatigue, constipation, ect)		14(5.1)
History of hospitalization	(n= 274)	
Previous admission		57 (20.8)
None		217 (79.2)
Symptoms and diagnosis for previous discharge	(n= 57)	
Cholecystitis		12 (21.0)
Hepatitis		5 (8.8)
Suspected Cholangiocarcinoma		18 (31.6)
Dyspepsia		9 (15.8)
Hepato-carcinoma cellulaire		11 (19.3)
Others		2 (3.5)
Patients underwent the U/S examination	(n= 274)	
Yes		274 (100.0)
Mass characteristics	(n= 274)	
Single mass		46 (16.8)
complex masses		228 (83.2)
Location of mass(es) in liver performed by U/S	(n= 274)	
Segment 1		
Yes		2 (0.7)
No		272 (99.3)
Segment 2		
Yes		3 (1.1)
No		271(98.9)
Segment 3		
Yes		4 (1.5)
No		270 (98.5)
Segment 4		
Yes		26 (9.5)
No		248 (90.5)
Segment 5		
Yes		40 (14.6)
No		234 (85.4)
Segment 6		
Yes		32 (11.7)
No		242 (88.3)
Segment 7		
Yes		47 (17.2)
No		227 (82.8)
Segment 8		
Yes		29 (10.6)
No		245 (98.4)
Undefined segment		
Yes		174 (63.5)
No		100 (36.5)

Data are no; (%) of subjects, CT scan- computerised tomography scan; U/S- ultrasonography

Table 8.3 Performance characteristics of laboratory among 274 suspected CCA cases

Liver function test		Total (n=274)	Number of patient with normal value	Numberof patient anormal value
WBC				
	Mean (SD)	13768.1 (6992.1)	4.1-10.9x10^3/µL	168 (62.7)
	Range	4.3-6.1 x10^3		
SGOT/AST(IU/l)				
	Mean (SD)	153.4(144.8)	0-35 IU/l	266 (97.1)
	Range	23-1022		
SGPT/ALT (IU/l)				
	Mean (SD)	112.1 (113.11)	0-45 IU/l	215 (78.5)
	Range	8.3-934		
Total bilirubine				
	Mean (SD)	23.5 (42.0)	2-17 µmol/l	256 (95.2)
	Range			
ALP				
	Mean (SD)	530.2 (617.4)	30-120 IU/l	241 (88.0)
	Range	14.7-4899		
GGT(IU/l)		(n=23)		
	Mean (SD)	166.4 (114.9)	0-30 IU/l	22 (95.7)
	Range	28.0-513.0		
AFP (IU/l)		(n=49)		
	Mean (SD)	10.32 (56.9)	0-44 ng/mL	1 (2.0)
	Range	0.02-400		
HBsAg-HBV		(n=268)		
	Yes			265 (98.9)
	No			3 (1.1)
HBcAg- HCV		(n=269)		
	Yes			266 (99.6)
	No			1 (0.4)
Stool exam		(n=274)		
	Yes			265 (96.7)
	No			9 (3.3)
Stool results		(n=265)		
Negative				220 (83.6)
Hookworm				4 (1.5)
Opisthorchis viverrini				33 (12.0)
Trichomonas hominis				1 (0.4)
Strongyroid stercolaris				5 (1.8)
Ascaris				2 (0.7)

Data are no; (%) of subject, otherwise indicated (standard deviation); WBC- white blood cell count, SGOP/ AST-Aspartate aminotransferase; SGPT/ALT-Alanine aminotransferase; ALP-Alkaline phosphatase; HBsAg- surface antigen of the Hepatitis-B-Virus (HBV) ; HBcAg- surface antigen of the Hepatitis-C-Virus (HCV) ; AFP-Alpha-Fetoprotein serum; GG- Gamma GT

8.5 Discussion

We investigated the incidence of suspected CCA cases using hospital records of reference hospitals in Lao PDR. To our knowledge this study has not been documented to date in Lao PDR. The diagnosis of CCA in Laos is likely to be very difficult due to lack of diagnostic tools and accessibility of treatment. All suspected cases admitted at the hospitals had undergone abdominal ultrasound, then CT or ERCP. Most of suspected cases admitted at the hospitals, in particularly from provincial hospitals need to refer their patient for further investigations at the central hospitals in Vientiane, for instances biologic marker test, CT scan and recently ERCP has been available at Mahosot Hospital, Vientiane Capital, Lao PDR. Our finding found that all records, patients were treated only symptomatic treatment for suspected CCA mitigation. None of further palliative treatment were not applicable, such as chemo- and radiotherapy, palliative plastic or metal stent (biliary stenting), PTBD (percutaneous biliary drainage), respectable tumor bypass which allow to relieve biliary obstruction and pain for the CCA patients (Aljiffry, 2009; Khan, 2005). PTBD and plastic or metal stent or pre-operative biliary drainage are widely used over the past few decades in the treatment of biliary diseases especially those due to unresectable malignant tumors for instance CCA, primary sclerosing cholangitis, cholangitis secondary (Aljiffry, 2009; Panpimanmas, 2011). At present, suspected CCA case numbers presented at the admission are accompanied with typical jaundice and right upper quadrant pain as confirmed in our studies. Some of them were seen annually through check-ups at the hospital.

In the diagnosis of CCA the first diagnostic imaging procedure is sonography which is the most useful tool for initial screening assessment guide to demonstrate the bile duct dilatation (Saini, 1997; Sharma, 1999). Abdominal computed tomography is the next examination for the extent to the tumour and the regional node involvement. In some case ultrasound can identify obstruction and ductal dilatation with providing a direct image of pathologic changes and in some cases may be sufficient to diagnose CCA. All suspected cases in our study had been initially examined by abdominal ultrasound, then CT (computed tomography), PTC (percutaneous transhepatic cholangiography), ERCP (endoscopic retrograde chanlangiopancreaticography), MRI (magnetic resonance imaging) and MRCP (magnetic resonance cholangiopancreatography). MRI along with MRCP provides more accurate diagnosis comprisable to invasive cholangiographic technique, namely ERCP or PTC (Manfredi, 2001; Varghese, 1999). This study showed

58.0%, 29.6% and 12.4 % of patients were admitted in the center, south and north at the referral hospital, accompanied with jaundice which is the main clinical manifestation of CCA. This observation was in line with the main cause of patient's hospitalization due to CCA that accounted for 60% of northeast hospital in Thailand (Mairiang, 2003; Sripa et al., 2010b). The majority of patients referred to central hospital (the central part of Laos) (i.e., Mahosot, Sethathirath and Friendship hospitals) which could be explained by affordability, reliable medical provider and enable to access the health care facility.

The prevalence of CCA liver increases according to the infection intensity of *O. viverrini* is the most exposure to CCA (Keiser and Uzinger, 2009; Sripa et al., 2010a; Sripa et al., 2010b). However, our findings were shown only 12% patients infected with *O. viverrini*. These numbers underestimate the true infection rates among patients admitted as hospitals have been conducted on fecal fresh examination which has only a low sensitivity. Viral hepatitis B and C has known to be prevalent in Southeast Asians and a risk factor for CCA (Honjo et al., 2005). The prevalence of hepatitis is unknown in Laos. This study reported 19.3% suspected hepatocarcinoma, of whom no case reported hepatitis B and C infection.

In short, we have a shortage of standard medical records. Number of records available keeping in archives at the hospitals was short. Quality of records depended on hospitals, facilities and medical. Hospitals had very limited data storage, which were mostly available only hard file. Little information on final diagnosis, treatment and evaluation of patients were performed. Demographic information were not originally defined the patients' residence when they admitted. Therefore, improvement of hospital information system as well as health information will provide crucial information on impact of hepatobiliary disease induced by *O. viverrini* in terms of crude incidence and spark interest to conduct the further broaden investigation.

8.6 Authors' contributions

PAS, PO conceived and designed the study; PAS, YV carried out the data collection; KA had the overall responsibility of data collection; PAS and PO carried out the data analysis and interpretation of the data and draft the manuscript with CH; PO, KA and CH assisted with manuscript revisions; all authors read and approved the final submitted manuscript; PAS and PO are guarantors of the paper.

8.7 Funding

The UNDP/WB/WHO Special Programme for Research and Training in Tropical Diseases, Joint Small Grants Programme for Operational Research in Communicable Diseases, Manila, Philippines [2010/92660-0]. PS and KP acknowledge financial support from the commission of research partnership with developing countries (KFPE). Furthermore, PS received support from the City of Basel and the Rudolf Geigy Foundation, respectively. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

8.8 Ethical approval

This study was approved by the Lao National Ethics Committee for Health Research (NECHR, N° 278/NECHR), Vientiane, Lao PDR, Ethical Committee of Canton of Basel-Stadt and Baselland (EKBB), Basel, Switzerland and Ethical committee board, World health organization Pacific Region, Manila, Philippines. Permission of data collection was provided by the MOH, the Provincial Health Department and the director committees of the hospitals of Lao PDR. All data collected will be anonymised in accordance with the standard operating procedures (SOPs) on the good clinical practice guidelines of the International Conference on Harmonization.

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9. Low Efficacy of Single-Dose Albendazole and Mebendazole against Hookworm and Parasitic Co-infection in Lao PDR

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This article has been accepted
PLos Neglected Tropical Disease (Published)

9.1 Abstract

Background: Albendazole and mebendazole are increasingly deployed for preventive chemotherapy targeting soil-transmitted helminth (STH) infections. We assessed the efficacies of single oral doses of albendazole (400 mg) and mebendazole (500 mg) for the treatment of hookworm infection in school-aged children in Lao PDR. Since *Opisthorchis viverrini* is co-endemic in our study setting, the effect of the two drugs could also be determined against this liver fluke.

Methodology/Principal findings: We conducted a randomized open-label, two-arm trial. In total, 200 children infected with hookworm (determined by quadruplicate Kato-Katz thick smears derived from two stool samples) were randomly assigned to albendazole (n=100) and mebendazole (n=100). Cure rate (CR; percentage of children who became egg-negative after treatment), and egg reduction rate (ERR; reduction in the geometric mean fecal egg count at treatment follow-up compared to baseline) at 21-23 days posttreatment were used as primary outcome measures. Adverse events were monitored 3 hours posttreatment. Single-dose albendazole and mebendazole resulted in CRs of 36.0% and 17.6% (odds ratio: 0.4; 95% confidence interval: 0.2-0.8; *P*=0.01), and ERRs of 86.7% and 76.3%, respectively. In children co-infected with *O. viverrini*, albendazole and mebendazole showed low CRs (33.3% and 24.2%, respectively) and moderate ERRs (82.1% and 78.2%, respectively).

Conclusions/Significance: Both albendazole and mebendazole showed disappointing CRs against hookworm, but albendazole cured infection and reduced intensity of infection with a higher efficacy than mebendazole. Single-dose administrations showed an effect against *O. viverrini*, and hence it will be interesting to monitor for potential ancillary benefits of preventive chemotherapy targeting STHs in areas where opisthorchiasis is co-endemic.

Clinical Trial Registration: Current Controlled Trials ISRCTN29126001

9.2 Author Summary

Parasitic worms remain a public health problem in developing countries. Regular deworming with the drugs albendazole and mebendazole is the current global control strategy. We assessed the efficacies of a single tablet of albendazole (400 mg) and mebendazole (500 mg) against hookworm in children of southern Laos. From each child, two stool samples were examined for the presence and number of hookworm eggs. Two hundred children were found to be infected. They were randomly assigned to albendazole (n=100) or mebendazole (n=100) treatment. Three weeks after treatment, another two stool samples were analyzed for hookworm eggs. Thirty-two children who received albendazole had no hookworm eggs anymore in their stool, while only 15 children who received mebendazole were found egg-negative. The total number of hookworm eggs was reduced by 85.3% in the albendazole and 74.5% in the mebendazole group. About one third of the children who were co-infected with the Asian liver fluke *Opisthorchis viverrini* were cleared from this infection following albendazole treatment and about one forth in the mebendazole group. Concluding, both albendazole and mebendazole showed disappointing results against hookworm, with albendazole performing somewhat better. The effect of these two drugs against *O. viverrini* should be studied in greater depth.

9.3 Introduction

Infections with the three common soil-transmitted helminths (STHs), *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm (*Ancylostoma duodenale* and *Necator americanus*), are a global public-health concern, particularly in areas where poor sanitation prevails [1,2]. STH infections are among the most widespread of the neglected tropical diseases (NTDs) [3]. Indeed, more than a billion people are currently infected with one or several STH species, even though growing efforts are underway to control these parasitic worm infections [4]. In terms of their estimated global burden, hookworm is the most important among the STHs, as it might cause the loss of over 20 million disability-adjusted life years (DALYs) among the estimated 600 million infected people worldwide [1,5]. Chronic hookworm infection cause intestinal blood loss and result in poor iron status and iron-deficiency anemia, particularly in children, and women in reproductive age [1,6,7]. As a consequence, permanent impairment, including delayed physical and cognitive development, has been described [8].

In the absence of a vaccine, the global strategy to control STHs and other NTDs is to reduce morbidity through repeated large-scale administration of anthelmintic drugs, a strategy phrased preventive chemotherapy [8]. At present, the World Health Organization (WHO) recommends four drugs against STH infections, whereas albendazole and mebendazole are the two most widely used for preventive chemotherapy [9]. In 2008, in the Western Pacific Region, 33.4 million children were given anthelmintic drugs [10]. According to the Lao national scheme for school deworming, there is a treatment round at the beginning of the first semester (September-December) and in the second semester (January-April). Mebendazole (single 500 mg oral dose) is annually distributed to all school-aged children since 2005 [11]. Recent efforts have been made to provide mebendazole also to preschool-aged children through the Expanded Program on Immunization (EPI) and vitamin A distribution [4,12]. However, the efficacy of mebendazole and albendazole against STH infections in Lao PDR remains to be determined, and such locally derived evidence is important to guide national treatment policies.

The liver fluke *Opisthorchis viverrini* is co-endemic in Lao PDR, and particularly high prevalences have been observed in the southern provinces [13-16]. Praziquantel is the current drug of choice against *O. viverrini* [3]. Previous work has shown that multiple

doses of albendazole also show some effect [17,18]. Hence, in areas where STHs and *O. viverrini* co-exist and preventive chemotherapy targeting STHs are under way, it will be interesting to monitor for potential ancillary benefits on opisthorchiasis.

The purpose of this study was to assess the efficacy of a single oral dose of albendazole (400 mg) and mebendazole (500 mg) against hookworm infection among school-aged children in Lao PDR. In addition, the effect on other STHs (i.e., *A. lumbricoides* and *T. trichiura*) and *O. viverrini* in co-infected children were assessed. Our study complements a recent investigation, done in the People's Republic of China comparing single and triple dosing with albendazole and mebendazole against the three common STHs [19].

9.4 Methods

9.4.1 Ethical Considerations and Treatment

The research protocol was approved by the Ethics Committee of Basel, Switzerland (EKBB; reference no. 146/08) and the Lao National Ethics Committee for Health Research (NECHR), Ministry of Health, Vientiane, Lao PDR (reference no. 170/NECHR). The trial is registered with Current Controlled Trials (identifier: ISRCTN29126001). Written informed consent was obtained from parents/legal guardians of eligible children. Participation was voluntary and children could withdraw from the trial at any time without further obligation.

At completion of the trial, all children of the two primary schools and participants who were still found positive for hookworm (or other STHs) were treated with albendazole (400 mg). *O. viverrini*-infected children were administered praziquantel according to national guidelines [20].

9.4.2 Study Area and Population

A randomized open-label trial was carried out between February and March 2009 in 2 primary schools (Oudomsouk and Nongbok Noi) in Batieng district, Champasack province, southern Lao PDR. Children in the 2 schools were treated with mebendazole 5-6 months prior to the start of our study. The schools are located approximately 15 km southeast of Pakse town, on the Bolaven plateau at an altitude of approximately 1000 m above sea level (geographical coordinates: 105°56'53" N latitude, 15°14'59" E

longitude). The rainy season lasts from May to mid-October. A census done in 2007 revealed that 43,651 people lived in the 95 villages of Batieng district (Dr. Nanthasane Vannavong, Champasack Provincial Health Department; personal communication). More than three-quarter of the households (77.5%) lack appropriate sanitation. Drinking water is primarily obtained from unprotected boreholes or wells. Most villagers live on subsistence rice farming and rubber plantations (Dr. Nanthasane Vannavong, Champasak Provincial Health Department; personal communication). Infections with STHs and *O. viverrini* are common in Batieng district; a recent study revealed infection prevalences above 50% and 20%, respectively [21].

9.4.3 Study Design

We designed a randomized, open-label trial comparing albendazole (single 400 mg dose) and mebendazole (single 500 mg dose) in the treatment of hookworm infection. The sample size was calculated based on results of a meta-analysis on the efficacy of current anthelmintic drugs against common STH infections, which reported cure rates (CR; defined as percentage of helminth-positive individuals who became helminth-egg negative after treatment) of 75% and 15% for albendazole (400 mg) and mebendazole (500 mg), respectively against hookworm infection [9]. In order to account for the large variation (uncertainty) of the observed efficacies of mebendazole in the individual studies (CRs of 8-91% were found in the six randomized controlled trials), we more than tripled the mean efficacy of mebendazole (50% instead of 15%). Assuming superiority of albendazole (1-tailed test) and taking into account a 90% power, and an alpha error of 5%, we obtained a sample size of 85 children per treatment group. Furthermore, we assumed a drop-out rate of 15%, which resulted in a total sample size of 200 hookworm-positive school-aged children.

9.4.4 Field and Laboratory Procedures

The teachers of the 2 primary schools, the children, and the staff of the National Institute of Public Health, Centre of Malaria, Parasitology and Entomology, Centre for Laboratory and Epidemiology, the Provincial Department of Health, the Provincial Hospital and the Malaria Station of Champassak, and the village authorities were informed 1 week in advance on the study aims and procedures. Potential risks and

benefits were explained to all children and their parents/guardians. An informed consent form was distributed to all parents/guardians and signed. Children assented orally.

At baseline screening the consenting children (n=465) of the 2 schools, aged 6-12 years, provided two fresh stool samples within a period of 3 days. Stool containers were filled by children and transferred to a laboratory in the early morning (between 08:00 and 09:00 hours). All collected specimens were worked up on the day of collection. From each stool sample, duplicate Kato-Katz thick smears were prepared on microscope slides, using standard 41.7 mg templates [22]. Kato-Katz thick smears were quantitatively examined under a light microscope for helminths with a 100x magnification. Slides were read within 30-45 min after preparation. A random sample of approximately 10% of the Kato-Katz thick smears were re-examined by 2 senior technicians for quality control purposes. In case of discrepancies (i.e., positive *vs.* negative results; egg counts differing by >10%), results were discussed with the respective technicians, and the slides re-examined until agreement was reached.

In addition, a questionnaire was administered to each participating child to obtain sociodemographic data (i.e., sex, age, parent's education and occupation, ethnic group, and sanitation infrastructure), and behavioral data (i.e., wearing shoes, sources of drinking water, food consumption, personal hygiene). Hookworm-positive children (defined by the presence of at least one hookworm egg in one of the quadruplicate Kato-Katz thick smears examined per child) were invited for treatment (n=200).

At enrollment, a clinical examination, which included measurement of weight (using an electronic balance measured to the nearest 0.1 kg), height (using a measuring tap fixed to the wall and measured to the nearest cm), and axcillary temperature (using battery-powered thermometers, measured to the nearest 0.01°C), anemia assessment (finger prick capillary blood sample) was conducted and a medical history taken. Children were excluded if they had fever, or showed signs of severe malnutrition. Additional exclusion criteria were the presence of any abnormal medical condition such as cardiac, vascular, pulmonary, gastrointestinal, endocrine, neurologic, hematologic (e.g., thalassaemia), rheumatologic, psychiatric, or metabolic disturbances, recent history of anthelmintic treatment (e.g., albendazole, mebendazole, pyrantel pamoate, levamisole, ivermectin,

and praziquantel), attending other clinical trials during the study, or reported hypersensitivity to albendazole or mebendazole.

At follow-up, 21-23 days after drug administration, 2 stool samples were collected from each child and transferred to a hospital laboratory within 1 hour after collection. Each stool specimen collected at follow-up was subjected to the same procedures as during the baseline survey. Hence, duplicate Kato-Katz thick smears were prepared from each stool sample, examined under a microscope within 30-45 min by experienced laboratory technicians, and helminth eggs were counted and recorded for each species separately. Quality control was in place as during the baseline survey.

9.4.5 Randomization

Children were randomly assigned to a single dose of albendazole (400 mg) or mebendazole (500 mg), using a block randomization procedure (six blocks each containing four treatment allocations), generated by an independent statistician who was not otherwise involved in the trial. The sequence of blocks was determined using a random number table. In addition, schools were decoded by a researcher to assign children either to albendazole or mebendazole. Eligible children were randomly assigned and allocated to treatment by an epidemiologist. Children and drug administrators were not blinded for drug treatment. Laboratory personnel and clinicians monitoring the adverse events were blinded throughout the study.

9.4.6 Drugs and Adverse Events

Albendazole (400 mg; Albendazole®, South Korea) was obtained from the Ministry of Health, Vientiane, Lao PDR. Mebendazole (500 mg; Vermox®, Italy) was donated by Johnson & Johnson Pharmaceuticals, provided through the Ministry of Health and the Ministry of Education, Vientiane, Lao PDR. At treatment day, both groups received the drugs under direct medical supervision on an empty stomach. Children were monitored for at least 3 hours after drug administration and asked to report for any drug-related adverse events using a standard questionnaire administered and graded by study physicians.

9.4.7 Statistical Analysis

Data were double-entered and validated in EpiData version 3.1 (Epidata Association; Odense, Denmark). Statistical analyses were performed with STATA, version 10.1 (Stata Corp, College Station, TX, USA). Efficacy was calculated for both intention-to-treat (ITT) and per-protocol (PP) analyses. ITT analysis was based on the initial treatment intent. PP analysis included only those children who had complete data records (i.e., quadruplicate Kato-Katz thick smear reading before and after treatment, and full treatment compliance).

Infections with hookworm, *A. lumbricoides*, *T. trichiura*, and *O. viverrini* were grouped into light, moderate, and heavy infections, according to WHO guidelines (for STHs) and cut-offs put forward by Maleewong et al. (for *O. viverrini*) [23-24]. Infection intensity classifications are as follows: hookworm, 1-1999 eggs per gram of stool (EPG) (light), 2000-3999 EPG (moderate), and ≥4000 EPG (heavy); *A. lumbricoides*, 1-4999 EPG (light), 5000-49,999 EPG (moderate), and ≥50,000 EPG (heavy); and *T. trichiura* and *O. viverrini*, 1-999 EPG (light), 1000-9999 EPG (moderate), and ≥10,000 EPG (heavy).

Primary outcome measures were CR and egg reduction rate (ERR), the latter defined as the positive group's reduction of geometric mean (GM) fecal egg count at posttreatment, divided by the GM fecal egg count at pretreatment, multiplied by 100. Additionally, changes in class of infection intensities were determined following treatment. Negative binomial regression was applied to compare ERRs observed between both treatment groups. A Wilcoxen test was employed for the matched pair's analysis. We determined egg reduction rate ratio (ERRR) and 95% confidence interval (CI). Pearson's χ^2-test and Fisher's exact test, as appropriate, were used to assess the baseline binary characteristics between the treatment arms. Statistical significance was estimated using a likelihood ratio test (LRT). *P*-value below 5% was considered significant.

9.5 Results

9.5.1 Study cohort

Four hundred sixty-five school-aged children were enrolled in the baseline screening. Two hundred children (43.0%), 130 boys and 70 girls with a parasitologically confirmed hookworm infection, were randomly assigned to one of the two treatments. Data of these 200 children were included in the ITT analysis. The remaining 265

children were excluded because they had no hookworm eggs in their stool (n=235) or provided only a single stool sample (n=30). Overall, One-hundred-and-seventy-one children (85.5%) had complete baseline data, received treatment and completed follow-up examinations, and hence were utilised for PP analysis. Twenty-nine children (14.5%) were lost to follow-up, 18 in the mebendazole and 11 in the albendazole group (Figure 1). One-hundred-and-seventy-one children were included in the primary analysis. Their parents most commonly had completed primary school only, namely 77.5% of parents for the albendazole group and 80.5% for the mebendazole group. The most common profession of patients' parents was farming with 49.4% and 62.2% for albendazole and mebendazole treatment groups, respectively. The 2 groups were similar in terms of household assets, source of drinking water and consumption of cooked foods as well as raw fish (data not shown). More specifically, the consumption of raw fish was reported by 61.8% and 58.5%, respectively, and included dishes like “Pa Dek” (fermented fish sauce), “Lap Pa” and “Koy Pa” (raw, fish-based dishes).

9.5.2 Baseline Characteristics

At baseline, characteristics of the two treatment groups were similar (Table 9.1), including age (albendazole recipients, mean (standard deviation, SD) age 8.4 (2.1) years; mebendazole recipients 8.7 (2.1) years), weight (mean (SD) 23.8 (5.8) kg and 25.0 (5.9) kg, respectively), height (mean (SD) 123.8 (11.0) cm; 126.9 (11.0) cm, respectively) and hemoglobin (Hb) concentration (mean (SD) 11.8 (10.1) mg/dl; 11.9 (10.4) mg/dl, respectively). In both treatment groups, children (82.0%) were diagnosed with a light hookworm infection, whereas the remaining children had moderate or heavy infection intensities. The hookworm GM fecal egg counts in the mebendazole and albendazole groups were 707.0 and 859.1 EPG, respectively (Table 9.2).

The overall infection rates of *A. lumbricoides, O. viverrini* and *T. trichiura* were 34.0%, 48%, 45.0% respectively. *O. viverrini* GM fecal egg counts were 84.9 EPG (albendazole) and 120.8 EPG (mebendazole) (Table 9.3).

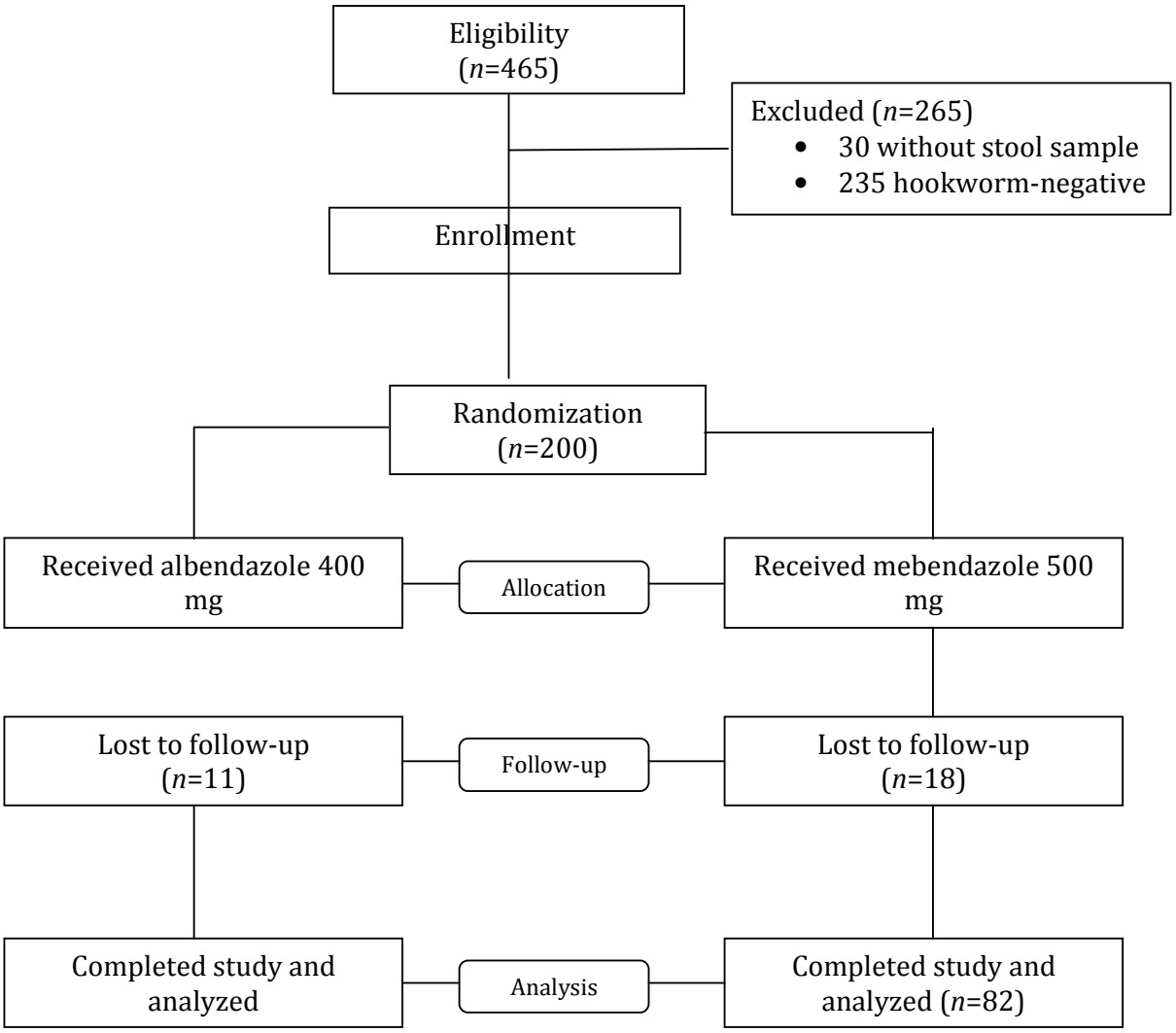

Figure 9.1: Flow chart detailing the study participation and compliance. Children who completed 2 stool samples were included in the final analysis for assessing the efficacy of single albendazole and mebendazole treatment against hookworm and concomitant helminth infections in Bachieng district, Champasack province, southern Lao PDR.

Table 9.1 Baseline characteristics

	Albendazole (*n*=89)	*Mebendazole* (n=82)
Boys/girls	56/33	49/33
Mean (±SD) age, y	9.0 (2.1)	9.0 (2.1)
Mean (±SD) weight, kg	24.0 (6.0)	25.2 (6.0)
Mean (±SD) height, cm	124.1 (11.0)	127.0 (11.0)
Mean (±SD) hemoglobin, mg/dL	11.85 (10.1)	12.04 (10.3)
Anemia (<11.5 mg mg/dL): n, % [a]	23 (57.5)	17 (42.5)
Latrine facility present	5 (5.6)	1 (1.2)
Parasitic infections		
Hookworm [b]		
Light (1-999 epg)	72 (80.9)	67 (81.7)
Moderate (1000- 9999 epg)	9 (10.1)	7 (8.6)
Heavy (>10 000 epg)	8 (9.0)	8 (9.7)
Co-infection with:		
Ascaris lumbricoides [b]		
Negative	61 (68.5)	53 (64.6)
Light (1-999 epg)	18 (20.2)	18 (22.0)
Moderate (1000- 9999 epg)	7 (7.9)	8 (9.8)
Heavy (>10 000 epg)	3 (3.4)	3 (3.7)
Trichuris trichiura [b]		
Negative	51 (57.3)	39 (47.6)
Light (1-999 epg)	38 (42.7)	43 (52.4)
Moderate (1000- 9999 epg)	4 (4.5)	0
Heavy (>10 000 epg)	0	0
Taenia spp		
Negative	78 (87.6)	79 (96.3)
Positive	11 (12.4)	3 (3.7)
Opisthorchis viverrini [c]		
Negative	44 (49.4)	50 (61.0)
Light (1-999 epg)	41 (46.1)	25 (30.5)
Moderate (1000- 9999 epg)	4 (4.5)	7 (8.5)
Heavy (>10 000 epg)	0	0

[a] According to guidelines put forth by WHO regarding definition of anemia [42]
[b] According to guidelines put forth by WHO [24], based on Kato-Katz thick smear examinations
[c] According to Maleewong and colleagues [23], based on Kato-Katz thick smear examinations
Data are no; (%) of subject, otherwise indicated (95% confidence interval); EPG, eggs per gram of stool; GM, geometric mean

9.5.3 Efficacy Against Hookworm

In the ITT analysis, the CRs of albendazole and mebendazole against hookworm infections were 32.0% and 15.0%, respectively. Overall, 124 children (73%) remained hookworm-egg positive; 68 treated with albendazole and 85 in the mebendazole treatment group. Si9.milar results were obtained with the PP analysis (Table 9.2). A statistically significant difference was observed when comparing the observed CRs using albendazole *vs.* mebendazole (OR=0.4; 95% CI 0.2-0.8; *P*=0.01). The hookworm GM fecal egg counts obtained at follow-up were 63.0 EPG in albendazole recipients and 147.3 EPG mebendazole recipients (ITT analysis 96.5 EPG and 210 EPG, respectively). The respective ERR for albendazole and mebendazole were 86.7% and 76.3% (ERRR 1.0; 95%CI 0.7-1.6; *P*=0.9). In children with moderate infection intensities (2000–3999 EPG), the effect of albendazole and mebendazole was significantly different (*P*=0.04).

9.5.4 Effect of Albendazole and Mebendazole Against *A. lumbricoides*, *T. trichiura*, and *O. viverrini*

Table 9.3 shows the effect of albendazole and mebendazole against *A. lumbricoides*, *T. trichiura* and *O. viverrini*. At baseline, GM infection intensities of *A. lumbricoides* were 1567 EPG in albendazole recipients and 1584 EPG in mebendazole recipients. Both albendazole and mebendazole treatments achieved high CRs above 90% and resulted in almost complete egg elimination. The CRs of albendazole and mebendazole obtained against *T. trichiura* were 33.3% and 27.9%, respectively. The respective ERRs were 67.0% and 66.0%. No statistically significant difference was observed for CR and ERR between the 2 treatments (OR=0.8; 95% CI 0.3-1.9; *P*=0.6 and ERRR=0.7; 95% CI 0.3-1.2, *P*<0.2). Finally, CRs against *O. viverrini* achieved with albendazole and mebendazole were 33.3% and 24.2%, respectively (OR=0.7; 95% CI 0.3-1.9; *P*=0.6). The respective ERRs were 82.1% and 78.2% (ERRR=0.8; 95% CI 0.2-3.9, *P*<0.8).

9.5.5 Adverse Events

Monitoring of children within 3 hours after drug administration revealed no drug-related adverse event, neither in the albendazole nor in the mebendazole group. Hence, both treatments were well tolerated.

Table 9.2 Per-protocol (PP) analysis of hookworm infection prevalence and cure rate of albendazole and mebendazole at baseline and follow-up

	Pretreatment		Posttreatment	
	Albendazole (*n*=89)	Mebendazole (*n*=82)	Albendazole (*n*=89)	Mebendazole (*n*=82)
No. of hookworm infected patients	89 (100)	82 (100)	57 (64.0)	67 (81.7)
No. of children cured (cure rate)	n.a.	n.a.	32 (36.0)	15 (17.6) [a]
Light infection (1-1999 EPG)	72 (80.9)	67 (48.2)	55 (61.8)	59 (72)
No. of children cured (cure rate)	n.a.	n.a.	17 (19.1)	8 (9.8) [b]
Moderate infection (2000-3999 EPG)	9 (18.0)	7 (46.7)	2 (2.2)	6 (7.3)
No. of children cured (cure rate)	n.a.	n.a.	7 (7.9)	1 (1.2) [c]
Heavy infection (≥ 4000 EPG)	8 (1.1)	8 (1.1)	0 (0)	2 (2.4)
No. of children cured (cure rate)	n.a.	n.a.	8 (9)	6 (7.3) [d]
GM fecal egg count (range), EPG	859.1 (699.0-1057.0)	707.0 (559.0-894.3)	63.0 (34.0-116.0)	147.3 (90.0-242.0)
Egg reduction rate	n.a.	n.a.	86.7%	76.3% [e]

[a]OR 0.4 [95% CI (0.2-0.8; *P*=0.01)] comparison of treatment outcomes between mebendazole *vs.* albendazole

[b]*P* =0.13; [c] *P*=0.04; [d] *P*=0.46

[e]ERRR 1.0 [95% CI (0.7-1.6; *P*=0.9)] comparison of treatment outcomes between mebendazole *vs.* albendazole

Note. Data are number; (%) of children, unless otherwise indicated (95% confident interval); GM, geometric mean; EPG, eggs per gram of stool; ERRR egg reduction rate ratio; OR odds ratio; n.a. not applicable

Table 9.3 Infection rate and cure rate of albendazole and mebendazole: *A. lumbricoides, T. trichiura, O. viverrini* at baseline and follow-up

	Pretreatment		Posttreatment	
Parasitic infection	Albendazole	Mebendazole	Albendazole	Mebendazole
A. lumbricoides (n=58)	(n= 28)	(n= 30)	(n= 28)	(n= 30)
No. of *A. lumbricoides* infected children	28 (100)	30 (100)	2 (7.1)	2 (6.7)
No. of patients cured (cure rate)	n.a.	n.a.	26 (92.9)	28 (93.3) [a]
GM fecal egg count (range), EPG	1567.0 (553.0- 4444.0)	1584.0 (528.0-4751.0)	0	0
ERR	n.a.	n.a.	100.0%	100.0% [b]
T. trichiura (n=82)	(n= 39)	(n= 43)	(n= 39)	(n= 43)
No. of *T. trichuris* infected children	39 (100)	43 (100)	26 (66.7)	31 (72.1)
No. of patients cured (cure rate)	n.a.	n.a.	13 (33.3)	12 (27.9) [c]
GM fecal egg count (range), EPG	94.1 (48.3-184.0)	65.2 (39.3-108.3)	75.0 (42.2-133.2)	48.0 (25.0-93.0)
ERR	n.a.	n.a.	67.0	66.0 [d]
O. viverrini (n=77)	(n= 45)	(n= 32)	(n=45)	(n= 32)
No. of *O. viverrini* infected children	45 (100)	32 (100)	30 (66.7)	25 (75.8)
No. of patients cured (cure rate)	n.a.	n.a.	15 (33.3)	8 (24.2) [e]
GM fecal egg count (range), EPG	84.9 (41.8-184.0)	120.8 (48.9-297.9)	73.0 (34.3-155.7)	114.4 (48.9-267.3)
ERR	n.a.	n.a.	82.1	78.2[f]

a OR 0.8 [95% CI (0.2-2.6; *P*=0.7) comparison of treatment outcomes between mebendazole *vs.* albendazole
b ERRR n.a.
c OR 0.8 [95% CI (0.3-1.9; *P*=0.6)] comparison of treatment outcomes between mebendazole *vs.* albendazole
d ERRR 0.7 [95% CI (0.3-1.2; *P*<0.2)] comparison of treatment outcomes between mebendazole *vs.* albendazole
e OR 0.7 [95% CI (0.3-1.9; *P*=0.6)] comparison of treatment outcomes between mebendazole *vs.* albendazole
f ERRR 0.8 [95% CI (0.2-3.9; *P*<0.8)] comparison of treatment outcomes between mebendazole *vs.* albendazole
Note. Data are number; (%) of children, unless otherwise indicated (95% confident interval); GM, geometric mean; EPG, eggs per gram of stool; ERRR, egg reduction rate ratio; OR odds ratio; n.a. not applicable

9.6 Discussion

This current head-to-head comparison of single-dose albendazole *vs.* mebendazole against hookworm infection in Lao school-aged children - to our knowledge the first trial in this Southeast Asian country - shows sobering results. Indeed, the standard single oral doses of albendazole (400 mg) and mebendazole (500 mg) that are recommended in preventive chemotherapy campaigns targeting STHs resulted in low CRs against hookworm infection. However, ERRs were moderate, 86.7 and 76.3% respectively.

A sizeable number of children were co-infected with *A. lumbricoides*, *T. trichiura* and *O. viverrini*, which allowed us to determine the effect of albendazole and mebendazole against these helminth species. With regard to *A. lumbricoides*, high efficacy of both drugs was confirmed. However, disappointing efficacy was confirmed against *T. trichiura* [25].

While the results obtained with mebendazole against hookworm and the efficacy observed with both drugs against *A. lumbricoides* and *T. trichiura* is in line with previous studies [19,26,27] and in agreement with overall CRs estimated through a meta-analysis [9], the low CR (36.0%) achieved with albendazole in the treatment of hookworm infection is somewhat surprising. Indeed, in the aforementioned meta-analysis, randomized controlled trials of single-dose albendazole (400 mg) revealed an overall CR against hookworm of 75% [9]. The reasons for the considerably poorer efficacy of albendazole observed in our study are unclear. Quality control of drug samples performed in our laboratories revealed that disintegration, dissolution and concentration of the albendazole tablets used in our trial were comparable to Zentel® (data not shown). The hookworm species (and strains) endemic in southern Lao PDR might be an explanation. However, there is a paucity of information on which hookworm species is predominant in Southeast Asia. Indeed, in our study setting the infection rates of *N. americanus* and *A. duodenale* are not known. Furthermore, recent studies documented that in Southeast Asia humans are at risk of acquiring *A. ceylanicum*, which is endemic in dogs and cats of the region and its importance in humans might be underestimated [28,29]. Hence, further analysis on the circulating parasite species is required to elucidate this issue. In addition, day to day variability in hookworm egg counts from individuals is a well described phenomenon [30]. Finally,

the study's sample size is rather small and therefore a few incidental effects such as failure of some children to swallow the pill correctly, might have contributed to low efficacy of albendazole for the treatment of hookworm infection. To sum up, differences in strain and species susceptibilities, host factors, and co-infections with other helminths are factors that might all play a role in explaining treatment failures [27,31].

However, at present, we cannot rule out that albendazole resistance is developing in our study setting. To date, nematode resistance in humans has not been reported. On the other hand, drug resistance is a major threat in veterinary public health [32,33]. The development of broad spectrum anthelmintic resistance, in particular resistance of nematodes to benzimidazoles, has been recognized in ruminants for decades [33,34]. Extensive studies on the underlying mechanisms of drug resistance have been carried out [35]. Further investigations on failure of the drugs to completely cure the patients are necessary in our study setting to substantiate this suspicion.

It is interesting to note that the 2 drugs employed, even at single oral doses, showed some effect against *O. viverrini*. Although CRs were low (33.3 and 24.2%), the moderate ERRs of 82.1 and 78.2% are encouraging. At present, praziquantel is the drug of choice against opisthorchiasis [36,37]. Studies carried out in the 1980s in *O. viverrini*-infected hamsters and patients infected with *O. viverrini* documented opisthorchicidal properties of albendazole and mebendazole [18,38]. However, long treatment courses of up to 7 days were recommended in view of these initial laboratory and clinical findings. Experiences with long treatment courses have been reported from a hospital-based randomized trial; albendazole given at dosages of 400 mg twice daily for 3 and 7 days resulted in CRs of 40% and 63%, respectively, and corresponding ERRs of 92% [18]. Furthermore, mebendazole in dosages of 30 mg/kg daily for 3 or 4 weeks resulted in a CR of 94% against *O. viverrini*. Long treatment courses compromise compliance, increase costs and are feasible for community-based control, which might explain that albendazole and mebendazole were not further promoted for *O. viverrini* treatment [38].

It should be noted that in our study Kato-Katz thick smears served as method for helminth diagnosis. However, this diagnosis approach does not allow differentiating the eggs of *O. viverrini* from minute intestinal flukes [39,40]. In addition, since the emphasis of our research was on hookworm, the efficacy of albendazole and mebendazole against

other STHs and *O. viverrini* could not be compared with the appropriate sample sizes. Finally, mostly light *O. viverrini* infections were present in our study and the sample of *O. viverrini* infected patients was not representative of the overall community as hookworm infection was the leading selection criteria. Hence, additional clinical investigations are warranted to assess the opisthorchicidal properties of albendazole and mebendazole in comparison to praziquantel.

Since the anthelmintic drug tribendimidine, in a recent open-label exploratory trial carried out in Lao PDR resulted in high CR and ERR against *O. viverrini* [41], a four-arm study might be designed, comparing praziquantel (treatment of choice) with tribendimidine, albendazole and mebendazole.

In conclusion, we have assessed the efficacy of albendazole and mebendazole against hookworm infection in Lao PDR and provide further evidence of effects of these drugs against other helminth co-infections. Both drugs showed a similar profile, with low efficacy against hookworm and, additionally, low efficacy against *T. trichiura* and high efficacy against *A. lumbricoides*. The low efficacy of single dose of albendazole against hookworm should be followed-up closely and further explored as this drug is widely used for mass drug administration to treat infections with STHs. Furthermore, the effects of the 2 drugs against *O. viverrini* warrants further investigations.

9.7 Acknowledgments

The authors are grateful to all school children and teachers for participating in the study. We are grateful to Dr. Samlane Phompida, Prof. Sithat Insisiengmai and Dr. Keoka Taisaiyavong for advice in conducting during field study. We thank doctors, technical staff and nurses for their kind assistance during the fieldwork. We would like to thank Ms. Carla Kirchhofer and Dr. Urs Duthaler for the quality evaluation of albendazole.

9.8 Funding

The trial was funded by the Swiss National Science Foundation, and the Swiss Agency for Development and Cooperation (project no. NF3270B0-110020). J.K. is grateful to the Swiss National Science Foundation for personal support (project no. PPOOA-114941). KFPE supported P.S. and K.P. Furthermore, P.S. and K.P. received support from the City of Basel and the Rudolf Geigy Foundation, respectively. The funders had no role in study

design, data collection and analysis, decision to publish, or preparation of the manuscript.

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10. A Randomized, Exploratory Open-label Trial on the Efficacy and Safety of Mefloquine, Artesunate, Mefloquine-artesunate, Tribendimidine and Praziquantel against *Opisthorchis viverrini*

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This article has been published in
The Lancet Infectious Disease 2011 Feb; 11(2):110-8. Epub 2011 Nov 24

10.1 Summary

Background. Praziquantel is the only drug available for treatment of *Opisthorchis viverrini*, although in-vivo studies point to activity of mefloquine, artesunate, and tribendimidine against this liver fluke. We aimed to assess the efficacy and safety of these drugs compared with that of praziquantel in patients with *O viverrini* infection.

Methods. We did a randomised open-label trial between February and April, 2010, in the Saysetha district, Attapeu Province, Laos. Eligible patients were school children aged 10–15 years who had *O viverrini* infections. Patients were randomly assigned to one of five different treatment groups by use of a computer-generated randomisation code. We assessed efficacy as cure rate and egg reduction rate in intention-to-treat and per-protocol analyses. The trial was registered with Current Controlled Trials, ISRCTN23425032.

Results. 125 children were randomly assigned: 25 received mefloquine, 24 artesunate, 24 mefloquine–artesunate, 27 tribendimidine, and 25 praziquantel. 19 patients were lost to follow-up. In the intention to treat analysis, 14 patients receiving praziquantel were cured compared with none with mefloquine, one with artesunate (odds ratio 0·03, 95% CI 0·004–0·29), one with mefloquine–artesunate (0·03, 0·004–0·29), and 19 with tribendimidine (1·87, 0·60–5·85). Egg reduction rate was 98·4% for praziquantel, 30·2% for mefloquine (egg reduction-rate ratio 1·61, 95% CI 0·21–0·72), 31·5% for artesunate (0·43, 0·23–0·80), 41·3% for mefloquine–artesunate (0·60, 0·31–1·10), and 99·3% for tribendimidine (1·00, 0·44–2·30). Most adverse events were mild or moderate and affected all treatment groups; serious adverse events—vertigo, nausea, vomiting, and anxiety—were reported only by patients taking mefloquine or mefloquine–artesunate.

Interpretation. Tribendimidine seems to be at least as efficacious as the drug of choice, praziquantel, for the treatment of O viverrini infections; both drugs were well tolerated. Mefloquine, artesunate, and mefloquine–artesunate did not show an effect. Tribendimidine should be further investigated with large clinical trials.

Funding. Swiss National Science Foundation, University of Basel.

10.2 Introduction

Opisthorchiasis is a neglected tropical disease caused by the liver fluke *Opisthorchis viverrini* that affects the poorest people in Cambodia, Laos, northeastern parts of Thailand, and Vietnam.[1,2] An estimated 67 million people are at risk, and 9 million are infected.[1] In Laos, the highest prevalence (50% in school children and up to 90% in adults) of *O viverrini* has been reported in villages adjacent to the Mekong river, particularly in the southern and central provinces.[3,4] Although most infections are asymptomatic, chronic *O viverrini* infection can cause obstructive jaundice, ascending cholangitis, cholecystitis, gallstones, hepatomegaly, and increased risk of cholangiocarcinoma.[5-7] Cholangiocarcinoma is a serious and fatal complication that is incurable at an advanced stage, hence early diagnosis and treatment is imperative.[6, 8, 9, 10]

Control of morbidity through periodic treatment with praziquantel is key for opisthorchiasis;[11,12] praziquantel is the only available drug for this infection, so if drug resistance evolves no active drug will exist unless other treatments are developed. The antimalarials artemether, artesunate (two semisynthetic derivatives of artemisinin), mefloquine, and tribendimidine (used as an anthelmintic drug in China) have opisthorchicidal properties in rodents.[13-15] Artesunate and artemether at a dose of 400 mg/kg given to hamsters infected with *O viverrini* resulted in worm-burden reductions of 77.6% and 65.5%, respectively.[13] Similarly, high worm-burden reductions were reported in hamsters given a single 300 mg/kg oral dose of mefloquine for juvenile and adult *O viverrini* in vivo.[15] Finally, a 400 mg/kg oral dose of tribendimidine achieved a worm-burden reduction of 95.7% in *O viverrini* infected hamsters. [14] We aimed to assess the efficacy and safety of mefloquine, artesunate, mefloquine–artesunate, and tribendimidine compared with that of praziquantel in patients with parasitologically confirmed *O viverrini* infection.

10.3 Methods

10.3.1 Patients

The study was done in the Saysetha district, Attapeu Province, Laos, from February to April, 2010. The province has an area of 10 320 km^2 and is the most southeasterly province of Laos. Prevalence of *O viverrini* is estimated to be about 21% in primary-school children in Attapeu.[4] The province has about 112 120 inhabitants, most of whom

belong to the ethnic group Lao Theung and are mainly engaged in subsistence rice cultivation.[16] The Saysetha upper and lower secondary school was selected for our study; 957 secondary-school children were enrolled during the academic year 2008–2009. A preliminary survey showed that the prevalence of *O viverrini* infection in this school was higher than 50% (Thongsom Keopaseurt, Provincial Hospital, Attapeu; personal communication).

The study was approved by the institutional research commission of the Swiss Tropical and Public Health Institute, (Basel, Switzerland), and the Ethics Committee of Basel (no 209/09). Ethical clearance was obtained from the National Ethics Committee, Ministry of Health, Vientiane, Laos (no 279/NECHR). The trial was registered with Current Controlled Trials, ISRCTN23425032. Permission for field work was provided by the Ministry of Health, the Provincial Health Department, and the Provincial and District Education Office (DEO). Written informed consent was obtained from the parents or legal guardians of each child. We also informed the participants and their parents that tribendimidine is currently registered only in China, and, as such, considered to be an investigational drug in Laos. We explained risk and benefits on the consent form in Lao.

10.3.2 Randomisation

Children who gave consent and met all study criteria were randomly assigned to one of the five different treatment groups by use of a computer-generated randomisation code. A random number sequence was generated with Stata version 10.1 by an independent member, who had no other involvement in the study. Patients were randomly assigned and allocated treatment by the pharmacologist according to the randomisation list in sequential order. Patients and study pharmacologist were not masked to treatment allocation, but the study clinicians and laboratory team were masked throughout the study.

10.3.3 Procedures

One week before the baseline screening survey the National Institute of Public Health, Centre of Malaria, Parasitology, and Entomology, Centre for Laboratory and Epidemiology, the Provincial Department of Health, the Provincial Hospital of Attapeu, and the teachers were informed about the study objectives, procedures, benefits, and

potential risks. 214 school children aged 10–15 years were invited to participate. From each consenting and participating child at least two stool samples were collected within 5 days consecutively. Children with a parasitologically confirmed *O viverrini* infection (at least two of four slides positive) had a full clinical examination, including measurement of weight (measured with an electronic balance to the nearest 0·1 kg), and axillary temperature (measured with battery-powered thermometers to the nearest 0·01°C). Additionally, a finger-prick blood sample was taken from each child for a rapid malaria test (Paracheck Pf, Orchid Biomedical System, Goa, India) and a urine sample from all girls for pregnancy testing (Innovacon, San Diego, CA, USA). Clinical malaria was defined as fever (axillary temperatures ≥37.5°C) and parasitaemia (≥100/μL).[17] Exclusion criteria were presence of clinical malaria, pregnancy, presence of any abnormal medical disorder (ie, hepatomegaly and splenomegaly, jaundice), history of any acute or severe chronic disease, psychiatric and neurological disorders, use of artesunate, artemether, any artemisinin-based combination therapy, mefloquine, or any anthelmintic treatment within the past month, and weight below 20 kg.

Mefloquine (mephaquine 250-mg/lactab) and mefloquine–artesunate (artequin) were obtained from Mepha AG (Aesch, Switzerland). Artesunate (50 mg tablets) was donated by Dafra Pharma (Turnhout, Belgium). Tribendimidine (200 mg tablets) was obtained from Shandong Xinhua Pharmaceutical Corporation (Zibo, Shandong, China); it is registered in China where its safety and efficacy against soil-transmitted helminths have been documented in thousands of patients.[18,19] Praziquantel (600 mg tablets) was purchased from Inresa (Bartenheim, France).

Mefloquine and mefloquine–artesunate were given according to recommended malaria treatment schedules. Mefloquine 25 mg/kg single-dose was given to patients with a bodyweight less than 30 kg or a split-dose spaced by 6 h for patients with a bodyweight above 30 kg (e.g, at bodyweight 30–34 kg, two lactabs were given followed by one lactab 6 h later). Mefloquine–artesunate was given as one tablet of 100 mg artesunate and one lactab mefloquine 250 mg once daily for 3 days consecutively. Mefloquine and praziquantel were given to the nearest half tablet according to the calculated dose per kg bodyweight. For artesunate we used a previously defined malaria treatment schedule (10 mg/kg as three split doses within 12 h).[20] Tribendimidine was given according to the manufacturer's instruction for the treatment of soil-transmitted

helminth infections: 200 mg (age below 14 years) or 400 mg (age above 14 years) as a single dose. Finally, praziquantel was given according to national policies in Laos: 75 mg/kg in two divided doses of 50 mg/kg and 25 mg/kg spaced by 6 h. All children received a biscuit and water before drug administration to improve tolerability and increase bioavailability.[21]

Children were supervised for at least 3 h after treatment and were asked to report any potential drug-related signs and symptoms at 24 h, 48 h, and 120 h after the first dose by use of a standardised questionnaire. A full clinical examination was done by a study physician in case children reported adverse events and appropriate treatment was given. Intensity of adverse events was graded as mild, moderate, severe, serious, or life-threatening, as judged by study physicians. At the end of the study, *O viverrini* egg-positive children who were enrolled in our study were treated with praziquantel (40 mg/kg). All school children received a single oral albendazole (400 mg) according to the national scheme for mass drug administration in Laos.[22, 23]

Filled stool containers were collected from children between 0800 h and 0900 h and replaced with empty containers to obtain at least two stool samples from each child within a period of 5 days. Stool containers were then taken to the laboratory at the provincial hospital. From each stool sample two Kato-Katz thick smears were prepared with the standard 41.7 mg template and were quantitatively examined with light microscopy for helminth eggs with a magnification of 100 times. Each Kato-Katz slide was read within 30–45 min after preparation. Number of *O viverrini* eggs and soil-transmitted helminths eggs (ie, *Ascaris lumbricoides*, hookworm, *Trichuris trichiura*, and *Taenia* spp) were counted and recorded for each parasite species separately. 10% of slides were re-examined for quality control by a senior microscopist.

Cure rates and egg reduction rates at 21–22 days after treatment were assessed as efficacy outcomes. Cure rate was defined as the percentage of the children excreting eggs before treatment, but in whom no eggs were identified when re-examined. Egg reduction rate was defined as the groups' reduction of geometric mean egg output after treatment divided by the geometric mean of the same patients before treatment, multiplied by 100.

Additionally, four stool samples (two pretreatment plus two posttreatment) were preserved in 10 mL sodium acetate-acetic acid-formalin solution, which contained

exactly 500 mg of stool for examination by the formalin-ether concentration technique; this allows differentiation of *O viverrini* and minute intestinal fluke infections.[24, 25] Specimens from patients, for which pretreatment and post-treatment samples could be preserved (per-protocol analysis), were shipped to a referral laboratory at the Khon Kaen University, Thailand. For analysis with the formalin-ether concentration technique, the sample was centrifuged, and the sediment analysed with light microscopy at magnifications of 40 and 100 times.[26]

10.3.4 Statistical analysis

Sample size was based on a suggested sample size of 12 patients per group for proof-of-concept trials, as recommended by Julious.[27] To account for patients dropping out we aimed to recruit 20–25 children per group. All data were double entered with EpiData software (version 3.1). Statistical analyses were done with Stata (version 10.1). Efficacy and safety were assessed with intention-to-treat and per-protocol analyses. Intention-to-treat was defined as an analysis based on the initial treatment intent and per-protocol analysis was defined as children who completed the entire clinical trial.

Prevalence of *O viverrini* was stratified according to the classification of infection intensities proposed by Maleewong and colleagues[28]—light infections (1–999 eggs per g of faeces), moderate (1000–9999), and severe (>10 000). Logistical regression models were used to examine cure rates of *O viverrini* infection and hookworm infection in different treatment groups (comparison of odds of parasite clearance between treatment groups). Negative-binomial regression was applied to compare egg reduction rates between the numbers of *O viverrini* eggs recovered from stool examination of patients treated with mefloquine, artesunate, mefloquine-artesunate, and tribendimidine with that for praziquantel.

Pearson's χ^2 test was applied to compare the baseline binary characteristics and proportion of reported adverse events between the treatment groups. Statistical significance was estimated with a likelihood-ratio test. Negative-binomial models were fitted to compare the number of adverse events in the treatment groups. Significance was defined as p-value less than 0.05.

10.3.5 Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

10.4 Results

Of 214 school children screened with the Kato-Katz method, 197 (92.1%) were *O viverrini* positive (Figure 10.1). We excluded 72 children (36.5%), because 70 provided only a single stool sample, one had fever, and another had splenomegaly. 125 patients were randomly allocated to five treatment groups and were included in the intention-to-treat analysis. Groups were not equal in size (24 children in the artesunate and mefloquine–artesunate group *vs* 27 in the tribendimidine group) because two patients were erroneously assigned to the tribendimidine group, instead of the artesunate and mefloquine–artesunate treatment group. Of 125 patients, 19 (15.2%) were lost to follow-up at the end of study. Four stool samples (two before treatment and two after treatment) were available from 106 patients for per-protocol analyses.

All baseline characteristics of treatment groups were similar, but there were more boys in the mefloquine treatment group (Table 10.1). Overall 63 boys and 62 girls, mean age 13.4 years (SD 1.4), were included in the study. Intensity of *O viverrini* infections was mild to moderate in most children. *O viverrini* geometric mean egg counts ranged from 609.1 to 3917.7 eggs per g of faeces. Overall prevalence of *A lumbricoides, T trichiura*, and *Taenia* spp was below 16.0%, hence these parasites were not included in the efficacy assessment. Prevalence of hookworm infection ranged from 71% to 83%. Results of analyses with the formalin-ether concentration technique confirmed the presence of *O viverrini* infection in all patients. *O viverrini* geometric mean baseline egg counts identified by this technique ranged from 82.5 to 639.0 eggs per g of faeces (n=106; data not shown). Co-infections with minute intestinal flukes in two patients and intestinal protozoa in nine patients were reported.

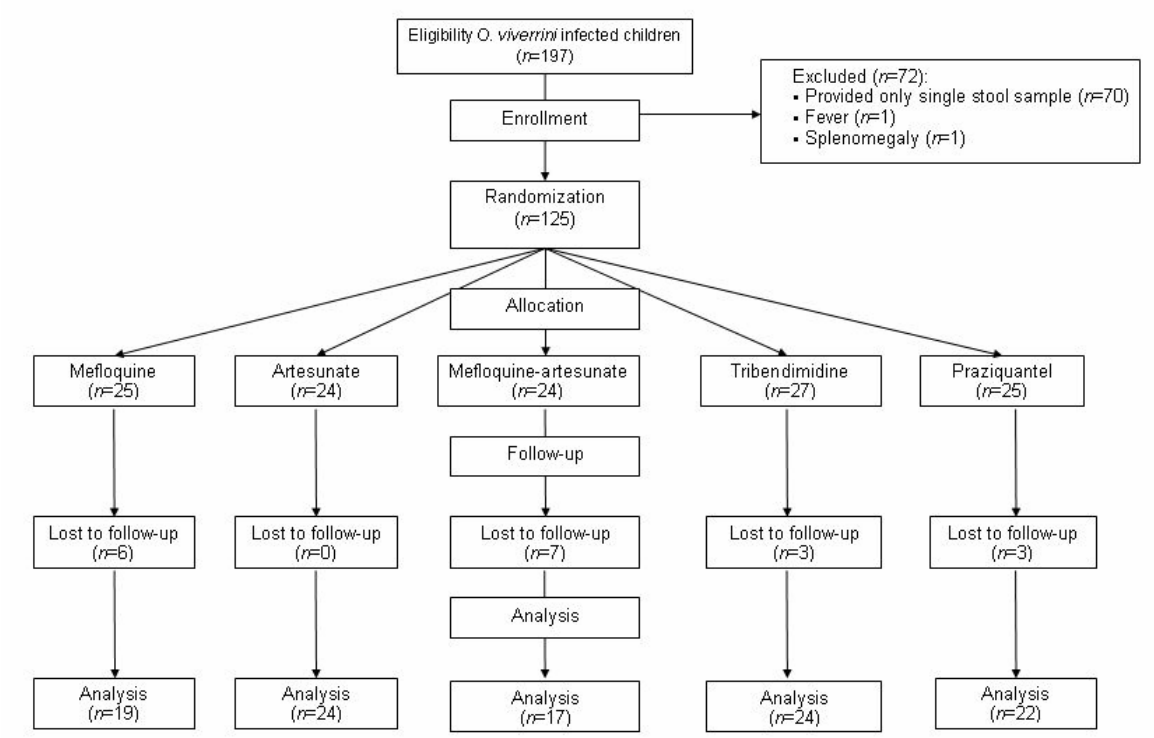

Fig.10.1 Trial profile

In the intention-to-treat analysis (Table 10.2), the highest cure rate was seen in patients treated with tribendimidine, followed by praziquantel. No significant difference was shown between the cure rates of tribendimidine and praziquantel (Table 10.3). No child receiving mefloquine was cured, and very low cure rates were calculated for artesunate and mefloquine–artesunate. Both tribendimidine and praziquantel treatments resulted in almost complete egg elimination (Table 10.2). By contrast, egg reduction rates were significantly lower for mefloquine and artesunate. The egg reduction rate was lower for mefloquine–artesunate, which was, however, not significant (Table 10.3).

Results of the per-protocol analysis (Kato-Katz data) were similar to those of the intention-to-treat analysis (Table 10.2, Table 10.3, Table 10.4). Analysis of stool samples with the formalin-ether concentration technique showed much higher cure rates (tribendimidine 96%, praziquantel 95%, mefloquine–artesunate 47%, artesunate 33%, and mefloquine 21%) and egg reduction rates (tribendimidine 99%, praziquantel 99%, mefloquine–artesunate 75%, artesunate 60%, and mefloquine 71%) than those obtained with the Kato Katz method. Although no significant difference was reported

between cure rates and egg reduction rates of tribendimidine and praziquantel (Table 10.3), these rates were significantly lower for the antimalarials mefloquine and artesunate. Additionally, the cure rate of mefloquine–artesunate was significantly lower; however, no significant difference was seen between egg reduction rates of mefloquine–artesunate and praziquantel (Table 10.3, Table 10.4).

Mefloquine, artesunate, mefloquine–artesunate, and praziquantel had no effect against hookworms, whereas tribendimidine achieved cure rates of 65.0% (both intention-to-treat and per-protocol analyses; (Table 10.2, Table 10. 4).

Adverse events were assessed at 3 h, 24 h, 48 h, and 120 h after the first dose (Table 10.5). No symptoms were reported before treatment. Most symptoms were mild 3 h after treatment, then increased in severity and subsided 48 h after treatment. In total, 92 (74%) mild adverse events, 47 (38%) moderate, 23 (18%) severe, 12 (10%) serious were reported (Table 6). No life-threatening adverse events were reported. 120 h after treatment, children were re-examined by the same physicians; none of them reported any adverse events.

Table 10.1 Demographic and laboratory baseline characteristics of 125 schoolchildren infected with *Opisthorchis viverrini* at inclusion

Characteristics	Drugs				
	Mefloquine	Artesunate	Mefloquine-artesunate	Tribendimidine	Praziquantel
	(*n* = 25)	(*n* = 24)	(*n* = 24)	(*n* = 27)	(n = 25)
Boys	16	13	12	10	12
Girls	9	11	12	17	13
Mean (SD) age, years	13.4 (1.2)	13.3 (1.6)	13.4 (1.6)	13.3 (1.3)	13.6 (1.3)
Mean (SD) weight, kg	39.5 (5.4)	38.8 (6.8)	38.0 (6.3)	40.6 (7.0)	39.4 (5.6)
Parasite infections					
Opisthorchis viverrini infection *					
Overall GM eggs per g of stool	1159.7	1368.0	1207.8	1968.1	1925.4
GM eggs per g of stool (range)	(609.1- 2208.0)	(745.3- 2510.9)	(715.1-2040.0)	(988.7 -3917.7)	(970.2-3821.2)
Number of light infection (1-999 eggs per g of stool)	14 (56%)	11 (46%)	11 (46%)	9 (33%)	11(44%)
Number of moderate infection (1000- 9999 eggs per g of stool)	9 (36%)	12 (50%)	11 (46%)	14 (52%)	9 (36%)
Number of heavy infection (>10 000 eggs per g of stool)	2 (8%)	1 (4%)	2 (8%)	4 (15%)	5(20%)
Co-infection with soil-transmitted helminths					
Hookworm	19 (76%)	20 (83%)	17 (71%)	20 (74%)	20 (80.0)
Ascaris lumbricoides	0 (0)	0 (0)	0 (0)	3 (11.1)	4 (16.0)
Trichuris trichiura	1 (4%)	1 (4%)	0 (0)	1 (3.7)	0 (0)
Taenia spp	2 (8%)	1 (4%)	1 (4%)	1 (3.7)	3 (12.0)

Data are no; (%) of subject, unless otherwise indicated. GM-geometric mean. * According to guideline's classification put forward by WHO, based on Kato-Katz analysis

Table 10.2 Intention-to-treat analysis of prevalence and cure rate of mefloquine, artesunate, mefloquine-artesunate, tribendimidine and praziquantel schoolchildren infected with *Opisthorchis viverrini* at follow-up, with Kato-Katz smear technique

	Intention-to-treat analysis				
	Mefloquine (*n* = 25)	Artesunate (*n* = 24)	Mefloquine-artesunate (*n* = 24)	Tribendimidine (*n* = 27)	Praziquantel (n = 25)
Opisthorchis viverrini					
Patient cured/ Patients infected	0/25 (0)	1/24 (4%)	1/24 (4%)	19/27 (70%)	14/25 (56%)
GM egg per g of Stool (range)	1052.2 (537.8- 2058.4)	1229.4 (625.1-2417.7)	653.9 (323.9-1320.1)	578.5 (47.7-7009.5)	159.9 (38.1- 671.2)
Egg reduction rate	30.2	31.5	41.3	99.3	98.4
Co-infection with hookworm					
Patient cured/ Patients infected	3/17 (18%)	4/20 (20%)	3/15 (20%)	11/17 (65%)	2/17(12%)

Note. Data are no; (%) of patients, unless otherwise indicated (95% confident interval). GM-geometric mean.

Table 10.3 Comparison of treatment outcome between groups

	Intention-to-treat analysis							
	MQ *vs* PZQ	*P*	AS *vs* PZQ	*P*	MQ-AS *vs* PZQ	*P*	TBD *vs* PZQ	*P*
Kato-Katz thick smear technique								
Opisthorchis viverrini								
OR	na	na	0.03 (0.004-0.29)	0.002	0.03 (0.004-0.29)	0.002	1.87 (0.60-5.85)	0.29
ERRR	0.40 (0.21-0.72)	0.003	0.43 (0.23–0.80)	0.008	0.60 (0.31-1.10)	0.08	1.00 (0.44-2.30)	0.98
Co-infection with hookworm								
OR	1.61 (0.23-11.09)	0.63	1.88 (0.30-11.78)	0.50	1.88 (0.27- 13.09)	0.52	13.75 (2.32-81.49)	0.004
	Per-protocol analysis							
	MQ *vs* PZQ	*P*	AS *vs* PZQ	*P*	MQ-AS *vs* PZQ	*P*	TBD *vs* PZQ	*P*
Kato-Katz thick smear technique								
Opisthorchis viverrini								
OR	na	na	0.02 (0.003-0.22)	0.001	0.04 (0.004-0.32)	0.003	2.17 (0.58-8.08)	0.25
ERRR	0.36 (0.19-0.68)	0.002	0.42 (0.22 -0.80)	0.008	0.54 (0.28-1.03)	0.06	1.00 (0.44-2.31)	0.98
Co-infection with hookworm								
OR	1.88 (0.27-13.09)	0.53	1.88 (0.30-11.78)	0.50	1.50 (0.18-12.46)	0.70	13.75 (2.32-81.49)	0.004
FECT technique								
OR	0.01 (0.001-0.13)	<0.001	0.02 (0.003-0.21)	0.001	0.04 (0.005-0.39)	0.005	1.10 (0.06-18.64)	0.95
ERRR	0.54 (0.43-0.67)	<0.001	0.81 (0.70-0.94)	0.009	0.87 (0.72-1.04)	0.14	1.00 (0.86-1.16)	0.99

Note. Data are odds ratios (OR, 95% confidence intervals) of parasite clearance; ERRR, egg reduction rate ratio; na, not applicable; MQ: mefloquine; AS: artesunate; MQ-AS Mefloquine-artesunate; TBD tribendimidine; PZQ Praziquantel

Table 10.4 Per-protocol analysis of prevalence and cure rate of mefloquine, artesunate, mefloquine-artesunate, tribendimidine and praziquantel schoolchildren infected with *Opisthorchis viverrini* at follow-up

	Per-protocol analysis				
	Mefloquine (*n* = 19)	Artesunate (*n* = 24)	Mefloquine-artesunate (*n* = 17)	Tribendimidine (*n* = 24)	Praziquantel (n = 22)
Kato-Katz thick smear technique					
Opisthorchis viverrini					
No. of patients cured (%)	0 (0)	1 (4.2)	1 (6.0)	19 (79.2)	14 (63.6)
GM epg (range)	1114.1 (498.9-2488.1)	1229.4 (625.1-2417.7)	669.1 (320.8-1395.7)	44.7 (11.6-171.7)	43.1 (16.6-111.7)
ERR (%)	28.7	31.5	36.6	99.3	98.4
Co-infection with hookworm					
No. patients of sole hookworm infection (*n* = 81)	(*n* = 15)	(*n* = 20)	(*n* = 12)	(*n* = 17)	(n = 17)
No. of patients cured (%)	3 (20.0)	4 (20.0)	2 (16.7)	11 (65.0)	2 (13.0)
FECT technique					
Opisthorchis viverrini					
No. of patients cured (%)	4 (21.1)	8 (33.3)	8 (47.1)	23 (95.8)	21 (95.5)
GM epg (range)	182.3 (77.0-433.5)	156.2 (82.2-297.0)	114.0 (69.2-187.3)	na	Na
ERR (%)	71.0	60.0	75.0	99.1	99.0

Note. Data are no; (%) of subject, otherwise indicated (95% confident interval); GM, geometric mean; epg, eggs per gram of stool; ERR, egg reduction rate; ; na, not applicable

Table 10.5 Clinical symptoms reported 3-48 hour after drug administration among 125 schoolchildren, stratified by treatment group

	No, (%) individuals with adverse event																			
	Mefloquine (n = 25)				Artesunate (n = 24)				Mefloquine-artesunate (n = 24)				Tribendimidine (n = 27)				Praziquantel (n = 25)			
Adverse event / Grade	3	24	48	At any time point	3	24	48	At any Time point	3	24	48	At any time point	3	24	48	At any time point	3	24	48	At any time point
Fatigue																				
Mild	3	4	2	7 (28.0)	2	3	2	6 (25.0)	4	5	7	12 (50.0)	2	3	2	5 (18.5)	5	8	2	11 (44.0)
Moderate	0	0	3	3 (12.0)	0	1	1	2 (8.3)	3	1	2	5 (20.8)	0	0	0	0	0	0	0	0
Severe	0	0	2	2 (8.0)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Serious	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Asthenia																				
Mild	0	4	0	4 (16.0)	0	2	0	2 (8.3)	0	4	0	2 (8.3)	0	1	1	1 (3.7)	0	0	1	1 (4.0)
Moderate	0	6	0	6 (24.0)	0	1	0	1 (4.2)	0	3	0	3 (12.5)	0	0	0	0	0	0	0	0
Severe	0	8	0	8 (32.0)	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1 (4.0)
Serious	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Headache																				
Mild	4	3	2	7 (28.0)	6	8	2	12 (50.0)	6	9	6	14 (58.3)	4	10	1	12 (44.4)	12	7	2	16 (64.0)
Moderate	1	2	4	6 (24.0)	1	2	1	4 (16.7)	1	3	4	8 (33.3)	0	0	0	0	0	2	0	2 (8.0)
Severe	0	1	3	3 (12.0)	0	1	0	1 (4.2)	1	1	1	1 (4.2)	0	0	0	0	0	2	0	2 (8.0)
Serious	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vertigo																				
Mild	6	3	1	9 (36.0) [a]	3	5	1	7 (29.2)	3	8	6	12 (50.0)	4	6	0	9 (33.4)	11	8	2	16 (64.0)
Moderate	1	13	4	15 (60.0)	1	0	0	1 (4.2)	1	7	4	10 (41.7)	0	1	1	1 (3.7)	0	2	0	2 (8.0)
Severe	0	0	3	3 (12.0)	0	1	0	1 (4.2)	1	1	0	1 (4.2)	0	0	0	0	0	0	0	0
Serious	0	1	1	1 (4.0)	0	0	0	0	0	1	0	1 (4.2)	0		0	0	0	0	0	0
Vomiting																				
Mild	0	1	0	1 (4.0)	0	1	0	1 (4.2)	0	2	3	4 (16.7)	0	1	1	1 (3.7)	0	2	1	2 (8.0)
Moderate	0	7	6	10 (40.0)	0	0	0	0	0	3	1	3 (12.5)	0	0	0	0	0	1	1	1 (4.0)
Severe	0	6	1	6 (24.0)	0	0	0	0	0	5	2	6 (25.0)	0	0	0	0	0	0	0	0
Serious	0	5	0	5 (20.0)	0	0	0	0	0	4	0	4 (16.7)	0	0	0	0	0	0	0	0
Nausea																				
Mild	2	4	1	6 (24.0)	2	3	2	5 (20.8)	7	6	6	14 (58.3)	3	7	0	9 (33.3)	5	5	1	10 (40.0)
Moderate	1	8	5	11 (44.0) [b]	0	0	1	1 (4.2)	0	5	3	8 (33.3)	1	0	1	2 (7.4)	1	0	1	2 (8.0)
Severe	0	2	1	2 (8.0)	0	0	0	0	0	3	0	3 (12.5)	0	0	0	0	0	0	0	0
Serious	0	2	1	2 (8.0)	0	0	0	0	0	2	0	2 (8.3)	0	0	0	0	0	0	0	0

a Significantly different from PQZ-treated children (p< .02); b Significantly different from PQZ-treated children (p< .007); c Significantly different from PQZ-treated children (p< .001)

Table 10.6 Summary of clinical symptoms recorded at 3-48 hour after drug administration, stratified by treatment group

Adverse event arisen after treatment	Treatment group					
	Mefloquine (*n* =25)	Artesunate (*n* =24)	Mefloquine-artesunate (*n* =24)	Tribendimidine (*n* =27)	Praziquantel (*n* =25)	Total (n=125)
At least 1 adverse event	22 (88.0)	16 (66.7)	23 (95.8)	20 (74.1)	20 (80.0)	101 (80.8)
Mild	18 (72.0)	15 (62.5)	20 (83.3)	19 (70.4)	20 (80.0)	92 (73.6)
Moderate	19 (76.0)	5 (20.8)	16 (66.7)	2 (7.4)	5 (20.0)	47 (37.6)
Severe	12 (48.0)	2 (8.3)	4 (16.7)	1 (3.7)	4 (16.0)	23 (18.4)
Serious	4 (8.3)	0	8 (33.3)	0(0)	0(0)	12 (9.6)

Note. Data are no; (%) of subject

At least one adverse event was reported by patients treated with artesunate (67%), tribendimidine (74%), praziquantel (80%), mefloquine (88%), and mefloquine–artesunate (96%; Table 6). No significant difference was identified in the frequency of any adverse event in tribendimidine, praziquantel, and artesunate treatment groups. Most reported events in the tribendimidine treatment group were mild and included headache, vertigo, nausea, and fatigue (Table 10.5). Vertigo and nausea were significantly more common in children treated with mefloquine (p=0.02 and p=0.007, respectively) than in any other treatment group. Additionally, dizziness was more common in patients who received mefloquine (p=0.02), and mefloquine–artesunate (p=0.001) than in those who were treated with praziquantel. 12 children treated with mefloquine or mefloquine–artesunate had serious adverse events including anxiety, nausea, vertigo, and vomiting, and were transferred to either the provincial or local hospital. These children received a full clinical examination and proper medical treatment, including parenteral transfusion, antiemetic drugs, and paracetamol or oral rehydration. Children were closely monitored, and after 48 h of treatment in hospital all children had recovered and could be discharged.

10.5 Discussion

In our open-label, randomised, phase 2 study, tribendimidine seems to be at least as efficacious as the standard treatment praziquantel for the treatment of *O viverrini* infection; both drugs were well-tolerated. Mefloquine, artesunate, and mefloquine–artesunate were not effective in patients with this infection. To our knowledge the efficacy of the antimalarial drugs mefloquine, artesunate, mefloquine–artesunate, and the anthelmintic drug tribendimidine, for the treatment of *O viverrini* infection has not been studied previously (see panel). Of note, another antimalarial drug, chloroquine, was historically used for treating opisthorchiasis; however, the cure rates and egg reduction rates were unsatisfactory.[29] Praziquantel served as reference, because it is the drug of choice for treatment of *O viverrini* infection.[23] Adverse events after praziquantel treatment are generally mild and transient, as confirmed by our study.[11] Single doses of 40 mg/kg praziquantel are widely used for community mass drug administration in southeast Asia. In Laos, such mass treatment was initially introduced in the 1980s in high-risk areas, under the close collaboration with the Lao Ministry of

Health and WHO.[30] In our study, split doses of 75 mg/kg praziquantel (75 mg/kg divided into two doses of 50 mg/kg and 25 mg/kg) were used, which is recommended for individual treatment and is the most effective regimen.[22] We reported only moderate cure rates after praziquantel treatment, which contrasts with previous studies reporting 96% and 100%.[31-33]

Single 200 mg or 400 mg oral doses of tribendimidine achieved higher cure rates and egg reduction rates than a double dose of praziquantel, although the difference was not significant. Tribendimidine is an amidantel derivative, first discovered and developed in China.[34] Preclinical and clinical studies to meet the international standard accepted by the US Food and Drug Administration (FDA) and European regulatory agencies are underway, with the ultimate goal of gaining approval for treatment of soil-transmitted helminthiases outside of China and inclusion in the WHO's essential medicines list. Tribendimidine has a broad spectrum of activity against intestinal nematodes (eg, *A lumbricoides, Enterobius vermicularis*, and the hookworms).[35] Single-dose oral tribendimidine is effective against *A lumbricoides* and hookworm, and shows promising activity against *Strongyloides stercoralis* and *Taenia* spp.[36] Our study confirms the good efficacy of tribendimidine for treatment of hookworm infections.

By contrast with recent laboratory findings,[13,15] mefloquine and artesunate, and mefloquine–artesunate showed no effect in the treatment of *O viverrini* infections. In a proof-of-concept study of efficacy and safety of these drugs against another trematode, *Schistosoma haematobium*, in Côte d'Ivoire, similarly low cure rates were reported for mefloquine (21%) and artesunate (25%); however, slightly higher egg reduction rates for mefloquine (74%) and artesunate (85%) were seen. Furthermore, promising results were shown with mefloquine–artesunate (cure rate 61%, egg reduction rate >95%).[37]

We differentiated *O viverrini* infections and other common foodborne trematodes by use of the formalin-ether concentration technique (Table 10.3, Table 10.4). Results from this technique confirmed the high efficacy of tribendimidine against *O viverrini* infection. Notably, the formalin-ether concentration technique showed higher cure rates of praziquantel and tribendimidine than did the Kato-Katz method, which could be explained by its lower sensitivity than the Kato-Katz thick smears. Our findings are consistent with those of Lovis and colleagues, [38] who showed a lower sensitivity of the formalin-ether concentration technique (49.4%) than of one Kato-Katz thick smear

(62.3%). Conversely, the low sensitivity of this technique contrasts with results from a study done in the south of Laos,[39] which had 96.8% sensitivity for the diagnosis of *O viverrini* infections.

The amount of stool used in the formalin-ether concentration technique is not of primary importance. However, Sayasone and colleagues [39] used purged stool samples as the reference gold-standard to calculate the validity of the formalin-ether concentration technique. Two previous studies done in Laos,[38,39] both using the same diagnostic methods to differentiate *O viverrini* from *O viverrini*-like parasites, showed that *O viverrini* often coexists with other foodborne trematodes, including minute intestinal flukes (e.g, *Haplorchis taichui*). In our study very few co-infections with minute intestinal flukes were detected.

Children treated with artesunate, or tribendimidine showed only mild adverse events, similar to those reported in previous studies.[20,36] Surprisingly, patients treated with mefloquine–artesunate in our study were more likely to experience adverse events than were schoolchildren treated with these drugs for *S haematobium* infection in Côte d'Ivoire, where only mild and transient adverse events were observed, with abdominal pain most common.[37] We cannot explain why mefloquine and mefloquine–artesunate were not tolerated in our study population, but *O viverrini* infection and other host factors might play a part.

10.6 Conclusions

Tribendimidine shows promising activity against *O viverrini* infection. The nematocidal and opisthorchicidal properties of this drug are very intriguing as there is huge geographical overlap of these parasites, and preventive chemotherapy is the mainstay of control. Once preclinical studies have been completed, and if the drug is registered outside China, large scale clinical studies should be done in *O viverrini* endemic settings. Additionally, a proof-of-concept trial with tribendimidine should be done in patients infected with *Clonorchis sinensis*, a closely related liver fluke. Furthermore, by contrast with in-vivo studies, antimalarial drugs seem to be ineffective for the treatment of *O viverrini* infections. Nonetheless, the use of antimalarials in areas in which malaria and liver fluke co-infections are common might have marginal benefits because these drugs slightly reduce *O viverrini* egg counts, as our study has shown. Moreover, studies of

tribendimidine–praziquantel combinations in hamsters infected with *O viverrini*, similar to those done in rats infected with *C sinensis*, [40] might be of interest, because combination chemotherapy is a useful strategy to delay the emergence of drug resistance.

Systematic review

We searched PubMed using the terms "*Opisthorchis viverrini*" OR "opisthorchiasis" AND "clinical trial" OR "cure rate" OR "egg reduction rate" OR "efficacy" AND "mefloquine" OR "tribendimidine" OR "artesunate". No previous studies, which assessed the efficacy and safety of mefloquine, artesunate, mefloquine-artesunate, and tribendimidine in patients with *O viverrini* infection were identified.

Interpretation

Our study provides the first evidence that tribendimidine, given at the recommended doses for treatment of soil-transmitted helminth infections (ie, 200 mg or 400 mg), seems to be an efficacious treatment for *O viverrini* infection. Mefloquine, artesunate, and mefloquine–artesunate cannot be recommended against *O viverrini* infections because of the low efficacies reported. Adverse events were most common in patients who were treated either with mefloquine or a mefloquine–artesunate combination.

10.7 Acknowledgments

We are indebted to teachers and school children at the Saysetha secondary school for their kind collaboration, commitment, and participation in our trial. We are grateful to Marcel Tanner for his continuous support. We thank Bounthavy Chaleunphon and staff of Provincial Hospital and Provincial Health Department, Vincent Amstutz and staff of Service Fraternel d'Entraide, the District Education Office, and the Provincial Government in Attapeu Province for their collaboration and active participation in management of drug-induced side-effects that occurred in patients. We thank Samlane Phompida and staff at the Centre for Malaria and Parasitology and Entomology, Vientiane, and also at Champasack malaria station for their kind support. Finally we thank Sithat Insisiengmai, (Ministry of Health, Laos) for his collaboration and recommendation.

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11. General discussion and conclusions

11.1 Overview

The paucity of information on severe morbidity of intestinal parasitic infections and scarcity of efficacies of their treatment in Lao PDR were motivation for the research conducted in this Ph.D. and govered the design of the different studies. In particular, I was interested by morbidity associated with potentially severe infections with *S. mekongi* and *O. viverri*, and while working discovered several cases of *C. philippinensis.* In addition, the need to critically assess the efficacy of widely used anti-helminthic drugs became evident. In this Ph.D. thesis particular attention is paid to *S. mekongi, O. viverrini* and hookworm. The aim of my research contribution was to determine the morbidity associated with food- and waterborne trematodiasis (*S. mekongi, O. viverrini,* mixed infections of *S. mekongi* and *O. viverrini*), soil-transmitted helminths (hookworm), and the intestinal nematode *C. philippinensis* in the most affected communities and patients in hospitals (Chapter 4-8) in Lao PDR. Additionally, we investigated the efficacy of the current drug of choice against soil-transmitted helminths, namely Albendazole (ABZ), *versus* Mebendazole (MBZ) in schoolchildren with a high prevalence of hookworms (Chapter 9). Finally, we assessed the safety and efficacy of mefloquine, artesunate, the combination mefloquine-artesunate and tribendimidine compared to standard treatment, praziquantel (PZQ) against *O. viverrini* (Chapter 10).

11.2 Document severe morbidity of parasitic infection: *C. philippinensis* and *S. mekongi*

Outbreaks and mortality of intestinal capillariasis of *C. philippinensis* occur mainly in Asia (Lu et al., 2006; Odermatt et al., 2010; Saichua et al., 2008). The parasite is often misdiagnosed, as eggs resemble those of *T. trichiura*. If not adequately treated, the infection may cause severe morbidity, which may even lead to death (Cross 1992; Odermatt et al., 2010; Saichua et al., 2008). No cases had previously been documented in Lao PDR. We identified described the first three cases here and thus provide new evidence on this parasites which is circulating in Lao PDR. Hence, there is a risk for outbreaks in humans (Chapter 4).

Our patients were admitted several times in different hospitals in critical conditions. Most of our cases were first misdiagnosed as *Trichuris* infection before the definite diagnosis was made. Stool examination of our patients revealed eggs of *C. philippinensis* and harboured more than two parasite species, including *Giardia lamblia, Entamoeba histolytica*, *Strongyloides stercoralis*, *O. viverrini*, and hookworm. Patients with long-standing history of diarrhoea may be suffering from *C. philippinensis*. This disease is difficult to differentiate from other causes for chronic diarrhea. We observed that repeated stool examination and well-trained laboratory personnel considerably improved sensitivity in identifying eggs and larvae of *C. philippinensis* (Chapter 4). Even so, excretion of *C. philippinensis* eggs with the stool may vary over time and make diagnosis unreliable. In addition to stool examination, endoscopic examination in combination with biopsy and/or intestinal aspiration might be a useful tool for diagnosis in case of absence of eggs in the stool (Sangchan et al., 2007; Tesana et al., 1983). All compounds of "zole" namely ABZ (400 mg) and MBZ (200 mg) are effective against *C. philippinensis* (Cross and Basaca-Sevilla, 1987; Singson et al., 1975). Our study showed that a single dose of 400 mg ABZ should be the drug of choice for treatment of *C. philippinensis* and that a long regimen is required for preventing the recurrence of this parasite (Chapter 4).

Transmission of waterborne *S. mekongi* is confined to the lower Mekong Basin. In Lao PDR, it includes the districts of Khong and Munlapamok in Champasack province, southern Lao PDR. *S. mekongi* was initially identified as a separate species from *S. japonicum* in the 1980s. A considerable number of severe cases were described from Laos in the initial years (Muth et al., 2010). Surprisingly, little is known with regard to the mortality due to haematemesis or other complications of *S. mekongi*. In the 1970s, severe manifestations of *S. mekongi* infection were mainly described from Lao immigrants in France (Muth et al., 2010). In Lao PDR, community- and hospital-based control efforts relying on praziquantel treatment were implemented for more than 20 years (Muth et al., 2010). By 1999, it was believed that *S. mekongi* is under control and a substantial reduction in infection rates was noted, predominantly in Cambodia (Muth et al., 2010; Urbani et al., 2002). No new severe cases have been reported and documented in the Lao health services since the 1980s until our study was conducted in 2006 (Hotez and Ehrenberg, 2010; Muth et al., 2010).

In our community helminth surveys, severe cases of *S. mekongi* were detected. The patients came forward as the survey teams were present in the villages on islands in the Khong district. Cases were traced yearly and their status evaluated. The severe clinical picture of *S. mekongi* seems to be re-emerging, since in our community helminth survey on Khong Island in 2006-2008 (Chapter 5) as we found six cases with very advanced stages of schistosomiasis. In a subsequent follow-up study, we found additional 3 severe cases between 2008 and 2010. Two cases died in 2008 due to rupture of the oesophageal varices. In addition to adult patients, children as young as 5 to 7 years old were diagnosed with these severe *S. mekongi* complications. In our recent surveys in three villages in Khong district showed that prevalence of *S. mekongi* and *O. viverrini* was 87.8% and 98.9%, respectively in schoolchildren (Sayasone et al, 2010), and 57% and 87%, respectively in adults (Chapter 5). Therefore, our case series on severe schistosomiasis provide further evidence that transmission of *S. mekongi* is ongoing in the endemic settings of Lao PDR and leads to re-emerging severe cases as documented by youngest severe cases. No case had been reported in Cambodia until the year 2000. However, an observation similar to the one in Laos has been made recently in Cambodia with a number of cases of *S. mekongi* appearing where MDA was conducted (Hotez and Ehrenberg, 2010; WHO 2009). However, sporadic cases were reported from travellers returning to their home country (Carmody et al., 2008; Houston et al., 2004).

The two case series on *C. philippinensis* and *S. mekongi* identified in communities and hospital attempted to understand where in Laos transmission was ongoing, and how patients evolve under anthelminthic treatments. Like comparable studies, the case series reports were not representative samples from the general population. Nonetheless, their evidence help bridge the gap between epidemiological studies on prevalence and current concrete hospital practices. We suggest including the diagnosis of *C. philippinensis* in the curriculum of laboratory personals. Also, specific training on diagnostic algorithms may increase awareness in the Lao health care providers on the severity of diseases associated with the two infections, and may enhance the accurate diagnosis and early treatment of *S. mekongi* and *C. philippinensis*. Early diagnosis, adequate treatment and correct case management will prevent severe consequences and death. Public health promotion

regarding hygiene and sanitation practices and avoidance of contact with infested water bodies needs to be reinforced.

11.3 Assessment of burden of parasitic infections and resolution of morbidity after treatment

A first set of hepatobiliary and splenic morbidity data (Chapter 6) pertaining to *S. mekongi* infection and co-infection with *O. viverrini* was obtained from a 2-year follow-up, community-based study launched in March 2006 in three villages in Khong district, Champasack province. The two flukes are co-endemic in southern districts of Lao PDR. Both parasites inflict liver morbidity and both can be treated by praziquantel (Keiser and Utzinger, 2007b). Regular treatment is recommended as the main public health intervention for both parasites (Keiser and Utzinger, 2009; Keiser and Utzinger, 2010). Yearly examination in the present study included the assessment of infection status by stool examination using Kato-Katz thick smear technique (Katz et al., 1972) and liver morbidity assessment by ultrasonographical examination (Chapter 6) as put forth by the WHO guideline on schistosomiasis related morbidity assessment (Niamey Working Group, 2000). The follow-up midpoint (2007) and endpoint studies (2008) assessed the infection status and the resolution dynamics of liver morbidity after a single oral dose of praziquantel treatment (40 mg/kg BW) in individuals infected with *S. mekongi* at the baseline.

To our knowledge, our studies (Chapter 6) consitute the first assessment of morbidity due to *S. mekongi* and co-morbidity with *O. viverrini* and its resolution dynamics after treatment since MDA was launched in the community. At baseline (2006), all the subjects were egg-positive for *S. mekongi* and 98.7% were infected with *O. viverrini*. Liver pathology was observed in a high prevalence of 37.1% of individuals infected with *S. mekongi*. No subject demonstrated signs of ascites and peripheral signs of decompensation. However, other symptoms, such as hepatomegaly (77.5%) and splenomegaly (82.2%) were observed At mid point (11 months after treatment), we found marked improvements of hepatosplenic morbidity and calcification around the portal tree had regressed, particularly among subjects with less advanced pathology at baseline. Nonetheless, advanced severe fibrosis

(Pattern E-F) may not quickly resolve following PZQ treatment. Two cases developed ascites and died due to rupture of oesophageal varices (Chapter 6). The endpoint (23 months after treatment) results showed slightly increased prevalence of *S. mekongi* (54.3%) and *O. viverrini* (74.2%). However, liver pathology remained unchanged compared to the midpoint evaluation. Overall, hepatosplenic lesions detected by US showed statistically significant improvement after treatment ($p<0.01$). However, in individuals with advanced periductal fibrosis the condition remained unchanged or reversed. Re-infection was detected at 11 month after treatment.

The second set of data were focusing on hepatobiliary morbidity with particular emphasis on cholangiocarcinoma (CCA) suspected lesions associated with *O. viverrini* infection comprised of two in-depth studies (hospital and community-based studies) conduceted in 2011 (Chapter 7-8). Field experience in Thailand has determined that infection of *O. viverrini* led to a wide range of hepatobiliary disease, including severe manifestations such as obstructive jaundice and ascending cholangitis (Mairiang and Mairiang, 2003; Mairiang et al., 2006; Mairiang et al., 2011; Sripa et al., 2007). Chronic infections due to *O. viverrini* are also known to provoke CCA, a malignant and highly lethal bile duct cancer (Shin et al., 2010; Sripa et al., 2007; Sripa and Pairojkul, 2008). One of the provinces in northeast Thailand was logged as the first elevated CCA incidence rate in the world (Sripa et al., 2010). The incidence of CCA in Laos in unknown although infection rates with *O. viverrini* are rampant.

Our community-based US survey in Saravan Province (Chapter 7), southern Lao PDR confirmed that patients infected with *O. viverrini* suffered from a substantial amount of hepatobiliar anormalities suspected lesions. A similar situation in *O. viverrini*- endemic areas was found in Thailand (Mairiang et al., 2006; Mairiang et al., 1992; Mairiang et al., 2011). Of 431 individuals screened with US, 5 patients (1.2%) had lesions suggesting CCA. The five suspected CCA cases were investigated by complete blood tests, serum bile marker tests including tumour antigen (i.e., carbohydrate antigen [CA] 19-9); cytokines (i.e., IL-6), proteases (i.e., plasminogen activator inhibitor) for detecting CCA cases (Sripa et al., 2009). However, the readings of tumor antigens varied from normal to slightly increased. Serum markers might not be able to detect such CCA cases in our population. We did not have any

rationale to explain why these makers were not increased in our suspected CCA population but it may imply clinical stage of CCA. Our study pointed out constraints in diagnosing CCA cases using this approach. None of our cases had been confirmed with histopathological results (with biopsy) and neither with CT scan, nor could ERCP be performed. High blood creatinine level was a contraindication for imaging diagnosis, namely ERCP, MRCP and CT scan. These imaging techniques include the use of iodinated radiocontrast through intravenous injection, which can lead to renal failure in patient with high blood creatinine.

Our hospital-based study in six referral hospitals found that there were 274 cases according to our criteria for diagnosis of suspected CCA (Chapter 8). Most records were kept in the hospital's repository for an average of 3.3 years. The 274 cases included records from 2006 to 2010. Most of these CCA suspected patients were admitted at the hospitals with the signs and symptoms characteristic for very advanced stages and referred from the provincial hospitals. A number of suspected CCA cases presented at the admission with jaundice and/or fever, and/or right upper quadrant pain as found in previous studies (Bhuddhisawasdi, 1996; Mairiang and Mairiang, 2003; Uttaravichien, 1999). All suspected cases admitted at the hospitals had undergone their first abdominal ultrasound, and only CT was available (Chapter 11). ERCP has been offered at Mahosot hospital, Vientiane capital, Lao PDR from 2011 onwards (Dr. Bouachanh Rasachack, pers. communication).

In Lao PDR, neither cancer registration, nor case management for CCA is available. Health facilities do not have the capacity to diagnose CCA and surgical and supportive treatment capacity are lacking. Hence, the incidence of CCA is unknown. However, given the high infection rates with *O. viverrini* which are similar (Chai et al., 2005; Rim et al., 2003; Sayasone et al., 2007) or beyond the rates in neighbouring Thailand, the incidence of CCA may be comparable to that found in north-eastern Thailand or even higher. The incidence of CCA and *O. viverrini* in Thailand has been calculated at 188 cases per 100 000 person-years from 1990-2001 (Sripa et al., 2007). With 1.2%, the prevalence of suspected CCA cases in our survey was exceedingly high. The situation in Saravan is believed to be highly critical. Despite a lack of definitive diagnosis for this lethal disease, our result on suspected CCA cases is in line with a recent finding on hepatobiliary morbidity due to *O. viverrini*,

screened by US (of ~ 800 subjects, 8 suspected CCA cases) (Dr. Bouasy Hongvanthong, pers. communication,). They call for further work on this bile duct cancer.

Recently, work on a new national cancer control programme has started in Lao PDR (Dr. Phetsamone, pers. communication). There is a good prospect that liver cancer including *O. viverrini*-induced CCA will be included in the national programme, allowing for establishing supportive treatment and control strategies. The resulting information will be crucial to develop intervention strategies, in particular the role of a cancer centre focusing on state-of-the-art diagnosis and treatment including surgery and palliative care for CCA patients. Cancer registration will lead to the better understanding of the epidemiology of CCA in Lao PDR (Parkin et al., 1993). It will also be essential to follow-up the prevalence of *O. viverrini* infection through large scale surveys.

We are conscious that our investigations have constraints. The limitations of the hospital-based study arise from the different diagnostic possibilities at the different health service levels and the shortage of diagnostic imaging (MRI and ERCP). Limitations of the community-based study include the relatively small number of study participants who met our selection criteria (Chapter 7). Furthermore, the health status of patients was not conducive to undergo CT and ERCP examination. Thus, for all suspected CCA cases we were unable to execute a final diagnosis or confirmation of CCA. Despite these shortcomings, our community- and hospital-based studies provide the first documents on the magnitude of morbidity associated with *O. viverrini* infection in Laos. Both studies underscore that chronic *O. viverrini* infection induces various hepatobiliary and -splenic abnormalities, and conclude that further clarifying research is urgently needed.

The high prevalence of morbidity and infections with both trematodes at the end point survey indicated limitations of MDA. As chemotherapy does not prevent re-infection and therefore on its own does not constitute sustainable control of helminths, a package intervention is required for long–term control of schistosomiasis and opisthorchiasis. The same probably also applies to the other neglected tropical diseases, notably STHs. A set of interventions including IEC to discourage the consumption of raw fish, to prevent direct contact with infected water bodies, and to promote hygiene and sanitation practices, targeting the young generation, improved sanitation and water supply and geospatial

approaches must be the cornerstones of schistosomiasis, opisthorchiasis, and STH and other target disease control (Utzinger et al., 2009; Sripa et al., 2010; Knopp et al., 2011b).
Since 2008 the Lao Ministry of Health with technical support from WHO has resumed the province-wide deworming programme in six selected provinces, of southern Lao PDR where *S. mekongi* and *O. viverrini* are endemic with prevalences higher than 25% (Dr. Keoka Taisaiyavong, pers. communication,). Additionally, a pilot package of intervention including improved hygiene and sanitation, access to clean water in combination with MDA and IEC might be a solution to interrupt the transmission and achieve sustainable control of food and waterborne trematode infections. A project has been set up in 2010 on Khong Island in collaboration with several partnerships including the National Institute of Public Health, Ministry of Health, Ministry of Health, the Swiss TPH and the International Development Research Centre to investigate this strategy and to assess its effectiveness (Somphou Sayasone, personal communication). Our studies further document and confirm the high magnitude of morbidity due to *S. mekongi* and *O. viverrini*. Large-scale community-based ultrasound studies with the possibility of advanced diagnostic procedures are warranted to investigate the true burden due to *S. mekongi* and *O. viverrini*-endemic settings. Our studies call for urgent concerted fluke control.

11.4 Assessment of efficacy of present anthelmintic drugs and potential candidates

Hookworm takes the first rank in terms of the global burden attributable to STHs (Utzinger et al., 2009). It shows increased severity in those who are affected with human immunodeficiency virus (HIV) and tuberculosis (Bethony et al., 2006; Keiser and Utzinger, 2009). A single dose of ABZ or MBZ is employed for school- or community-based morbidity control of STHs in highly endemic areas. It is also administered to vulnerable populations (schoolchildren and reproductive women) through regular school deworming and mass drug administration programmes in Lao PDR (Jex et al., 2011). Resistance to anthelminthic drugs in animals has been described for decades. Nevertheless, the mechanism is unclear (Geerts and Gryseels, 2006; Wolstenholme et al., 2004). Single doses of ABZ and MBZ have been considered critical as drug resistance might develop (Albonico et al., 2004). In

addition, low efficacies of MBZ against hookworm infections were reported from Mali, Tanzania, and Vietnam (Albonico et al., 2003; De et al., 1977; Flohr et al., 2007). ABZ resulted in low cure rates against hookworm infections in a review and in Ghana (Horton, 2000; Humphries et al., 2011b). Consequently, there is a hot debate in respect to the low efficacy of benzimidazole against hookworm infections in humans (Humphries et al., 2011a; Montresor et al., 2011). No information on the efficacy of these drugs was collected in Lao PDR before the study conducted in the framework of the present Ph.D.

We investigated the efficacy of a single oral dose of ABZ or MBZ against hookworm in an endemic area of STHs in Laos (Chapter 9). In our trial, CRs and ERRs of ABZ proved superior over MBZ (P=0.01). However, both anthelminthics ABZ and MBZ, showed disappointing cure rates of 30.0 % and 17.6 %, respectively against hookworm in our study. According to a recent meta-analysis, the respective cure rates of ABZ and MBZ were 72% and 15% (Keiser and Utzinger, 2008). CR of ABZ (36%) found in our study was 2 times lower compared to a meta-analysis and a recent study carried out in China (CR of ABZ was 69% in treatment of hookworm) (Steinmann et al., 2011).

The low cure rates of ABZ in our study might be attributable to the differences in strain between hookworm species, namely *N. americanus* and *A. duodenale*. Both species are common worldwide (Brooker et al., 2004) but in Southeast Asia, species identification has never been done (Jex et al., 2011). A zoonotic helminth, *A. ceylanicum* is also known to infect human in this region (Jirraanankul et al., 2011; Traub et al., 2008). Consequently, our study participants could have been infected with any of these parasites. Secondly the study's sample size is relatively small due to day to day variation of hookworm egg counts. Thirdly a few incidental effects such as failure of some children to swallow the pill correctly, might theorically have happened and contributed to low efficacy of ABZ. However, we cannot rule out that drug resistance indeed exists as the drugs have been used for decades. Therefore, drug efficacy - particularly of single-dose administration - should be monitored carefully and integrated into further chemotherapy campaigns.

In view of the low CR obtained with ABZ and MBZ against hookworm infections in Laos (Chapter 9), there is a pressing need to conduct large scale trials that provide new data regarding the efficacy of commonly used anthelminthics against hookworm infections in

different settings, so that the most efficacious drugs can be used in control programmes and emerging resistance can be detected at an early stage. Therefore, further studies should take into account the sample size in order to deal with variability of egg shedding, and the circulating parasite species. The issue is of particular concern as these drugs are widely used for mass drug administration, not only for STHs, but also for lymphatic filariasis control (WHO, 2002). Another issue is the diagnostic test employed. In the absence of a gold standard, the Kato-Katz thick smear technique is the most widely used parasitological diagnostic test for hookworm, recommended by WHO (WHO, 1991).

Kato-Katz is a direct stool examination technique and inexpensive tool, widely deployed in the large scale epidemiological survey for diagnosis of helminths. However, Kato-Katz has limitations in detecting hookworm egg. Low sensitivity was observed, in particular of light infections. The novel FLOTAC technique might offer an alternative. It is a qualitative and quantitative stool examination technique with a high sensitivity to detect hookworm eggs (Utzinger et al., 2008). In addition to its high sensitivity in detecting human hookworm infections, FLOTAC allows detecting multi-species parasite infections with superior sensitivity over the traditional Kato-Katz thick smear and ether-based concentration techniques (Keiser et al., 2010a; Knopp et al., 2011a). It is important to note that *O. viverrini* eggs in the stool samples were diagnosed by the Kato-Katz thick smear technique and FECT (Formalyl ether concentration technique). The data of FECT, which is a good method to differentiate between *O. viverrini* and other intestinal trematodes (i.e, minute intestinal flukes), suggested that Kato-Katz technique (3 stool samples examined with duplicate Kato-Katz slides) showed higher sensitivity than FECT (Chapter 10). These findings might be contradictory to previous studies employing the same procedures (Sayasone et al., 2009). No explanation can currently be offered to elucidate the discrepancy. PCR might be an alternative gold standard for *O. viverrini* diagnosis. Nonetheless, also this technique has some limitations: it is time-consuming, and needs well-trained staff as well as expensive equipment. Hence it is currently not feasible to apply it in the field studying resource-constrained settings. There is thus a need for further innovation and standardization of diagnostic tools (Bergquist et al., 2009).

A broad spectrum anthelminthic developed by Chinese scientists, tribendimidine, might be a new candidate for hookworm treatment and control (Ren et al., 1987). In the absence of vaccine, elimination of hookworm might be possible by means of improving socioeconomic conditions, strengthening of hygiene and sanitation and targeted treatment (drug treatment of infected individuals only) as a successful public health program in the southern USA and a trend from morbidity control toward transmission control in Zanzibar show (Brown, 1976; Knopp et al., 2011b; Utzinger et al., 2008). The same activities and mechanisms are invoked as the explanation of a marked reduction of the hookworm prevalence in Southeast Asia (de Silva et al., 2003).

In addition to the primary outcome, we have generated supportive evidence that ABZ and MBZ, even at single oral doses, have opisthorchicidal properties against *O. viverrini*. Although cure rates were relatively low in our study population, promising egg reduction rates were observed, corroborating previous studies (Jaroonvesama et al.,1981; Pungpark et al., 1984). Similar cure rates and ERRs were observed for other parasites (i.e., *Ascaris lumbricoides* and *Trichuris trichura*) as reported in meta-analysis (Keiser and Utzinger, 2008). Both drugs resulted in high CRs and ERRs against *A. lumbricoides* and were less effective against *T. trichura*. Other studies also reported comparable outcomes (Olsen et al., 20009; Steinmann et al., 2011). However, due to the selection bias, i.e. only persons with hookworm infection were recruited into the study, conclusions can be drawn.

A host of parasitic infections are of public health concern worldwide and their areas of endemicity often geographically overlap in tropical and sub tropical countries, in particular Southeast Asia (Steinmann et al., 2008a; Steinmann et al., 2010). Today, very few anthelminthic agents have been approved for large scale administration to human. The pharmaceutical industry lacks incentives to develop new anthelmintic drugs for use in humans (Utzinger and Keiser, 2004). Attempts have been made to piggy back on existing drugs, where clinical information is already available, and which thus need not undergo the long and expensive process of drug development (Nawaka and Hudson, 2006). Following this strategy, the antimalarials artemether and artesunate, mefloquine and the Chinese anthelminthic tribendimidine were tested for activity against *O. viverrini* in a hamster model (Keiser et al., 2006; Keiser and Utzinger, 2007a; Keiser et al., 2008). However, the

efficacy of none of these compounds had been investigated in *O. viverrini* infected patients. Following a similar line of investigation, the experience related to the antimalarial drugs mefloquine and artesunate was used to investigate activity against *Schistosoma haematobium* in infected schoolchildren, and in a recent proof of concept study on treatment of chronic fascioliasis (Keiser et al., 2010b; Keiser et al., 2011).

The Chinese anthelminthic tribendimidine has been approved in 2004 for human use in China (Xiao et al., 2005). Tribendimidine is a derivative of amidantel and has been developed by the National Institute of Parasitic Disease in Shanghai, China, since the 1980s (Ren et al. 1987). Currently, efforts are ongoing to register tribendimidine for the treatment of soil-transmitted helminthiases with international authorities such as WHO and major regulatory agencies including FDA and EMEA. The artemisinins are the basic components of modern malaria treatment (White, 2008). Mefloquine is an orally administered antimalarial drug developed by the Walter Reed Army Institute of Research in 1988 and used both for prophylaxis and treatment against malaria (Palmer et al., 1993).

Tribendimidine treatment resulted in promisingly high cure rate (CR) and egg reduction rate (ERR) against *O. viverrini* in our exploratory trial. This drug might thus be an alternative for liver fluke treatment (Sripa B et al., 2011). Hence, large scale studies are warranted to confirm its efficacy and safety. It might be worth to study a chemotherapy combination, because this might delay the development of drug resistance (Olliaro and Tailor, 2004). A phase three four-arm study might be designed, comparing standard treatment, praziquantel (treatment of choice) with tribendimidine, ABZ and MBZ (Dr. Somphou Sayasone, pers communication).

In chapter 10, on the basis of an intention-to–treat analysis, a 400 mg single dose of tribendimidine achieved better cure (70.4%) and egg reduction rates (99.3%) against *O. viverrini* than a double dose of praziquantel (CR=56.0%, ERRs=98.4%). However, this finding was not statistically significant since only a small number (n=125) of children was included in our exploratory study (p=0.29). Both intention-to-treat and per-protocol analysis indicated tribendimidine cured hookworm infections at a promising rate (CR=65%, *P*=0.004) (Chapeter 10) and even much higher compared to ABZ treatment against hookworm in the light of our previous trial (Chapter 9). In our proof of concept

study, mefloquine and artesunate resulted in low CR of *O. viverrini*. However, promising results of ERRs were seen in children taking mefloquine-artesunate.

Adverse events were observed in all treatment arms, mostly of mild to moderate intensity. Single dose tribendimidine is generally well tolerated in the present study and had proven to be safe at a dose of 200-400 mg in settings where multiple intestinal helminth species are co-endemic (Steinmann et al., 2008b). Surprisingly, serious adverse events were recorded after mefloquine treatment, and in the combination treatment of mefloquine-artesunate. Dizziness, nausea, vertigo, vomiting and headache were the most common adverse events observed in patients taking mefloquine and a combination of mefloquine and artesunate.

11.5 Conclusions

In the previous discussion sections main conclusions were given in each section. In the following the main conclusions are given again.

The series of severe cases demonstrate that adequate diagnosis andcase management of infections with *C. philippinensis* are required in Laos. The severe *S. mekongi* cases warrant particular attention to re-emergence of *S. mekongi* in the community of Khong and adjacent districts. Furthermore, regular deworming programmes particularly among school-and pre-school aged children in *S. mekongi* and *O. viverrini* endemic communities should be implemented to reduce severe hepatosplenic pathogenesis and prevent chronic consequences in adulthood.

O. viverrini, *S. mekongi* are common parasitic infections of major public health significance in rural areas of Lao PDR. Our study, using US and stool examination, provides an insight into the problems related to morbidity due to *S. mekongi* and *O. viverrini* and co-infections. These parasites induce a wide range of hepato-biliary and -splenic pathogenesis in the affected communities in southern Lao PDR. Furthermore, our studies document that severe morbidity and in particular CCA is associated to high degree with *O. viverrini* infection. It is of particular concern in endemic settings as well as across the country. Appropriate cancer care facilities (including treatment by surgery, chemotherapy, radiotherapy and palliative care) and cancer registration are necessary in order to assess the prevalence of CCA and

ethical difficulties on case management. Our evidence on morbidity due to both parasites could be used as a basis for a rational design and implementation of control strategies.

The efficacy of different anthelminthic drugs has to be regularly assessed in order to guide policy recommendations and take timely action should resistance develop. Today, ABZ and MBZ have low efficacy if applied in MDA campaigns. Further investigation using sensitive diagnostic approaches are required for the detection of helminths. Large-scale trials investigating the efficacy and safety of tribendimidine should be a priority as this drug showed nematocidal and opisthorchicidal properties in our study. It is very intriguing to focus on this drug as there is huge geographical overlap of these parasites and preventive chemotherapy is the mainstay of control.

Preventive chemotherapy and primary prevention including public health promotion like health education, safe water supply, hygiene and sanitation as well as integrated approaches to enhance synergies between programmes are needed. A system of monitoring and surveillance is required. Regular training of doctors, health care provider, nurses and laboratory staff is necessary in order to timely diagnose infections, dispense appropriate treatment, and properly manage severe cases both at community and central hospital level.

11.6 Further research needs

1.-It is particularly important to implement large scale community-based ultrasound survey s with the aim of enabling comparisons between regions

2.-Concerted action on health promotion needs to be re-inforced for the prevention of complications due to helminth infections.

3.-Development of accurate diagnostic tools and approaches for the assessment of helminth infections, particularly light residual infections after treatment are needed.

4.-Large scale assessments of safety and efficacy of tribendimidine for the treatment of *O. viverrini* and helminth co-infections, are logic next steps given the promising results obtained in this study.

5.-A phase three four-arm study might be designed, comparing standard treatment, praziquantel (treatment of choice) with tribendimidine, ABZ and MBZ.

6.-Evaluation of safety and efficacy of combination chemotherapy (i.e., praziquantel-tribendimidine against *O. viverrini*) as a useful strategy to delay the emergence of drug resistance as in malaria treatment (i.e., artemisinin- based therapy)

7.-A clinical trial assessing efficacy and safety of TBD against C*lonorchis sinensis* is warranted.

8.-Studies of tribendimidine-praziquantel combinations in hamsters infected with *O viverrini* might be of interest.

11.7 Recommendations

1.-Develop a surveillance system and monitoring of severe case and proper case management

2.- Recent control efforts are insufficient and need to be consolidated including regular MDA, case detection and improved sanitation and health services

3.-Strengthen human resource capacities on dealing with diagnosis and treatment of CCA, for instance education of health professionals, lab personal and surgeon on liver surgery and radiology

4.-Design and establishing a cancer registry including CCA as well as other cancers

5.-Assessment of the efficacy of current drug used for mass drug campaigns (i.e, ABZ, MBZ and PZQ)

6.-Develop CCA cancer registration as well as other organ-related cancers with a protocol for diagnostic algorithms and cancer treatment (palliative care, surgery and so on) with an aim of estimating the CCA burden in Lao PDR

7.-Administer regular MDA programmes with a set of interventions (health education, engineering development, sanitation and hygiene) for control of liver fluke in other *O. viverrini*-endemic provinces

8.-Systemic monitoring of drug efficacy and early detection of drug resistance on current helminth control programmes

9.-Enforcement of long-term sustainable control programmes on improved infrastructure, health education, changing behaviours, sanitation, and safe water supply.

11.8 References

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12. Appendix – Curriculum vitae

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Marital status Married
Nationality Lao
Language Lao (Mother tongue), Thai (very good oral), English (very good), French (very good)

Academic qualifications and work experience

Oct. 2007-Dec. 2011	Ph.D. in Epidemiology and Public Health, Swiss Tropical and Public Health Institute (Swiss TPH)
Oct. 2010	An official staff, National Institute of Public Health, Ministry of Health, Lao PDR
Nov. 2006-March. 2007	Research assistant, Institut de la Francophine pour la Médecine Tropicale (IFMT), Lao PDR
Oct. 2006	Master of Tropical Medicine and International Health, IFMT M.Sc. thesis supervised by Prof. med. Dr. Michael Strobel, former director of IFMT "Risk factor of bladder stone in children aged 5-16 years, Khammouane Province, Lao PDR" (honourable award)
1999-2004	B.Sc (diploma in Lao and French), University of Health Sciences and l'Agence Univertiaire de la Francophonie
2001-2002	Attended medical year 5, Faculty of Medicine, University of Lille 2, France B.Sc Thesis supervised by Prof. Dr. Michael Strobel, former director of IFMT and Dr. Anan Sackpraseut, Chief of Gyneco-Obstetric Department, Mahosot Hospital. Vientiane capital, Lao PDR "Induced abortion among young women admitted to Mahosot hospital, Vientiane capital, Lao PDR"
1996-1998	Chanthabuly high school, Vientiane Capital, Lao PDR

Oral presentations, Posters at Scientific Conferences

Dec. 2011	American Society of Tropical Medicine and Hygiene 60th Annual Meeting " Nine severe schistosomiasis mekongi in Southern Lao's People Democratic Republic" (Poster presentation)
Oct. 2011	7th European Congress on Tropical Medicine and International Health "A randomized, exploratory open-label trial on efficacy and safety of mefloquine, artesunate, mefloquine-artesunate, tribendimidine and praziquantel against *Opisthorchis viverrini*" (Poster presentation)
Mar. 2011	The International Conference of Liver fluke, Khon Kean, Thailand "A randomized, exploratory open-label trial on efficacy and safety of mefloquine, artesunate, mefloquine-artesunate, tribendimidine and

praziquantel against Opisthorchis viverrini" (Oral presentation)

Oct. 2010 Swiss Society of Tropical Medicine and Parasitology, Annual Congress 2010, Spiez, "Nine severe schistosomiasis mekongi in Southern Lao's People Democratic Republic" (Oral presentation)

Oct. 2008 Swiss Society of Tropical Medicine and Parasitology, Annual Congress 2008, Vevey

Dec. 2006 Joint International Tropical Medicine Meeting 2006 and 6th Asia-Pacific Travel Health Conference (JITMM 2006-6th APTHC), "Food-borne trematodiasis in Lao PDR" (Oral presentation) and "Risk factor of bladder stone in children aged 5-16 years, Khammouane Province, Lao PDR" (Oral presentation)

Oct. 2006 District Health System in South-East Asia The Regional Seminar was held by Ministry of Health, Lao PDR and Belgian Technical Cooperation, in collaboration with international partners and South-Eeast Asian countries, Vientiane Capital, Lao PDR, "Use and misuse of private pharmacies: survey among consumers in Vientiane Capital and Vientiane province" (Oral presentation)

Publications (published and in press)

Year 2008

Soukhathammavong P, Sayasone S, Harimanana AN, Akkhavong A, Thammasack S, Phoumindr N, Choumlivong K, Choumlivong K, Keoluangkhot V, Phongmany S, Akkhavong K, Hatz C, Strobel M, Odermatt P. *Three cases of intestinal capillariasis in Lao People's Democratic Republic*. Am J Trop Med Hyg. 2008 Nov;79(5):735-8. (Published)

Year 2009

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