Control approaches for *Opisthorchis viverrini* and co-infections in Lao PDR

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Basel, den 20. Juni 2017

Prof. Dr. Martin Spiess
Dekan

Dedicated to my beloved family

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List of Abbreviations

CCA	Cholangiocarcinoma
CLTS	Community-led total sanitation
DHO	District health office
EIDs	Emerging and re-emerging infectious diseases
EKBB	Ethical Committee of Canton of Basel-Stadt and Baselland
EPG	Eggs per gram
FECT	Formalin ether concentration technique
IEC	Information, education and communication
KAPB	Knowledge, attitudes, perception and behaviour
KAP	Knowledge, attitudes and practice
MDA	Mass drug administration
MIF	Minute intestinal trematode
NECHR	National Ethics Committee for Health Research
NIOPH	National Institute of Public Health
PAMS	Partnership Actions for Mitigating Syndromes (NCCR North-South, Switzerland)
PHO	Provincial health office
PCR	Polymerase chain reaction
SAF	Sodium acetate acetic-acid formalin
STH	Soil-transmitted helminthiasis
Swiss TPH	Swiss Tropical and Public Health Institute

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Summary

Background: In Lao People's Democratic Republic (Lao PDR) helminth infections are highly prevalent. All groups of helminths, including nematodes, trematode and cestodes can be found throughout the country. Besides high rates and intensity of infections, most individuals are infected with several different species of intestinal parasitic infections. As in many other countries, a main underlying factor for high worm infestations is the scarce economic resources which lead to a lack of appropriate sanitary facilities, and hygiene related education and adequate behaviour. Therefore, the parasite infection is common among the rural population. However in Laos, there is an additional, particular risk factor responsible for the populations, high worm-load; namely the consumption of raw or insufficiently cooked foodstuff such as raw meat, fish and vegetables. This habit is deeply culturally rooted, and widespread in the Lao population. In this PhD thesis, we tested and determined the appropriate control approaches for *Opisthorchis viverrini (O. viverrini)* and other important helminth infections such as *Strongyloides stercoralis (S. stercoralis)* and *Schistosoma mekongi (S. mekongi)*. The tested approaches compose of latrine promotion programme, eco-health intervention study and novel urine test for *S. mekongi* diagnosis in Khong District, Champasack Province, Lao PDR.

Goal and specific objectives: The present PhD study aimed to develop the appropriate control approaches for *O. viverrini* infection and co-infections in Lao PDR. Four specific objectives were pursued: **i).** To assess *S. stercoralis* infection and the risk of infection among the populations on three islands in Khong district, Champasack province, Southern Laos; **ii).** To define *O. viverrini*, *S. mekongi* and STH infections in humans in the ecological environment of Khong district, Champasack province where their potential animal reservoir, and intermediate hosts are living in close connectivity; **iii).**To compare the diagnostic tools for detection of *S. mekongi* infection in Lao People's Democratic Republic and neighbouring country Cambodia; and **iv).** To assess the impact of improved sanitation and its use on the transmission of intestinal helminth infections in highly endemic areas, three islands in Khong district, Champasack province, Southern Laos.

Methodology: Both cross-sectional and cohort studies were used in this PhD thesis's research. All data of this PhD thesis were obtained from community-based studies.

First study of this PhD research was latrine intervention which was called as latrine study, conducted in March 2011 to January 2013 on three islands, i.e. Donlong, Donthan and Donlieng island located in the Mekong River in Khong district, Champasack province, southern Laos. Given an experimental pre-test and post-test with one control group was used to assess the effects of latrine in the study villages on preventing of helminth infections particularly *O. viverrini* and *S. mekongi*. Household-based promoting latrine construction was conducted. Two stool samples were collected per study participants within a five day period. Each sample was examined by using Kato-Katz (KK) thick smears and Baermann technique. There were three different phases of the study which described elsewhere in material and methods section of Chapter 7.

A baseline study of ecohealth intervention for *O. viverrini*, *S. mekongi* and STH was conducted in October 2011 and August 2012 on Done Khon and Done Som islands. Household members aged two years and older and potential animal reservoir hosts, i.e., dogs, cats, pigs and buffaloes, from selected households were enrolled and examined for helminth infections. For *O. viverrini*, snails of the genus *Bithynia* spp. were collected with a scoop from water bodies near the study villages (e.g., ponds, canals, and rice fields). *Cyprinoid* freshwater fish were captured from the same selected water bodies as well as from the Mekong using fishing net. For *S. mekongi*, *N. aperta* snails were hand-picked from the rocky area of the Mekong River. Two KK thick smears, formalin ether concentration technique (FECT), the shedding test and the pepsin enzyme digestion technique were performed for human, animal reservoir hosts, *Bithynia* spp. and *N. aperta* snail hosts and *Cyprinoid* fish, respectively to assess their infections.

The evaluation of the new diagnostic techniques for *S. mekongi* was conducted between February and April 2016 in *S. mekongi*-endemic villages in Lao PDR and Cambodia. Urine and serum samples were obtained from each study participant to be tested for *Schistosoma* infection by POC-CCA, UCP-LF CAA and ELISA assays. We collected three stool samples from each participant during five consecutive days. Stool samples were subjected to examination by duplicate KK thick smears examined under a light microscope.

Principle findings/results: Our first report was from the first phase of latrine study which emphasized on epidemiology of *S. stercoralis* infection on Mekong islands, showed 41.0%

overall prevalence rate. The infection rate did not differ between the islands (Donlong 44.1% vs. Donthan/Donlieng 38.2%, p=0.107). The highest infection rate was observed with *O. viverrini* (72.2%), followed by hookworm (56.1%) and *S. mekongi* (12.8%). *Trichuris trichiura* (3.3%), *Ascaris lumbricoides* (0.3%) and *Taenia* spp. (0.3%). The most important risk factor was sex. Male study participants had a significantly higher risk for a *S. stercoralis* infection than female participants taking into account the age of the study participants (adjusted OR 1.97, 95% CI 1.45–2.67).

The baseline result of our ecohealth intervention revealed that human infection rates with *O. viverrini,* hookworm, *S. mekongi, T. trichiura*, *A. lumbricoides* and *Taenia* spp. were 60.7%, 44.1%, 22.2%, 4.1%, 0.6% and 0.1%, respectively. Heavy intensity infections were 4.2%, 3.6% and 1.8% for *O. viverrini*, *S. mekongi* and hookworm, respectively. . *O. viverrini* infection rate among dogs and cats were 25.0% and 53.1%, respectively. *S. mekongi* infection rates among dogs were 14.7%.. Prevalence of *O. viverrini* and *S. mekongi* in snails was 0.3% and 0.01%, respectively. Overall prevalence of *O. viverrini* infection in fresh water fish was 26.9%, with the highest infection rates occurring in *Hampala dispa* (87.1%), *Cyclocheilichthys apogon* (85.7%) and *Puntius brevis* (40.0%). Illiteracy and lower socioeconomic status increased the risk of *O. viverrini* infection, while those aged 10–16 years and possessing latrines at home were less likely to be infected. Household dogs and cats that consumed raw fish were significantly and positively associated with *O. viverrini* infection of the household members. For *S. mekongi*, children under 9 years old were exposed significantly to this infection, compared to older age groups.

Study on comparison of novel and standard diagnostic tools for the detection of *S. mekongi* infection in Lao PDR and Cambodia which was carried out between February and April 2016. Stool microscopy by KK thick smear revealed an overall prevalence of *S. mekongi* of 6.4% (one case in Cambodia and 23 cases in Lao PDR), while that of *O. viverrini*, hookworm, *T. trichiura*, *A. lumbricoides* and *Taenia* spp. were 50.4%, 28.1%, 3.5%, 0.3% and 1.9%, respectively. In total, 377 urine and serum samples were tested for *S. mekongi* infection. In the urine samples, the tests for CCA and CAA detected *S. mekongi* infections in 21.0% and 38.7% of the study participants, respectively. In the serum samples, the CAA assay revealed a prevalence of 32.4%, while a combination of the CAA assay in serum and in urine

revealed a prevalence of 43.2%. There was a difference between the two study locations with a higher prevalence reached in the samples from Lao PDR.

Study on assessment the effects of latrine in the study villages on preventing of helminth infections particularly *O. viverrini* and *S. mekongi* which was performed by using an experimental pre-test and post-test with one control group. In the intervention villages, the helminth infection rates at baseline for *S. mekongi*, *O. viverrini*, hookworm, *A. lumbricoides*, *T. trichiura*, *Enterobius vermicularis*, *S. stercoralis* and *Taenia* spp. were 28.6%, 79.5%, 48.8%, 0.3%, 3.5%, 0%, 43.1% and 0%, respectively. At follow-up they were reduced to 22.6%, 68.2%, 26.2%, 2.5%, 1.1%, 0.4%, 31.1% and 0.4%, respectively. Reduction in prevalence of four important helminthiasis were significantly observed (baseline vs follow-up, *P-value*), *S. mekongi* (28.6% vs 22.6%, *P-value* <0.001)*, O. viverrini* (79.5% vs 68.2%, *P-value* <0.001), Hookworm (48.8% vs 26.2%, *P-value* <0.001) and *S. stercoralis* (43.1% vs 31.1%, *P-value* <0.001) in the intervention villages. While *S. mekongi* infection in the control villages was increased (1.8% vs 2.6%, *P-value* =0.74) and other helminths were decreased *O. viverrini* (71.8% vs 59.9%, *P-value* =0.027), Hookworm (65.6% vs 38.3%, *P-value* <0.001) and *S. stercoralis* (38.3% vs 34.8%, *P-value* =0.001).

Conclusion/Significance: *O. viverrini, S. mekongi*, and STH particularly *S. stercoralis* were still high prevalence in Mekong islands as well as the multiparasitism was observed in all studies of this PhD thesis works. There is a pressing need to design and implement an integrated helminth control intervention on the Mekong Islands in southern Lao PDR. An appropriate integrated control approach involving interventions targeting human behaviour, animal reservoirs, and environmental modification, health education and improved access to clean water and adequate sanitation might improve the effectiveness of interventions and lead to the elimination of infections. Furthermore, the new diagnostic tool CCA, CAA and ELISA were evaluated and showed a substantially higher prevalence estimates for *S. mekongi* compared to Kato-Katz thick smears. Active schistosomiasis mekongi in Lao PDR and Cambodia might thus have been considerably underestimated previously. Hence, sustained control efforts are still needed to break transmission of *S. mekongi*. The pivotal role of highly sensitive diagnostic assays in areas targeting elimination cannot be overemphasised.

Zusammenfassung

Einleitung: In Laos sind Helminthen Infektionen äusserst häufig. Alle Helminthen-Gruppen sind endemisch, wie die Nematoden, Trematoden und Zestoden. Neben den hohen Infektionsraten und Infektionsintensitäten, sind auch viele Bewohner mit mehreren Helminthen-Arten infiziert. Wie in vielen anderen Ländern sind die zugrundeliegenden Faktoren die geringen finanziellen Ressourcen, die zu einer Abwesenheit von angemessener Sanitären Anlagen, Hygiene, Bildung und Verhalten führen. Aus diesem Grund sind die parasitären Infektionen in der ländlichen Bevölkerung extrem häufig. Hingegen gibt es in Laos ein weiteres charakteristisches Merkmal der Bevölkerung, das zu einem grossen Risiko von Wurminfektionen führt, nämlich der weitverbreitete und kulturell tief verwurzelte Konsum von rohen oder ungenügend gekochten Nahrungsmitteln, wie Fisch, Fleisch und Gemüse. In dieser Doktorarbeit haben wir angepasste Kontrollmassnahmen für die Bekämpfung vom Leberegel *Opisthorchis viverrini* und anderen wichtigen Helminthen Infektionen, wie *Strongyloides stercoralis*, *Schistosoma mekongi,* untersucht. Zu den getesteten Programme gehörten ein Latrine Programm, eine Eco-Health Intervention und neue diagnostische Methoden für *S. mekongi.*.

Ziele der Arbeit: Die vorliegende Dissertation hatte zum Ziel Bekämpfungsmassnahmen gegen *O. viverrini* Infektionen und Ko-Infektionen in Laos zu testen. Dabei wurden vier spezifische Ziele verfolgt: **i).** das Messen der *S. stercoralis* Infektion und Infektionsrisiko in der Bevölkerung auf den Mekong Inseln des Khong Distrikts, Champasack Provinz, Laos; **ii)** Das Abschätzen der Infektionen mit *O. viverrini*, *S. mekongi* und boden-übertragenen Helminthen (STH) in Menschen, potentiellen Tierreservoirs und Zwischenwirten in einem Gebiet im Khong Distrikt; **iii).** Der Vergleich von Diagnosemethoden für *S. mekongi* Infektionen im südlichen Laos und nördlichen Kambodscha; und **iv).** Das Messen der Auswirkung einer Latrinen-Intervention auf die Übertragung der intestinalen Helminthen auf einer hoch-endemischen Mekong Inseln des Khong Distrikts, Champasack Provinz, Laos.

Methodologie: Für diese Dissertation wurden alle Daten in Querschnittsstudien von ausgewählten Dörfern erhoben.

Die erste Studie dieser Dissertation ist eine Latrinen-Interventionsstudie, die von März 2011 bis Januar 2013 auf den drei Mekong Inseln, Donlong, Donthan und Donlieng, im

Khong Distrikt, Champasack Provinz, Laos, durchgeführt wurde. Die Interventionsstudie folgte einer vor-nach Intervention Logik mit einer Kontrollgruppe. Studienteilnehmer wurden beim Start und Ende auf Helminthen Infektionen untersucht. Nach der Startuntersuchung wurden Probanden mit Infektionen behandelt. In Interventionsdörfern wurde den Haushalten eine Unterstützung für den Bau von Latrinen zugesprochen. Die Helminthen Infektionen wurden mit Hilfe der Kato-Katz Methode in zwei Stuhlproben pro Proband bestimmt.

Zwischen Oktober 2011 und August 2012 wurde der erste Teil der Eco-Health Studie durchgeführt. Dies beinhaltete die Untersuchung der Studienteilnehmer auf die Infektionen mit *O. viverrini, S. mekongi* und STH auf den Mekong Inseln Done Khon und Done Som. Haushaltsmitglieder älter als zwei Jahre wurden in die Studie aufgenommen sowie auch potentielle Tierreservoire, wie Hunde, Katzen, Schweine und Büffel, und auf eine Helminthen Infektion untersucht. *Bithynia* Schnecken wurden mit der Scooping Technik in verschiedenen Wasserstellen (z.B. Teiche, Kanäle, Reisfelder) gesucht und auf eine Infektion mit *O. viverrini* untersucht. *Cyprinoide* Fische wurden in den gleichen Wasserstellen inklusive dem Mekong gesucht und ebenfalls auf eine *O. viverrini* Infektion untersucht. *Neotricula aperta* Schnecken wurden auf den Felsen der Mekong-Ufer gesucht und auf eine *S. mekongi* Infektion untersucht. Mit der Kato-Katz und der Formalin-Ether Konzentration Techniken wurden die Helminthen im Stuhl der Probanden und der Reservoir-Tiere diagnostiziert. Für die Diagnose einer Schneckeninfektion wurde ein Zerkarien-Ausscheidungstest verwendet. Die Pepsin-Enzym-Verdauungstechnik wurde verwendet um Meta-Zerkarien im Fisch zu identifizieren.

Die Evaluation der neuen diagnostischen Techniken für eine aktive *S. mekongi* Infektion wurde zwischen Februar und April 2016 in *S. mekongi* – endemischen Dörfern in Laos und Kambodscha durchgeführt. Urin und Serum wurde von allen Studienteilnehmern auf eine *Schistosoma* – Infektion mittels POC-CCA, UCP-LF CAA und ELISA untersucht. Ausserdem gab jeder Studienteilnehmer drei Stuhlproben die mittels Kato-Katz Technik untersucht wurden.

Resultate: Unser erstes Manuskript stammt aus der ersten Phase der Latrinenstudie und fasst die epidemiologischen Resultate der Infektionen zusammen. Im Speziellen bespricht

es die Epidemiologie der *S. stercoralis* Infektion, die mit einer Prävalentz von 41.0% sehr häufig war. Es bestand kein statistisch signifikanter Unterschied der *S. stercoralis* Infektionen zwischen den Inseln (Donlong 44.1% vs. Donthan/Donlieng 38.2%, p=0.107). Aber Männer aller Altersklassen hatten ein fast doppeltes Risiko an einer *S. stercoralis* Infektion zu leiden als Frauen (adjusted OR 1.97, 95% CI 1.45–2.67). Die höchsten Infektionsraten wurden für *O. viverrini* (72.2%) gefunden, gefolgt von Hakenwurm Infektionen (56.1%) und *S. mekongi* (12.8%). *Trichuris trichiura* (3.3%), *Ascaris lumbricoides* (0.3%) und *Taenia* spp. (0.3%) wurden ebenfalls diagnostiziert.

Die erste Querschnittstudie der EcoHealth Intervention zeigte in den Studienteilnehmern hohe Helminthen Infektionsraten mit *O. viverrini* (60.7%), Hakenwurm (44.1%), *S. mekongi* (22.2%), *T. trichiura* (4.1%), *A. lumbricoides* (0.6%) und *Taenia* spp. (0.1%). Hohe Infektionsintensitäten wurde für *O. viverrini* (4.2%), *S. mekongi* (3.6%) und Hakenwürmer (1.8%) gefunden. Zudem wurde *O. viverrini* in Hunden (25.0%) und Katzen (53.1%) diagnostiziert. *S. mekongi* Infektionen wurden auch in 14.7% der Hunde gefunden. Die Prävalenz der *O. viverrini* und *S. mekongi* Infektionen in den Schneckenwirten waren 0.3%, beziehungsweise 0.01%. In 26.9% der untersuchten Fische wurde eine *O. viverrini* Infektion festgestellt. Dabei wurden die höchsten Infektionsraten in *Hampala dispa* (87.1%), *Cyclocheilichthys apogon* (85.7%) und *Puntius brevis* (40.0%) identifiziert. Ungebildete Studienteilnehmer und solche mit einem tiefen sozio-ökonomischen Status hatten ein grösseres Risiko für eine *O. viverrini* Infektion, während dem junge Studienteilnehmer im Alter von 10-16 Jahren ein reduziertes Infektionsrisiko hatten. Hunde und Katzen, die mit rohen Fisch gefüttert wurden, wurden häufiger in Haushalten gefunden deren Mitglieder mit *O. viverrini* infiziert waren. *S. mekongi* Infektionen wurden vor allem in jungen Studienteilnehmern (< 9 Jahren) diagnostiziert.

Die Studie zur Evaluation der neuen Diagnostiktechniken einer *S. mekongi* Infektion zeigte die folgenden Resultate. Die Stuhluntersuchungen mittels Kato-Katz Test ergaben eine Prävalenz von 6.4% (1 Fall in Kambodscha und 23 Fälle in Laos). Des Weiteren wurden Infektionen mit *O. viverrini* (50.4%), Hakenwürmern (28.1%), *T. trichiura* (3.5%), *A. lumbricoides* (0.3%) und *Taenia* spp. (1.9%) diagnostiziert. Insgesamt wurden 377 Urin und Serum Proben auf eine *S. mekongi* Infektion untersucht. Im Urin wurde eine Infektion mit CCA in 21.0% und CAA in 38.7% der Probanden nachgewiesen. Im Serum ergaben

32.4% der CAA Tests ein positives Resultat. Die Kombination aller CAA Tests im Serum und Urin ergaben eine Prävalenz von 43.2%. Alle Diagnosemethoden identifizierten höhere Infektionsraten in Laos als in Kambodscha.

Die Latrinen Interventionsstudie ergab die folgenden Resultate. In der Baseline Studie wurden Helminthen-Infektionen in beträchtlichen Prävalenzen gefunden. Die folgenden Helminthen wurden diagnostiziert: *S. mekongi* (28.6%), *O. viverrini* (79.5%), Hakenwürmer (48.8%), *A. lumbricoides* (0.3%), *T. trichiura* (3.5%) und *S. stercoralis* (43.1%). In den Interventionsdörfern waren die Prävalenzraten von vier Helminthen nach dem Latrinenbau signifikant tiefer, nämlich von *S. mekongi* (28.6% vs. 22.6%, P <0.001), *O. viverrini* (79.5% vs. 68.2%, P <0.001), Hakenwürmer (48.8% vs. 26.2%, P <0.001) und *S. stercoralis* (43.1% vs. 31.1%, P <0.001). In den Kontrolldörfern stieg die *S. mekongi* Prävalenz an (1.8% vs. 2.6%, P=0.74) und die Prävalenzen sanken für *O. viverrini* (71.8% vs. 59.9%, P=0.027), Hakenwürmer (65.6% vs. 38.3%, P <0.001) und *S. stercoralis* (38.3% vs. 34.8%, P=0.001).

Schlussfolgerungen: Infektionen mit *O. viverrini, S. mekongi* und STH, insbesondere *S. stercoralis,* und Mehrfachinfektionen wurden in allen Studien dieser Dissertation diagnostiziert. Es besteht eine hohe Notwendigkeit ein integriertes Helminthen-Kontrollprogramm auf den Mekong Inseln zu entwickeln. Ein solches Programm muss Interventionen im Menschen (Prävention, Behandlung, Gesundheitserziehung, Hygiene), im tierischen Reservoir (Behandlung) und in der Umgebung (Sanitation) beinhalten. Des Weiteren haben die neuen diagnostischen Tests wie CCA, CAA und ELISA eine höhere Sensitivität als der zurzeit gebrauchte Kato-Katz Test. Schistosomiasis in Laos und Kambodscha ist höchst wahrscheinlich sehr viel häufiger als angenommen. Demzufolge sind verstärkte und anhaltende Anstrengungen nötig um die Übertragung dieses Parasiten zu unterbrechen. Dabei spielen die sensitiven Diagnosemöglichkeiten eine entscheidende Rolle.

ສະຫຼຸບຫຍໍ້

ປະຫວັດຄວາມເປັນມາ: ການຊຶມເຊື້ອໜອນກາຝາກໃນປະເທດລາວແມ່ນມີອັດຕາສູງ. ທຸກກຸ່ມຂອງໜອນກາຝາກປະກອບມີພວກໜອນກາຝາກໂຕກົມ, ແປໃບໄມ້ ແລະ ແປຂໍ້ ແມ່ນສາມາດພົບໄດ້ທົ່ວໄປຂອງປະເທດ. ຄຽງຂ້າງກັບອັດຕາການຕິດເຊື້ອ ແລະຄວາມຮຸນແຮງຂອງການຕິດເຊື້ອທີ່ສູງ, ຄົນສ່ວນຫຼາຍ ຍັງຕິດເຊື້ອໜອນກາຝາກພ້ອມໆກັນຫຼາຍຊະນິດນຳອີກ. ຄືກັນກັບປະເທດອື່ນໆ, ປັດໃຈຫຼັກສຳຫຼັບອັດຕາການຕິດເຊື້ອທີ່ສູງ ແມ່ນການຂາດເຂີນທາງດ້ານເສດຖະກິດ ເຊິ່ງນຳໄປສູ່ການຂາດເຂີນສິ່ງອຳນວຍຄວາມສະດວກທາງດ້ານສຸຂະພິບານ ແລະ ສຸຂະອະນາໄມ ທີ່ກ່ຽວພັນເຖິງການສຶກສາ ແລະພຶດຕິກຳທີ່ມີຢູ່. ດັ່ງນັ້ນ, ການຊຶມເຊື້ອກາຝາກຈຶ່ງພົບເຫັນຫຼາຍ ນຳປະຊາຊົນເຂດຊົນນະບົດ. ເຖິງຢ່າງໃດກໍ່ຕາມສຳຫຼັບປະເທດລາວ ແມ່ນຍັງມີປັດໃຈເສີມອີກ ໂດຍສະເພາະແມ່ນປັດໃຈທີ່ເປັນຄວາມຮັບຜິດຊອບໃນໂຕຂອງປະຊາກອນເອງ ທີ່ເຮັດໃຫ້ມີການຕິດເຊື້ອໜອນກາຝາກຈຳນວນຫຼາຍ ຄືການບໍລິໂພກອາຫານດິບ ແລະ ບໍ່ສຸກດີ ເຊັ່ນ: ການກິນຊີ້ນດິບ, ປາດິບ ແລະ ຜັກດິບ. ນິໄສດັ່ງກ່າວນີ້ເປັນການຝັງເລິກທາງດ້ານວັດທະນະທຳ ແລະ ກະຈາຍໄປທົ່ວປະຊາຊົນລາວ. ຢູ່ໃນບົດນິພົນປຣິນຍາເອກສະບັບນີ້, ພວກເຮົາໄດ້ທົດສອບ ແລະ ກຳນົດວິທີການທີ່ເໝາະສົມໃນການຄວບຄຸມເຊື້ອກາຝາກໃບໄມ້ໃນຕັບຊະນິດ *Opisthorchis viverrini (O. viverrini)* ແລະ ການຊຶມເຊື້ອໜອກກາຝາກທີ່ສຳຄັນອື່ນໆ ເຊັ່ນ: ເຊື້ອກາຝາກໂຕກົມອ່ຽນ *Strongyloides stercoralis (S. stercoralis)* ແລະ ເຊື້ອກາຝາກໃບໄມ້ໃນເລືອດ *Schistosoma mekongi (S. mekongi)*. ວິທີການທີ່ໄດ້ທົດສອບປະກອບມີ (latrine promotion programme), ການສຶກສາດ້ານສຸຂະພາບສິ່ງແວດລ້ອມ (eco-health intervention study) ແລະ ວິທີກວດຢ່ຽວແບບໃໝ່ເພື່ອບົ່ງມະຕິການຕິດເຊື້ອກາຝາກໃບໄມ້ໃນ ເລືອດ *S. mekongi* ຢູ່ທີ່ເມືອງໂຂງ, ແຂວງຈຳປາສັກ, ສປປ ລາວ.

ເປົ້າໝາຍ ແລະ ຈຸດປະສົງສະເພາະ: ການສຶກສາລະດັບປຣິນຍາເອກນີ້ ໄດ້ຕັ້ງເປົ້າເພື່ອພັດທະນາ ວິທີການອັນເໝາະສົມໃນການຄວບຄຸມເຊື້ອກາຝາກໃບໄມ້ໃນຕັບຊະນິດ *O. viverrini* ແລະ ການຕິດເຊື້ອຮ່ວມ ຢູ່ໃນ ສປປ ລາວ. ປະກອບມີ 4 ຈຸດປະສົງສະເພາະຄື: **i).** ປະເມີນການຊຶມເຊື້ອໜອນກາຝາກກົມອ່ຽນ *S. stercoralis* ແລະ ປັດໃຈສ່ຽງຕ່ຳການຕິດເຊື້ອ ຢູ່ນຳປະຊາຊົນໃນ 3 ດອນ ຂອງເມືອງໂຂງ, ແຂວງຈຳປາສັກ, ພາກໃຕ້ຂອງປະເທດລາວ; **ii).** ກຳນົດອັດຕາການຕິເຊື້ອ *O. viverrini*, *S. mekongi* ແລະ ໜອນກາຝາກທີ່ຕິດຕໍ່ຜ່ານໜ້າດິນ ຢູ່ໃນຄົນທີ່ອາໃສຢູ່ໃນສະພາບແວດລ້ອມຂອງເມືອງໂຂງ, ແຂວງຈຳປາສັກ ບ່ອນທີ່ມີສັດທີ່ເປັນຮັງເກັບເຊື້ອພະຍາດ (animal reservoir) ແລະຜູ້ຮັບໂຕກາງທີ່ດຳລົງຊີວິດຢູ່ຢ່າງໃກ້ຊິດກັນ; **iii).** ສົມທຽບເຄື່ອງມືການບົ່ງມະຕິ ເພື່ອຊອກຫາການຕິດເຊື້ອກາຝາກໃບໄມ້ໃນເລືອດ ຢູ່ ສປປ ລາວ ແລະ ປະເທດເພື່ອນບ້ານກຳປູເຈຍ; ແລະ **iv).** ປະເມີນຜົນຂອງການປັບປຸງ

ສຸຂະພິບານ ແລະ ການນຳໃຊ້ມັນຕໍ່ການສົ່ງຜ່ານການຕິດເຊື້ອໜອນກາຝາກໃນລຳໄສ້ ຢູ່ໃນເຂດທີ່ມີການລະບາດສູງ, ຢູ່ສາມດອນຂອງເມືອງໂຂງ, ແຂວງຈຳປາສັກ, ພາກໃຕ້ຂອງລາວ.

ວິທີວິທະຍາ: ຮູບແບບການສຶກສາແບບ cross-sectional ແລະ cohort studies ໄດ້ຖືກນຳໃຊ້ເຂົ້າໃນບົດຄົ້ນຄວ້າປຣິນຍາເອກນີ້. ຂໍ້ມູນທຸກຢ່າງໃນບົດນິພົນນີ້ ແມ່ນໄດ້ມາຈາກການສຶກສາໃນລະດັບຊຸມຊົນ.

ການສຶກສາໂຕທຳອິດຂອງບົດນິພົນນີ້ແມ່ນ ການເຮັດວິດຖ່າຍ (latrine intervention), ເຊິ່ງເຮັດໃນເດືອນມີນາ ປີ 2011 ເຖິງ ເດືອນມັງກອນ ປີ 2013 ຢູ່ໃນສາມດອນ ຄື: ດອນໂລງ, ດອນຖານ ແລະ ດອນລຽງ ເຊິ່ງຕັ້ງຢູ່ຕາມລຳແມ່ນ້ຳຂອງ ຂຶ້ນກັບເມືອງໂຂງ, ແຂວງຈຳປາສັກ, ພາກໃຕ້ຂອງລາວ. ດ້ວຍການໃຊ້ວິທີການທົດລອງແບບກ່ອນແລະຫຼັງ (experimental pre-test and post-test) ຮ່ວມກັບມີໜຶ່ງກຸ່ມຄວບຄຸມ ເພື່ອໃຊ້ປະເມີນຜົນທີ່ໄດ້ຮັບຂອງການໃຫ້ວິດຖ່າຍແກ່ປະຊາຊົນໃນໝູ່ບ້ານ ໃນການປ້ອງກັນການຕິດເຊື້ອໜອນກາຝາກ ໂດຍສະເພາະແມ່ນເຊື້ອໃບໄມ້ໃນຕັບ *O. viverrini* ແລະ ເຊື້ອໃບໄມ້ໃນເລືອດ *S. mekongi*. ການສົ່ງເສີມໃຫ້ມີການສ້າງວິດຖ່າຍ ໃນລະດັບຄົວເຮືອນໄດ້ຖືກດຳເນີນການ. ມີການເກັບຕົວຢ່າງອາຈົມສອງຄັ້ງຕໍ່ຜູ້ຮ່ວມການຄົ້ນຄວ້າຜູ້ໜຶ່ງ ພາຍໃນໄລຍະເວລາຫ້າມື້ຕິດຕໍ່ກັນ. ຕົວຢ່າງອາຈົມຖືກກວດດ້ວຍວິທີ Kato-Katz (KK) thick smears ແລະ Baermann technique. ການຄົ້ນຄວ້ານີ້ປະກອບມີ 3 ໄລຍະ ເຊິ່ງໄດ້ກ່າວໄວ້ແລ້ວໃນພາກທີ 7.

ການສຶກສາເບື້ອງຕົ້ນຂອງ ecohealth intervention ສຳຫຼັບ *O. viverrini*, *S. mekongi* ແລະ STH ໄດ້ດຳເນີນການໃນເດືອນຕຸລາ ປີ 2011 ແລະ ເດືອນສິງຫາ ປີ 2012 ຢູ່ດອນຄອນ ແລະ ດອນໂສມ. ສະມາຊິກຄົວເຮືອນທີ່ມີອາຍຸແຕ່ 2 ປີຂຶ້ນໄປ ແລະ ສັດທີ່ເປັນຜູ້ຮັບເກັບພະຍາດ (animal reservoir hosts) ຄື: ໝາ, ແມວ, ໝູ ແລະ ຄວາຍ ຂອງຄົວເຮືອນທີ່ຖືກເລືອກ ແມ່ນໄດ້ຮັບການກວດເພື່ອຊອກຫາການຕິດເຊື້ອໜອນກາຝາກ. ສຳຫຼັບເຊື້ອໃບໄມ້ໃນຕັບ *O. viverrini*, ຫອຍໃນຕະກູນ genus *Bithynia* spp. ໄດ້ຖືກເກັບດ້ວຍວິທີຊ້ອນເອົາຈາກແຫຼ່ງນ້ຳໃກ້ໝູ່ບ້ານ ເຊັ່ນ: ໜອງ, ຮ່ອງຕະຄອງ ແລະຕາມທົ່ງນາ. ປາ *Cyprinoid* ກໍ່ໄດ້ຖືກຈັບຈາກແຫຼ່ງດຽວກັນ ແລະ ເອົາມາຈາກນ້ຳຂອງນຳອີກ ດ້ວຍວິທີໃຊ້ແຫ່ຫາປາ. ສຳຫຼັບ *S. mekongi*, ຫອຍ *N. aperta* ໄດ້ຖືກເກັບດ້ວຍມືຕາມໂງ່ນຫີນຂອງແມ່ນ້ຳຂອງ. ວິທີ KK thick smears, formalin ether concentration technique (FECT), shedding test and the pepsin enzyme digestion technique ໄດ້ຖືກນຳມາໃຊ້ກວດສຳຫຼັບຄົນ, ສັດຜູ້ຮັບເກັບພະຍາດ, ຫອຍ *Bithynia* spp. ແລະ *N. aperta* ແລະ ປາ *Cyprinoid*, ຕາມລຳດັບ ເພື່ອປະເມີນການຕິດເຊື້ອ.

ການປະເມີນວິທີການບິ່ງມະຕິແບບໃໝ່ສຳຫຼັບການຕິດເຊື້ອໃບໄມ້ໃນເລືອດ *S. mekongi* ໄດ້ຖືກດຳເນີນການໃນລະຫວ່າງເດືອນກຸມພາ ແລະ ເມສາ ປີ 2016 ໃນໝູ່ບ້ານເຂດລະບາດຂອງເຊື້ອໃບໄມ້ໃນເລືອດ ຂອງປະເທດລາວ ແລະ ກຳປູເຈຍ. ຕົວຢ່າງຢ່ຽວ ແລະ ເລືອດ ໄດ້ເອົາມາຈາກຜູ້ເຂົ້າຮ່ວມ ເພື່ອມາກວດຊອກຫາການຕິດເຊື້ອໃບໄມ້ໃນເລືອດ ດ້ວຍວິທີທົດສອບແບບ POC-CCA, UCP-LF CAA ແລະ ELISA assays. ພວກເຮົາເກັບຕົວຢ່າງອາຈົມ ສາມຄັ້ງພາຍໃນຫ້າມື້ຕິດຕໍ່ກັນຈາກຜູ້ເຂົ້າຮ່ວມການສຶກສາ. ຕົວຢ່າງອາຈົມໄດ້ຖືກກວດສອງແຜ່ນສະໄລດ໌ ຕໍ່ອາຈົມແຕ່ລະຄັ້ງ (duplicate KK thick smears examined).

ການຄົ້ນພົບທີ່ສຳຄັນ/ຜົນທີ່ໄດ້ຮັບ: ລາຍງານທຳອິດຂອງພວກເຮົາແມ່ນ ມາຈາກການຄົ້ນຄວ້າໄລຍະທີໜຶ່ງຂອງການສຶກສາການໃຫ້ວິດຖ່າຍ (latrine study) ໂດຍເນັ້ນໃສ່ດ້ານລະບາດວິທະຍາຂອງເຊື້ອໂຕກົມອ່ຽນ *S. stercoralis* ຢູ່ໃນເກາະດອນຂອງແມ່ນ້ຳຂອງ, ເຊິ່ງພົບວ່າມີການຕິດເຊື້ອເຖິງ 41.0%. ອັດຕາການຕິດເຊື້ອບໍ່ມີຄວາມແຕກຕ່າງລະຫວ່າງເກາະດອນ (ດອນໂລງ 44.1% vs. ດອນຖານ/ດອນລຽງ 38.2%, p=0.107). ການຕິດເຊື້ອທີ່ສູງກວ່າໝູ່ແມ່ນ *O. viverrini* (72.2%), ຕາມມາດ້ວຍ hookworm (56.1%) ແລະ *S. mekongi* (12.8%). *Trichuris trichiura* (3.3%), *Ascaris lumbricoides* (0.3%) ແລະ *Taenia* spp. (0.3%). ປັດໃຈສ່ຽງທີ່ສຳຄັນໃນການຕິດເຊື້ອໂຕກົມອ່ຽນແມ່ນເພດ. ພົບວ່າຜູ້ຊາຍມີຄວາມສ່ຽງສູງກວ່າຜູ້ຍິງທີ່ຈະຕິດເຊື້ອໂຕກົມອ່ຽນ *S. stercoralis* (adjusted OR 1.97, 95% CI 1.45–2.67).

ຜົນການສຶກສາເບື້ອງຕົ້ນຂອງການຄົ້ນຄວ້າ ecohealth intervention ສະແດງໃຫ້ເຫັນວ່າ ການຕິດເຊື້ອໃນຄົນຂອງເຊື້ອ *O. viverrini,* hookworm, *S. mekongi, T. trichiura*, *A. lumbricoides* ແລະ *Taenia* spp. ແມ່ນ 60.7%, 44.1%, 22.2%, 4.1%, 0.6% ແລະ 0.1%, ຕາມລຳດັບ. ອັດຕາການຕິດເຊື້ອໃນລະດັບທີ່ຮຸນແຮງມີ 4.2%, 3.6% ແລະ 1.8% ສຳຫຼັບ *O. viverrini*, *S. mekongi* ແລະ hookworm, ຕາມລຳດັບ. ອັດຕາການຕິດເຊື້ອ *O. viverrini* ຢູ່ໃນໝາ ແລະ ແມວ ແມ່ນ 25.0% ແລະ 53.1%, ຕາມລຳດັບ. ອັດຕາການຕິດເຊື້ອ *S. mekongi* ຢູ່ໃນໝາແມ່ນ 14.7%. ອັດຕາການຕິດເຊື້ອ *O. viverrini* ແລະ *S. mekongi* ຢູ່ໃນຫອຍແມ່ນ 0.3% ແລະ 0.01%, ຕາມລຳດັບ. ອັດຕາການຕິດເຊື້ອລວມທັງໝົດຂອງ *O. viverrini* ຢູ່ໃນປາແມ່ນ 26.9%, ເຊິ່ງມີອັດຕາການຕິດເຊື້ອສູງກວ່າໝູ່ຢູ່ໃນປາສູດ *(Hampala dispa)* (87.1%), ປາດອກງົ້ວ *(Cyclocheilichthys apogon)* (85.7%) ແລະ ປາຂາວມ້ວ *(Puntius brevis)* (40.0%). ການກິກໜັງສື ແລະ ລະດັບເສດຖະກິດຕ່ຳ ແມ່ນເພີ່ມຄວາມສ່ຽງໃນການຕິດເຊື້ອ *O. viverrini*, ແລະ

ກຸ່ມອາຍຸແຕ່ 10–16 ປີ ແລະ ຜູ້ທີ່ມີວິດຖ່າຍໃນເຮືອນຈະເຮັດໃຫ້ມີອັດຕາການຕິດເຊື້ອຕ່ຳ. ຄົວເຮືອນທີ່ມີໝາ ແລະແມວທີ່ກິນປາດິບມີຄວາມສຳພັນຢ່າງມີໃນສຳຄັນກັບການຕິດເຊື້ອ *O. viverrini*. ສຳຫຼັບ *S. mekongi*, ກຸ່ມເດັກນ້ອຍທີ່ມີອາຍຸລຸ່ມ 9 ປີ ແມ່ນມີໂອກາດສຳພັດກັບເຊື້ອກາຝາກຢ່າງມີໃນສຳຄັນ ເມື່ອສົມທຽບໃສ່ກຸ່ມອາຍຸທີ່ສູງກວ່າ.

ການຄົ້ນຄວ້າການສົມທຽບລະຫວ່າງເຄື່ອງມືການບ່ຽງມະຕິແບບໃໝ່ ແລະ ແບບມາດຕະຖານ ໃນການກວດຫາການຕິດເຊື້ອກາຝາກໃບໄມ້ໃນເລືອດ *S. mekongi* ໃນປະເທດລາວ ແລະ ກຳປູເຈຍ ເຊິ່ງໄດ້ດຳເນີນການໃນລະຫວ່າງເດືອນກຸມພາ ແລະ ເດືອນ ເມສາ ປີ 2016. ຜົນການກວດອາຈົມດ້ວຍວິທີ KK thick smear ສະແດງໃຫ້ເຫັນວ່າ ອັດຕາການຕິດເຊື້ອລວມຂອງ *S. mekongi* ແມ່ນ 6.4% (ມີ 1 ກໍລະນີຢູ່ກຳປູເຈຍ ແລະ 23 ກໍລະນີຢູ່ ສປປ ລາວ), ສ່ວນການຕິດເຊື້ອ *O. viverrini*, hookworm, *T. trichiura*, *A. lumbricoides* and *Taenia* spp. ແມ່ນ 50.4%, 28.1%, 3.5%, 0.3% ແລະ 1.9%, ຕາມລຳດັບ. ໃນທັງໝົດ 377 ຂອງຕົວຢ່າງຍ່ຽວ ແລະ ເລືອດ ໄດ້ຖືກກວດເພື່ອຊອກຫາການຕິດເຊື້ອ *S. mekongi*. ຢູ່ໃນຕົວຢ່າງນ້ຳຍ່ຽວ, ການກວດແບບ CCA ແລະ CAA ກວດພົບການຕິດເຊື້ອ *S. mekongi* 21.0% ແລະ 38.7%, ຕາມລຳດັບ. ຢູ່ໃນຕົວຢ່າງເລືອດ, ການກວດແບບ CAA assay ສະແດງໃຫ້ເຫັນອັດຕາການຕິດເຊື້ອແມ່ນ 32.4%, ໃນຂະນະທີ່ຜົນກວດລວມຈາກຕົວຢ່າງເລືອດ ແລະ ຍ່ຽວ ຂອງ CAA (combination of the CAA assay) ສະແດງໃຫ້ເຫັນອັດຕາການຕິດເຊື້ອ *S. mekongi* ແມ່ນ 43.2%. ແລະມີຄວາມແຕກຕ່າງກັນລະຫວ່າງສອງສະຖານທີ່ການສຶກສາ ຄືຢູ່ ສປປ ລາວ ມີອັດຕາການຕິດເຊື້ອໃບໄມ້ໃນເລືອດສູງກວ່າ.

ການຄົ້ນຄວ້າເພື່ອປະເມີນຜົນຂອງການໃຫ້ວິດຖ່າຍແກ່ປະຊາຊົນໃນໝູ່ບ້ານ ເພື່ອປ້ອງກັນການຕິດເຊື້ອໜອນກາຝາກ ໂດຍສະເພາະແມ່ນ *O. viverrini* ແລະ *S. mekongi* ເຊິ່ງດຳເນີນການດ້ວຍວິທີ ທົດສອບກ່ອນ ແລະ ຫຼັງ ຮ່ວມກັບໜຶ່ງກຸ່ມບ້ານຄວບຄຸມ. ໃນບ້ານທົດລອງ (intervention villages) ການຕິດເຊື້ອໜອນກາຝາກ ໃນການສຶກສາເບື້ອງຕົ້ນ (at baseline) ສຳຫຼັບ *S. mekongi*, *O. viverrini*, hookworm, *A. lumbricoides*, *T. trichiura*, *Enterobius vermicularis*, *S. stercoralis* ແລະ *Taenia* spp. ແມ່ນ 28.6%, 79.5%, 48.8%, 0.3%, 3.5%, 0%, 43.1% ແລະ 0%, ຕາມລຳດັບ. ສ່ວນຜົນການຕິດເຊື້ອໜອນກາຝາກໃນການສຶກສາໄລຍະຕິດຕາມກວດຄືນ (at follow-up) ເຊິ່ງມັນໄດ້ຫຼຸດລົງ 22.6%, 68.2%, 26.2%, 2.5%, 1.1%, 0.4%, 31.1% ແລະ 0.4%, ຕາມລຳດັບ. ການຫຼຸດລົງຂອງ 4 ເຊື້ອໜອນກາຝາກໂຕສຳຄັນແມ່ນສາມາດສັງເກດ

ເຫັນໄດ້ຢ່າງມີໃນສຳຄັນດັ່ງນີ້ (baseline vs follow-up, *P-value*), *S. mekongi* (28.6% vs 22.6%, *P-value*<0.001), *O. viverrini* (79.5% vs 68.2%, *P-value*<0.001), Hookworm (48.8% vs 26.2%, *P-value*<0.001) ແລະ *S. stercoralis* (43.1% vs 31.1%, *P-value*<0.001) ຢູ່ໃນໝູ່ບ້ານທີ່ເຮັດການທົດລອງ. ໃນຂະນະທີ່ການຕິດເຊື້ອ *S. mekongi* ໃນບ້ານຄວບຄຸມແມ່ນເພີ່ມສູງຂຶ້ນ (1.8% vs 2.6%, *P-value*=0.74) ແລະ ການຕິດເຊື້ອກາຝາກ ໂຕອື່ນໆແມ່ນຫຼຸດລົງ *O. viverrini* (71.8% vs 59.9%, *P-value*=0.027), Hookworm (65.6% vs 38.3%, *P-value*<0.001) ແລະ *S. stercoralis* (38.3% vs 34.8%, *P-value*=0.001).

ສະຫຼຸບຜົນການຄົ້ນຄວ້າ/ຄວາມສຳຄັນ: *O. viverrini, S. mekongi*, ແລະ STH ໂດຍສະເພາະແມ່ນ *S. stercoralis* ຍັງຄົງມີອັດຕາການຕິດເຊື້ອທີ່ສູງ ຢູ່ໃນເກາະດອນຂອງແມ່ນ້ຳຂອງ ພ້ອມທັງການຕິດເຊື້ອແບບພ້ອມກັນຫຼາຍຊະນິດ (multiparasitism) ແມ່ນຍັງພົບເຫັນໄດ້ໃນທຸກໆການຄົ້ນຄວ້າຂອງບົດນິພົນປຣິນຍາເອກສະບັບນີ້. ມັນມີຄວາມຈຳເປັນທີ່ຈະຕ້ອງມີການອອກແບບ ແລະ ດຳເນີນການສຶກສາແບບແຊກແຊງແບບເຊື່ອມສານໃນການຄວບຄຸມເຊື້ອໜອນກາຝາກ ຢູ່ໃນເກາະດອນຂອງແມ່ນ້ຳຂອງ ທາງພາກໃຕ້ຂອງ ສປປ ລາວ. ວິທີການຄວບຄຸມແບບເຊື່ອມສານທີ່ເໝາະສົມ ທີ່ມີເປົ້າໝາຍໃສ່ໃນການແຊກແຊງພຶດຕິກຳຂອງຄົນ, ສັດທີ່ເປັນຜູ້ຮັບເກັບພະຍາດ (animal reservoirs), ແລະ ການດັດແປງສະພາບແວດລ້ອມ, ສຸຂະສຶກສາ ແລະ ປັບປຸງໃຫ້ມີການເຂົ້າເຖິງແຫຼ່ງນ້ຳສະອາດ ແລະ ການສຸຂະພິບານ (sanitation) ອາດຈະສາມາດເພີ່ມປະສິດທິຜົນຂອງການເຮັດແຊກແຊງ ແລະອາດນຳໄປສູ່ການກຳຈັດການຕິດເຊື້ອໄດ້. ອີກຢ່າງ, ເຄື່ອງມືໃນການບົ່ງມະຕິໃໝ່ CCA, CAA ແລະ ELISA ໄດ້ຖືກປະເມີນ ແລະສະແດງໃຫ້ວ່າອັດຕາການຕິດເຊື້ອ *S. mekongi* ແມ່ນສູງ ເມື່ອສົມທຽບໃສ່ການກວດແບບ Kato-Katz thick smears. ດັ່ງນັ້ນກໍລະນີຄົນເຈັບພະຍາດຫອຍເມືອງໂຂງ ຫຼື ພະຍາດໃບໄມ້ໃນເລືອດທີ່ຕິດເຊື້ອ ໃນປະເທດ ລາວ ແລະ ກຳປູເຈຍແມ່ນຖືກປະເມີນຕ່ຳກວ່າຄວາມເປັນຈິງໃນການສຶກສາທີ່ຜ່ານມາ. ຈຶ່ງເຮັດໃຫ້ຄວາມພະຍາຍາມໃນການຄວບຄຸມແບບຢືນຢົງແມ່ນຈຳເປັນ ເພື່ອຢຸດການສົ່ງເຊື້ອກາຝາກ *S. mekongi*. ດັ່ງນັ້ນ ບົດບາດຂອງວິທີການບົ່ງມະຕິທີ່ໃຫ້ຄວາມແມ່ນຢຳສູງ ຕໍ່ເປົ້າໝາຍຂອງການກຳຈັດເຊື້ອແມ່ນບໍ່ສາມາດເບິ່ງຂ້າມໄດ້.

1. Introduction

1.1. Multiparasitic infections in Lao PDR

In Lao PDR, opisthorchiasis and schistosomiasis are major public health importance for helminthiasis which may lead to be fatal hepatobiliary diseases such as cholangiocarcinoma (CCA) and liver cirrhosis due to chronic opisthorchiasis and schistosomiasis, respectively (Aye Soukhathammavong et al., 2015; Sayasone et al., 2012; Sripa et al., 2011b; Sripa et al., 2009; Urbani et al., 2002). Moreover, other helminthic infections are also prevalent and coexisted with *O. viverrini* infection such as soil-transmitted helminthiasis (STH) and other food-borne trematodes, particularly several species of minute intestinal flukes (MIF) (families Heterophyidae and Lecithodendriidae) which *Haplorchis taichui* was the predominant species (Chai et al., 2013; Chai et al., 2007; Chai et al., 2009; Chai et al., 2005b; Laymanivong et al., 2014; Rim et al., 2003; Sayasone et al., 2011; Sayasone et al., 2009a; Vonghachack et al., 2015). Furthermore, *S. mekongi* has been recognised as a coexisted infection with *O. viverrini* in a restricted area only in the islands of Mekong river in Khong district and Mounlapamok district, Champasack province (Sayasone et al., 2011; Sayasone et al., 2012; Vonghachack et al., 2015).

1.2. Sanitation, behaviour and related issues for helminthic infections in Lao PDR

Lacking sanitation and water supply are related to the transmission of infectious diseases, including parasitic infections such as food-borne trematodiasis, schistosomiasis and STH (Gelaye et al., 2014; Pruss-Ustun et al., 2014; Strunz et al., 2014). In rural parts of Lao PDR, sanitation coverage is very low. Overall the coverage of improved sanitation is less than 50% with a large difference between urban and rural area. The sanitation coverage is more than 80% in urban centres and 40% in rural areas of the country (UNICEF, 2009). In our setting areas, Khong district compounds of a large number of islands in the Mekong River where the intermediate hosts (snail and fish) of two important parasites (*O. viverrini* and *S. mekongi*) are abundant therefore poor sanitation by open defecation practices may support the transmission between infected parasite human waste and their intermediate hosts. In 2010, our study villages of the Khong district approximately less than 40% of the households had latrines availability. There is a real need to increase access to adequate sanitation and eco-health intervention such as human and animal host assessment for

parasitic infections with combination of sanitation improving/animal control and health education as the approaches for sustainable parasitic diseases control in this country.

Regarding to Lao people's behaviour of open defecation in the environment which is rampant and the cause for parasitic transmission as well as raw food eating behaviour as a deep root cultural food consumption in many places of the country, therefore multiparasitism is highly prevalent in the country, particularly in the southern part of the country such as Khong district, Champasack province, where all above mentioned groups of parasitic infections are highly endemic (Aye Soukhathammavong et al., 2015; Forrer et al., 2012; Sayasone et al., 2011; Sayasone et al., 2012; Vonghachack et al., 2015).
Moreover, developing and validating of new techniques for parasitic diagnosis are also required and challenge in this country.

1.3. *Opisthorchis viverrini* and its infection

O. viverrini is food-borne trematode and prevalent in Southeast Asia including Cambodia, Lao PDR, Thailand and Vietnam (Andrews et al., 2008; Keiser and Utzinger, 2009; Sripa et al., 2010). The global estimate for the number of people infected with *O. viverrini* is that 10 million people, while approximately 2 million and 8 million people infected in Lao PDR and Thailand, respectively (Keiser and Utzinger, 2009; Sripa et al., 2010).

O. viverrini is the parasite of dog and cat. Human is an accident definitive host whereas dog and cat serve as reservoir host of the parasite (Harinasuta and Harinasuta, 1984; Upatham and Viyanant, 2003; Wykoff et al., 1965). Although dog and cat are the important reservoir hosts, another fish eating mammals are the definitive host as well. Human and animal acquire infection by eating raw/undercooked fish or raw/insufficient cooked pickled fish containing metacercariae cysts (Harinasuta and Harinasuta, 1984; Keiser and Utzinger, 2005, 2009). Aquatic snails act as first intermediate hosts which are *Bithynia* spp. whereas, freshwater fish of the family of *Cyprinidae* acts as the second intermediate host (Chanawong and Waikagul, 1991; Harinasuta and Harinasuta, 1984).

The life cycle of *O. viverrini* is complex and has several hosts for developing into adult worms (Figure 1). In brief, *O. viverrini* adult worms live in the biliary system of definitive hosts. Then embryonated eggs pass out in faeces. On reaching water the eggs are eaten by

aquatic snails, the first intermediate host (*Bithynia* spp.). In the snail the miracidia hatch and develop further through the stages of sporocysts, rediae and cercariae in six to eight weeks. The cercariae then leave the snail, penetrate into susceptible freshwater fish (family of *Cyprinidae*), encyst in the muscle and develop into metacercariae and become an infective stage within in six weeks. As mention above human and mammal animal are infected by eating raw, undercooked fish harboring metacercariae and after ingestion the metacercariae excyst in the duodenum or jejunum and then migrate to the bile duct. The parasites become mature within four weeks and begin to produce eggs. The life span of the fluke is over 25 years in human (Harinasuta and Harinasuta, 1984; Kaewkes, 2003; Keiser and Utzinger, 2009; Sripa et al., 2010).

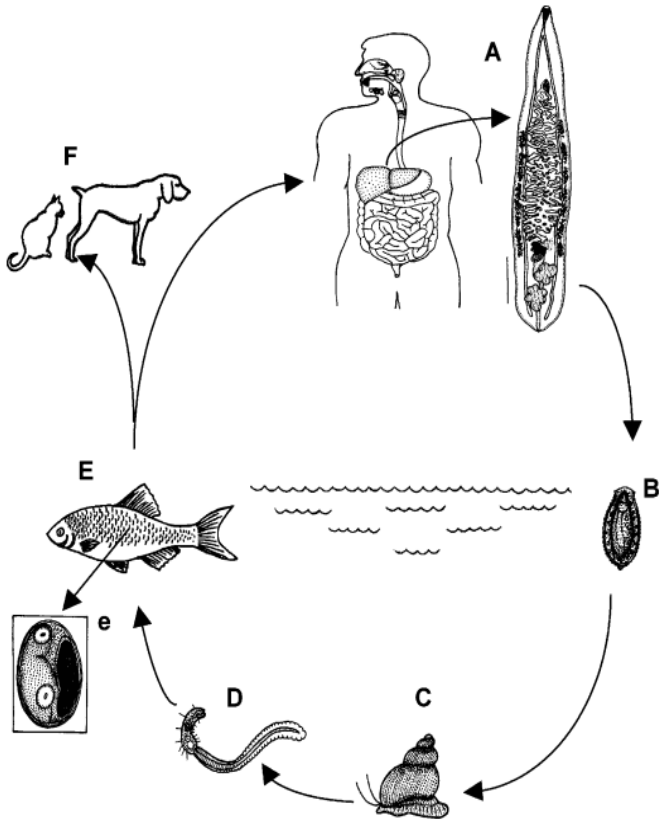

Figure 1.1. Life cycles of liver flukes (*Opisthorchis viverrini*) (Kaewkes, 2003)
(A) adult worm in bile duct; (B) embryonated egg; (C) first intermediate host; *Bithynia* snail;
(D) cercaria; (E) second intermediate host (*cyprinoid* fish) and metacercaria (e);
(F) reservoir host, dog and cat.

Infection with *O. viverrini* is often asymptomatic, particularly light intensity of the infection. Symptomatic cases of opisthorchiasis generally experience pain in the right upper quadrant, diarrhoea, loss of appetite, indigestion and fullness, whereas 5–10% of infected individuals had weakness or malaise, flatulence or dyspepsia and abdominal pain, and 5% had hepatomegaly (Sripa et al., 2010). Severe cases may present with weakness, lassitude, weight loss, ascites and oedema. Complications may include cholangitis, obstructive jaundice, intra-abdominal mass, cholecystitis and gallbladder or intrahepatic stones. The severity of disease is associated with both intensity and duration of infection. Moreover, many studies showed that *O. viverrini* infection is associated with Cholangiocarcinoma (Keiser and Utzinger, 2009; Sripa et al., 2011b; Sripa et al., 2010; Sripa and Pairojkul, 2008).

1.4. Epidemiology of *Opisthorchis viverrini* infection in Lao PDR

O. viverrini infection is high prevalent and major public health problem in Lao PDR which distributed through the central to the south of the country. It is mainly distributed in the south of the country (Chai et al., 2005b; Forrer et al., 2012; Giboda et al., 1991; Lovis et al., 2009; Rim et al., 2003; Sayasone et al., 2007; Sayasone et al., 2009b; Sripa et al., 2011b; Sripa et al., 2010). It estimated that over 2 million people are infected with *O. viverrini* in Lao PDR (Keiser and Utzinger, 2009; Sripa et al., 2010). Rim and colleagues showed 10.9% of *O. viverrini* infection in the nation large-scale survey on parasite infections among Lao schoolchildren, varied by region with higher prevalent in lower central and southern part of the country (Rim et al., 2003). Nevertheless, the prevalence of *O. viverrini* infection may be underestimate or overestimate the real infections among the population duo to single Kato-Katz thick smear examination and the technique was not possible to discriminate minute intestinal fluke eggs from *O. viverrini* eggs (Lovis et al., 2009; Rim et al., 2003). Furthermore, the *O. viverrini* prevalence in general population shown 43.7% and the provinces in southern part had the highest *O. viverrini* infection rate than the north (Yoon et al., 2014). Recent studies among southern population revealed *O. viverrini* infection rate was more than 80% (Forrer et al., 2012; Phongluxa et al., 2013; Sayasone et al., 2011; Sayasone et al., 2015a; Soukhathammavong et al., 2011; Yoon et al., 2014).

Consuming of raw foodstuff such as raw meat, fish and vegetables are very common practice among Lao population. Particularly, the consumption of raw or insufficiently cooked fish was reported 75.1% in Saravan Province (Sayasone et al., 2007). Several traditional dishes prepare from freshwater fish which are the sources of liver fluke infection (Phongluxa et al., 2013; Xayaseng et al., 2013). Therefore, main source of infection for *O. viverrini* is freshwater fish. This habit is deeply culturally rooted, and widespread in the Laotian population. Moreover, increasing of age has been found linkage to high prevalent and intensity of *O. viverrini* infection (Sayasone et al., 2007; Sithithaworn et al., 2006; Strandgaard et al., 2008). Cholangiocarcinoma is also expected due to *O. viverrini* infection in Lao PDR as occurred in neighbouring country Thailand, but the high quality data is scarce and needed further in-depth clinical and epidemiological research (Keiser and Utzinger, 2009; Sripa et al., 2011b; Sripa and Pairojkul, 2008).

1.5. Diagnosis of *Opisthorchis viverrini*

Today, there are three main approaches for diagnosis of food-borne trematodiasis such as direct parasitological diagnosis, immunodiagnosis and molecular diagnostic approach (Keiser and Utzinger, 2009). However, direct parasitological examinations for detecting of eggs in faeces, bile or duodenal fluids are the gold standard diagnosis for *O. viverrini* and other trematodes infection, with these widely used methods include the Kato-Katz thick smear, Stoll's dilution and the quantitative FECT (Keiser and Utzinger, 2009; Sripa et al., 2010). Among them, Kato-Katz thick smear is frequently and applicably used in the field and large-scale studies as its simplicity, rapidity and inexpensiveness, while FECT is laboratory-based technique which requires more reagents and particularly centrifuge machine.

Although Kato-Katz thick smear is applicable in the field and large-scale investigations, its low sensitivity in light infection is considered to increase the number of slide reading or multiple stool samples of the same person (Sayasone et al., 2015b), whereas FECT and PCR have been shown more sensitivity in light infection better than Kato-Katz thick smear (Hong et al., 2003; Keiser and Utzinger, 2009; Lovis et al., 2009).

1.6. Other trematode infections

1.6.1. Food-borne trematode infections

Food-borne trematodes have been described approximately 6,000 digenean species, including liver fluke, intestinal fluke and lung fluke, but only a few are medical important. Human and animal acquire infected when ingesting raw, pickled, or undercooked aquatic product harbouring metacercariae or when drinking contaminated water. In brief, the life cycle of common five food-borne trematodes including intestinal flukes (*Echinostoma hortense, Fasciolopsis buski,* and *Heterophyes heterophyes*), a liver fluke (*Clonorchis sinensis*), and a lung fluke (*Paragonimus westermani*) shows in figure 2 (Keiser and Utzinger, 2009). Globally estimation for the number of people infected with *C. sinensis* is 35 million, almost half of whom (15 million) are Chinese. More than 20 million people are infected with *Paragonimus* spp. Estimates for *Fasciola* spp. infections range between 2.4 million and 17 million. Approximately 1.2 million people are infected with *O. felineus*. The estimated 40 to 50 million people are infected with one or several species of intestinal flukes (Keiser and Utzinger, 2007, 2009; Lun et al., 2005).

In Southeast Asia, *O. viverrini, C. sinensis, Fasciola* spp., *Paragonimus* spp. and intestinal flukes are considered to be medical important food-borne trematodes (Sripa et al., 2010). In Lao PDR, the trematodes of the families Heterophyidae and Lecithodendriidae often coexist with *O. viverrini* infection in one patient which the most common of mixed infections was *H. taichui* (Chai et al., 2013; Chai et al., 2005b; Lovis et al., 2009; Sato et al., 2010; Sayasone et al., 2007; Sayasone et al., 2009b).

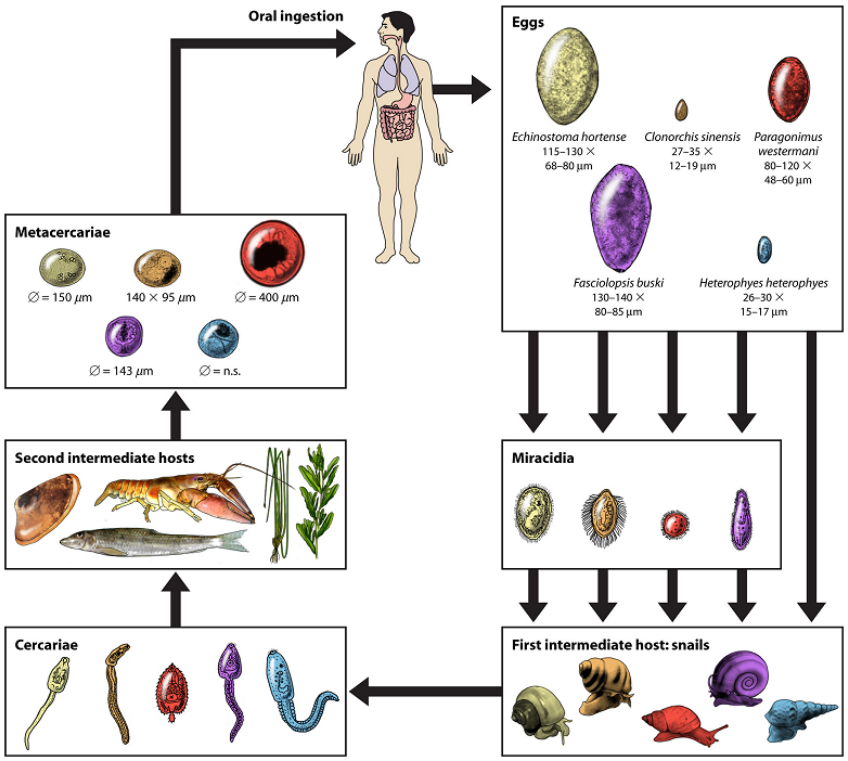

Figure 1.2. Life cycles of five different food-borne trematodes: *Echinostoma hortense*, *Fasciolopsis buski*, *and Heterophyes heterophyes*, *Clonorchis sinensis*, and *Paragonimus westermani* (Keiser and Utzinger, 2009)

1.6.2. Water-borne trematode infections

Water-borne trematodes, including blood fluke, liver fluke and intestinal fluke which less common than food-borne trematodiasis. The most important parasite of this group is family Schistosomatidae. Five species of schistosomes are important agents of disease, which is called schistosomosis or bilhaziosis in human. These are *Schistosoma haematobium, S. intercalatum, S. japonicum, S. mansoni and S. mekongi*. Two of these, blood fluke *S. japonicum and S. mekongi* are endemic in Asia such as China, Indonesia and the Philippines for *S. japonicum* and Cambodia, Lao PDR and Thailand for *S. mekongi* (Muth et al., 2010; Zhou et al., 2010).

Human serves as one of the definitive hosts of schistosomes and be infected by penetration of schistosome cercariae. Cercariae had emerged from the snail which serves as first intermediate host, then swim in the water where they can survive for about 24 hours. On contact with wet skin of the definitive host, the cercariae cast off their tails then penetrate

the skin. The parasite travel around the body via the circulation carried through the heart to the lungs of the definitive host. After a few days of essential development in the lungs, the schistosomula return to the heart and go to the liver. Within 5-6 weeks, the worms develop to full maturity, mate and migrate to the mesenteric veins of the small or large intestine and the veins of the vesicle plexus where the females start laying eggs (Cheever et al., 1994). Schistosomes have no second intermediate host. Several animals are found to serve as the reservoir host of *S. japonicum*, e.g. dog, cat, cattle, buffaloes, sheep, goat, horse, pig, mice and rate. Infection in human and animal may persist indefinitely, and as long as 47 years has been recorded in one instance (Miyazaki, 1991).

For *S. mekongi*, reservoir hosts can be found in dog and pig (Lorette et al., 1983; Strandgaard et al., 2001) while buffalo is suspected as potential host but its involvement has not yet to be proven (Muth et al., 2010). The snail vector was found to be *Neotricula aperta* including alpha, beta and gamma race strain, the aquatic snails living in the Mekong River and its tributaries (Davis et al., 1976; Kitikoon and Schneider, 1976; Temcharoen, 1976). Whereas, only *Gamma N. aperta* has been reported being infected with *S. mekongi* in the nature (Kitikoon et al., 1973). The most technique has been used to diagnose *S. mekongi* infection is Kato-Katz method which has low sensitivity, particularly a single stool examination as well as when infection intensities are low (Bergquist et al., 2009a; Urbani et al., 2002). Therefore, repeated Kato-Katz stool examination and the development/validation of new techniques should be considered (Muth et al., 2010).

Novel, highly sensitive tests for schistosomiasis include the up-converting phosphor *(*UCP*)* lateral flow (LF) test for the schistosome circulating cathodic antigen (CCA) and the corresponding anodic antigen (CAA). Both tests work for *S. mansoni* and *S. haematobium*, infections (Stothard et al., 2014) and very recently shown to be effective for indicating infection also by *S. japonicum* and *S. mekongi* (van Dam et al. 2015a; 2015b). Sensitivity is not only about 7 times better than Kato-Katz, but these tests can detect circulating antigens from all schistosome species in the urine. However, these CCA and CAA test had been validated again in the endemic areas.

1.7. Soil-transmitted helminthiasis

Soil-transmitted helminthiasis (STH) means that eggs or larvae of helminthes develop and become infective stage after a period of incubation in soil (Setasuban, 1986). Human acquires the infection of STH by ingestion of eggs containing infective larvae or penetration of infective larvae stage into the skin regarding to their species. The four important species of STH consist of *Ascaris lumbricoides (A. lumbricoides), Trichuris trichiura (T. trichiura),* hookworm and *Strongyloides stercoralis (S. stercoralis)* which was found worldwide (Bethony et al., 2006; Keiser and Utzinger, 2008). STH is a public health and economic impact in the developing countries, particularly in the tropical and subtropical regions (Chan et al., 1994).

Mode of transmission of *A. lumbricoides* and *T. trichiura* is similar. Their route of infection is orally. Transmission occurs by ingestion the fully embryonated eggs from soil that contaminate the hands, fingernails or fresh vegetables. Unembryonated eggs shed by worms with the faeces require about 1-2 weeks developing into infective eggs in the soil (Bethony et al., 2006; De Silva et al., 2003). On ingestion, the infective eggs of *A. lumbricoides* pass through the stomach, and then hatch out leaving larvae in the small intestine. They burrow into the wall of intestine and enter into the circulation via the heart, the lungs where they remain in the alveoli for several days during which they grow and molt twice. They are then carried up to the trachea; pass over the epiglottis, down along the esophagus to the stomach and small intestine where they molt for the fourth and finally then develop into adults. Whereas, the infective eggs of *T. trichiura* are digested in the small intestine, and larvae emerge via the polar plugs. They temporarily enter the nearby crypts of the intestine, and then migrate to the superficial luminal epithelium, later passing down to the cecum and undergo molting, developing into adult worms around in the three months (Bethony et al., 2006; Garcia, 2007; WHO, 2002b). Hookworm and *S. stercoralis*, human is infected by penetration of their infective larvae from contaminated soil which called filariform larvae. Then, the larvae reach the intestinal habitat after a migration that includes the bloodstream, lungs, trachea and oesophagus. Whereas, *S. stercoralis* differs from other intestinal helminths in the larvae already hatch in the intestinal lumen where infective stages can develop, enabling autoinfection and, therewith, indefinite persistence of infection (Altintop et al., 2010).

1.8. The animal hosts of *Opisthorchis viverrini* and other helminthes

The animal hosts of *O. viverrini* comprise of aquatic snails, freshwater fish and fish eating mammals which act as first intermediate, second intermediate and definitive hosts, respectively. The first intermediate host of *O. viverrini*, *Bithynia* spp. includes *Bithynia (Digoniostoma) siamensis goniomphalos, Bithynia siamensis funiculata* and *Bithynia siamensis siamensis*. Within them, *Bithynia funiculata* was highly susceptible to *O. viverrini* than others (Chanawong and Waikagul, 1991; Harinasuta and Harinasuta, 1984; Keiser and Utzinger, 2009; Wykoff et al., 1965). *Bithynia siamensis funiculata, B. siamensis goniomphalos* and *B. siamensis siamensis* is distributed in northern, northeast and central Thailand, respectively (Upatham and Viyanant, 2003; Wykoff et al., 1965). In Thailand, the prevalence of *O. viverrini* cercariae in *Bithynia* snail was extremely low which approximately 0.083–1.6% (Kaewkes, 2003). While, there is very scarce data pertaining *Bithynia* snail infection with *O. viverrini* cercariae in Lao PDR. However, Ditrict and colleague reported that 0.09% of *B. siamensis goniomphalus* were infected by cercariae of *O. viverrini* in Nam Ngum water reservoir where situates in the central of Lao PDR (Ditrich et al., 1992; Ditrich et al., 1990). The second intermediate host of *O. viverrini* is well known that freshwater fish in the family *Cyprinidae* are the major second intermediate hosts of *Opisthorchis* spp. and *C. sinensis*. (Kaewkes, 2003; WHO, 1995). In Lao PDR, cyprinoid fish have been reported as the second intermediate host of *O. viverrini* through the country (Ditrich et al., 1990; Manivong et al., 2009; Rim et al., 2008a; Sayasone et al., 2007). The high prevalence of *O. viverrini* metacercariae infection among cyprinoid fish was increased during dry season (November to April) in Namdone, Nampakane and Mekong river, Khammouane Province (Manivong et al., 2009). Dog and cat are known as reservoir hosts of *O. viverrini*, but a little known of their prevalence in Lao PDR. However, a study from the neighboring country such as Thailand reported the prevalence of *O. viverrini* infection in cat and dog, 36.4% and 3.8%, respectively (Enes et al., 2010).

Only *Gamma N. aperta* has been recognised to be infected with *S. mekongi* in the nature with the prevalence of 0.3% (Kitikoon et al., 1973; Muth et al., 2010; Urbani et al., 2002). While dog and pig serve as the important reservoir hosts for transmission, but it is still lacking of epidemiological information of these animal reservoir hosts.

1.9. Control approaches for FBT and other helminth infections in Lao PDR

From our knowledge, many factors have been involved the endemicity of opisthorchiasis and other helminthiasis comprising sanitation, consumption behaviour, education, environment and poverty. Control of human liver fluke infection can be facilitated by treatment of human and animal reservoirs for reducing the excretion of eggs, improved sanitation for preventing eggs from reaching water sources and health promotion including information, education and communication (IEC) to discourage consumption of raw fish and to improve sanitary practices (Jongsuksuntigul and Imsomboon, 2003; Keiser and Utzinger, 2009; Sripa et al., 2010).

Almost Food and water borne trematodes involve intermediate host pathway but elimination of an intermediate host, such as snails, is difficult to achieve, since they are widespread and a part of the environment. Molluscicides probably also kill fish who share the environment (Sripa et al., 2010). Although, a 10-year control program in southern Laos had been have mass treatment and health education, only moderately reduced the prevalence of *O. viverrini* infection. The limited success of the program was related with lack of public awareness about the disease combined with inadequate sanitation and high infection risk (Phongluxa et al., 2013; Sripa et al., 2010; Strandgaard et al., 2008) whereas control of STH involves sanitation improving in the communities. Given the sanitation facilities and their proper utilization play a key-role in the increase and maintenance of adequate hygienic conditions in a community therefore, environmental sanitation can be carried out successfully by providing latrines and health education combine with MDA in the endemic areas for both Food and water borne trematodes and STH (Sripa et al., 2010). Moreover, school health control programs for STH were reported their success in many countries of Southeast Asia included Lao PDR (Kobayashi et al., 2005) which targeting to young generation for changing their behaviour on good hygiene and defecation practice.

1.10. Identified research needs

Today, the prevalence of opisthorchiasis viverrini is still high among Lao population (Forrer et al., 2012; Lovis et al., 2009; Phongluxa et al., 2015; Rim et al., 2003; Sayasone et al., 2007; Sayasone et al., 2012). *O. viverrini* infection is considered to be not only a medical and public health problem but also an economic impediment to the country. Although, a

10-year control program in southern Laos had been have mass treatment and health education, only moderately reduced prevalence of *O. viverrini* infection (WHO, 2011). The limited success of the programme was related with lack of public awareness about the disease combined with inadequate sanitation and high infection risk due to poverty (Phongluxa et al., 2013; Sripa et al., 2010; Strandgaard et al., 2008). Therefore, sanitation facilities are important role to increase and maintenance of adequate hygienic conditions in the communities, particularly in rural communities of Lao PDR. This parasite is responsible for the development of a fatal liver cancer (cholangiocarcinoma) (Sripa et al., 2011b). Furthermore, Mekong schistosomiasis is endemic in our setting areas (*S. mekongi*) leading to intestinal and liver diseases. Although treatment is available re-infection rates are high as the main transmission route through open defecation is not altered. It is high public health importance to explore how appropriate sanitation can be installed in these settings. These experiences may trigger the promotion of similar approaches in other settings of Lao PDR.

In addition, other eco-health control approaches are needed to define and assess the proper intervention for sustainable controls of opisthorchiasis viverrini and other important helminthiasis in Lao PDR, consisting of chemotherapy, sanitation improvement, and health education. Measurement of infection in their hosts comprising intermediate, definitive, reservoir host is really important to success the parasitic controls which refer to define and assess the environmental contamination among the animal hosts (Keiser and Utzinger, 2009; Sripa et al., 2010; WHO, 1995).

Schistosomiasis, the WHO Roadmap for elimination of neglected tropical diseases (NTDs) and the WHO Regional Action Plan for NTDs in the Western Pacific Region for 2012-2016 have targeted these areas for elimination of schistosomiasis as a public health problem by 2016 (WHO, 2012a). Because of the preventive chemotherapy programmes implemented in endemic areas in Cambodia and Lao PDR make individuals harbouring mainly light-intensity infections likely to be missed by the standard Kato-Katz diagnostic test used so far (Zhu et al., 2014) resulting in imprecise assessment of the impact of preventive chemotherapy and other interventions. Therefore, highly sensitive tests for schistosomiasis (*S. mekongi*) UCP-LF CCA and CAA are necessary to validate as a step for this success in Lao PDR.

This PhD thesis would like to maximize the number of control approach studies purposing to develop and define the appropriate tools against food-borne trematodiasis, particularly *O. viverrini* infection in Lao PDR and other important helminthiasis as its co-infections such as schistosomiasis mekongi.

2. Goal and Objectives

2.1. The goals

The goals of the recent PhD thesis are to develop and define the control approaches of food-borne trematodiasis, *Opisthorchis viverrini* infection in Lao PDR; and other important helminthiasis, particularly in *S. mekongi* and *S. stercoralis* as its co-infections.

2.2. The specific objectives

- To assess *S. stercoralis* infection and the risk of infection among the populations on three islands in Khong district, Champasack province, Southern Laos.
- To define *O. viverrini*, *S. mekongi* and STH infections in humans, in the ecological environment of Khong district, Champasack province where their potential animal reservoir, and intermediate hosts are living in close connectivity.
- To compare the diagnostic tools for detection of *Schistosoma mekongi* infection in Lao People's Democratic Republic and neighbouring country Cambodia.
- To assess the impact of improved sanitation and its use on the transmission of intestinal helminth infections in highly endemic areas, three islands in Khong district, Champasack province, Southern Laos.

3. Approach and Methodology

The PhD thesis was conducted within the frame of the existing and productive parasitic research partnership between the Swiss Tropical and Public Health Institute (Swiss TPH), Basel, Switzerland and The National Institute of Public Health (NIOPH), Vientiane Capital, Lao PDR. It is composed of four studies such as (i) assessment of *S. stercoralis* infection and its risk among the populations on three islands in Khong district, Champasack province; (ii) define *O. viverrini*, *S. mekongi* and STH infections in humans, in the ecological environment of Khong district, Champasack province where their potential animal reservoir, and intermediate hosts are living in close connectivity; (iii) comparison the diagnostic tools for detection of *S. mekongi* infection in Lao People's Democratic Republic and Cambodia; and (iv) assessment the impact of improved sanitation and its use on the transmission of intestinal helminth infections in highly endemic areas, three islands in Khong district. Each study is individual explained which gives information on the study area, study subjects, approach and methods used as following.

3.1. Assessment of *S. stercoralis* infection and the risk of infection

This study was obtained from the baseline survey in phase one of the latrine intervention programme. We aimed to assess the *S. stercoralis* infection and the risk of infection among the populations on three islands in Khong district, Champasack province, Southern Laos. We conducted a cross-sectional study on three islands in Khong district.

3.1.1. Study population

The study was conducted in March 2011 on three islands, i.e. Donlong, Donthan and Donlieng island are located in Mekong River in Khong district, Champasack province, southern Laos. The study islands represent typical islands of the Khong districts. The studied villages were selected based on the Provincial Health Office report as a very low proportion of households with latrines. Twenty to thirty households were chosen from the households list of the head of the village, using a simple random sampling procedure. All household members aged 2 years or older were invited to participate in the study.

3.1.2. Field procedures and laboratory examinations

A household and an individual questionnaire were administered. Two stool samples were collected per study participants within a five day period. Each sample was examined by using Kato-Katz thick smears technique (Katz et al., 1972a) and Baermann technique (Garcia and Bruckner, 2001). The stool samples were stored at ambient temperature and transferred to the laboratory of the Khong district Hospital within 2–3h post-collection where they were further processed. Kato-Katz and Baermann tests are described in detail elsewhere (Khieu et al., 2013a; Sayasone et al., 2011) (Garcia and Burckner, 2001). For Baermann test, the centrifuged sediment was examined under a microscope for the present of *S. stercoralis* larvae (L1-stage). A single Kato-Katz thick smear was prepared for each stool sample and examined within 1h of preparation. Helminth eggs were counted and recorded separately to obtain species-specific infection intensity estimates.

3.2. Define *O. viverrini*, *S. mekongi* and STH infections in humans, in the ecological environment of Khong district, Champasack province

We employed an eco-health approach to study *O. viverrini*, *S. mekongi* and STH infections in human and animal reservoir hosts as well as in the intermediate molluscs and fish hosts on Mekong islands of Southern Lao PDR where multiple helminth infections are highly prevalent and their hosts are living in close connectivity.

3.2.1. Study design, area and population

The study followed the logic of a cross-sectional study. It was carried out between October 2011 and August 2012 in two islands (Done Khon (Khon island) and Done Som (Som island)) of the Khong district, Champasack province, southern Laos. The islands are located in the Mekong River and are highly endemic for *S. mekongi, O. viverrini* and STH. In each selected village, about 30 households were randomly selected from household list available at village office. All members of the selected households aged ≥ 2 years and present on the survey day were invited to participate in the study. Animal reservoir hosts of the household, i.e., dogs, cats, pigs and buffaloes, from selected households were also enrolled and examined for helminth infections. In addition, intermediate hosts for *O. viverrini, Bithynia* spp. snails (1st intermediate host) and *Cyprinoid* fish (2nd intermediate host), and

for *S. mekongi, Neotricula aperta* were collected in selected sites of the study villages and examined for their infection.

3.2.2. Field procedures and laboratory examinations

In each village, a house, school or temple was identified as field study station. In each selected household, two questionnaires were administered to head of household and individual. Eligible study participants were invited to submit two stool samples over consecutive days to our research team for parasitological analysis. The first stool container was handed to the study participant on the registration day with detailed explanation on stool collection. The second empty container was handed out after study participants returned the first filled container. Two Kato-Katz thick smears (Katz et al., 1972a) were prepared from each stool sample and examined under light microscopes by an experienced technician within 1 hour after preparation. Eggs were counted and recorded for each helminth species separately. Smears were allowed to clear for 30 min after set-up.

We collected the faecal samples from potential domestic reservoir animals, i.e., cats, dogs, pigs and water buffaloes, if present. The rectal enema inducing method using Sodium Chloride (NaCl) solution and petroleum jelly lubricant was used for small animal (e.g., cats, dogs and pigs) to collect the fresh faecal samples (Enes et al., 2010). In water buffaloes, the faecal sample was collected by rectal swap. The collected faecal samples were immediately preserved in 10% of formalin and transported to the National Institute of Public Health (NIOPH) in Vientiane Capital for further examination using formalin ether concentration technique (FECT) (Ebrahim et al., 1997).

Bithynia spp. and *Neotricula aperta* snails were examined for the presence of cercariae using shedding test, previously described by Sri-Aroon and colleagues (Sri-Aroon et al., 2005; Sri-Aroon et al., 2007). Snails of the genus *Bithynia* spp. were collected by the scooping method (Kitikoon et al., 1981) from canals, natural stream and ponds and swampy areas, in which *Cyprinoid* fish were collected. *N. aperta* (Davis et al., 1976) were collected from the rocky banks of the Mekong River where water was frequently used by villagers for their daily purposes. Submerged stones were dredged and snails were hand-picked from them (Kitikoon et al., 1981).

Cyprinoid freshwater fish were collected from natural ponds, streams, rice fields, and irrigation cannels surrounding the study villages. For each fish, the length and weight were measured and recorded. Species identification was done using a guideline available at FishBase's website . Fish digestion was performed using pepsin enzyme digestion technique (WHO, 1995). The residue was examined for the presence of *O. viverrini* metacercariae. The metacercariae were counted and recorded for each infected fish.

3.3. Comparison the diagnostic tools for detection of *S. mekongi* infection in Lao People's Democratic Republic and Cambodia

The UCP-LF CCA and CAA techniques were validated to assess *S. mekongi* infection in the villages of *S. mekongi*-endemic villages in Lao PDR and Cambodia. We compared a set of available assays to get a handle on the real prevalence and intensity of infection in the areas in Cambodia and Lao PDR.

3.3.1. Study design, area and population

A cross-sectional study was conducted between February and April 2016 in *S. mekongi*-endemic villages in Lao PDR and Cambodia. Four villages, two in each of the endemic districts of Lao PDR and Cambodia, respectively, were selected. The villages Som VenOok and Ban Yai VeunSom in Khong district, Champasack province in southern Lao PDR were selected together with the villages Kbal Chuor and Sre Khoeun in Kratié province in northern Cambodia. All household members older than 6 years were enrolled. They were asked to fill in a questionnaire pertaining to demographic details and risk factors for infection, information on hygiene, disease knowledge and anthelminthic drugs taken during the latest 6 months. In Lao PDR, about 200 individuals living in Som VenOok and Ban Yai VeunSom situated on islands in the Mekong River were approached about the study. The study households were randomly selected from a list of households of the two villages. In Cambodia, according to the 2008 census, the total population was 2,339 people (1,602 in Kbal Chuor and 737 in Sre Khouen). Between 120 and 130 individuals were randomly selected from 30 to 35 households in each study village.

3.3.2. Field procedures and laboratory examinations

Repeated stool examination for intestinal helminth infections and liver flukes were conducted and examined by triplicate Kato-Katz thick smears examined under light microscope (Katz et al., 1972b). Furthermore, from each study participant urine and serum samples were obtained to be tested for *Schistosoma* infection by POC-CCA, UCP-LF CAA and ELISA. Serum and urine samples were transferred to one place for local collection in each country wherefrom they were eventually shipped to speciality laboratories at Swiss Tropical and Public Health Institute (Swiss TPH) in Basel, Switzerland and Leiden University Medical Center (LUMC) in The Netherlands.

3.3.3. Detection of *S. mekongi* antibodies and circulating schistosome antigens

Schistosoma serology was performed by ELISA at Swiss TPH using *S. mansoni* adult worm extract (AWE) and *S. mansoni* soluble egg antigen (SEA) (Ampah et al., 2016; Nickel et al., 2015).

Detecting of circulating schistosome antigens, UCP-LF CAA assay for serum and urine was carried out at LUMC. The POC-CCA test devices were obtained from Rapid Medical Diagnostics (Pretoria, South Africa) and tests were performed according to the manufacturer's description. The amount of urine analysed per strip was 30 µL applied by pipetting, rather than one droplet. Test results were visually interpreted, including distinction of trace-signals (Corstjens et al., 2014; van Dam et al., 2015b).

3.4. Assessment the impact of improved sanitation and its use on the transmission of intestinal helminth infections in highly endemic areas, three islands in Khong district, Champasack province, Southern Laos

PAMS project was carried out in Khong District, Champasack Province, Lao PDR purposing to improve sanitation by providing latrines in selected four villages of Donlong Island (intervention villages), Khong District. Other villages on neighbouring islands were served as control. We aimed to assess the impact of improved sanitation and its use on the transmission of *S. mekongi*, *O. viverrini* and other intestinal helminth infections in highly endemic areas of three islands in Khong district, Champasack province, Southern Laos.

3.4.1. Study design, area and population

We conducted an intervention study in three islands, i.e. Donlong, Donthan and Donlieng Island located in the Mekong River in Khong district, Champasack province, southern Laos where after a base-line assessment of helminth infection and a mass-drug administration (MDA) with antihelminthic drug latrines were constructed. Donlong island composes of four villages, namely Haulong, Longsong, Longkang and Hanglong village which were the intervention village group whereas Donthan and Donlieng islands compose of one village each namely Donthan and Donlieng village, respectively which were the control village group. Details of village population were described elsewhere (Vonghachack et al., 2015).

An experimental pre-test and post-test with one control group was used to assess the effects of latrine in the study villages on preventing of helminth infections particularly *O. viverrini* and *S. mekongi*. Household-based promoting latrine construction was conducted. There were three different phases of the study as described below and illustrated in Figure 4.1.

First, a cross-sectional baseline survey was carried out in March 2011 to assess intestinal parasitic infections, and people's knowledge, attitudes, perception and behaviour (KAPB) about latrine, personal hygiene and raw food consumption in both intervention and control villages. After the cross-sectional study, all inhabitants aged 4 years and above from the intervention and control villages were offered treatment as mass drug administration approach (MDA), with praziquantel (single 40 mg/kg oral dose) and albendazole (single 400 mg oral dose) (MoH., 2004). It was called first MDA.

Second, in the intervention villages each household committed to construct a latrine. Within a period of 9 months more than 300 were constructed. The project subsidized the lining of the pit and the slab while the rest of the construction was conducted by the household members. After the latrine construction, all individuals living in both settings (control and intervention areas) received another full MDA, with praziquantel and albendazole. Those infected with *S. stercoralis* were treated with a single 200µg/kg dose of ivermectin in this phase (Satoh and Kokaze, 2004; Suputtamongkol et al., 2011).

Finally, a follow-up survey was carried out 12 months later after the second MDA, using the identical survey methodology as at base-line.

3.4.2. Field and laboratory procedures

Within the baseline and follow up surveys, parasitological methods were used the same process. Two stool samples were collected per study participants within a five day period. Each sample was examined by using Kato-Katz thick smears technique (Katz et al., 1972a) and Baermann technique. Kato-Katz and Baermann tests are described in detail elsewhere (Khieu et al., 2013; Sayasone et al., 2011) (Garcia and Bruckner, 2001). Helminth eggs were counted and recorded for each species separately to obtain species-specific infection intensity estimates.

3.4.3. Risk factors assessment by questionnaire

The questionnaire was used to collect information at the individual level, e.g. personal perception and behaviour about latrines and their construction, the use of toilets in daily life, and personal hygiene. Raw and insufficiently cooked food consumption behaviour (fish, pork, beef, and vegetable) and daily life activities were also investigated. The questions on socio-economic status (SES) were assessed at the household level. Head of the family who could be either husband or wife were asked to provide the information on household assets including electric devices, engines (motorcycle, truck, engine boat,…), agricultural land and livestock ownership, construction material of house and latrines.

4. Epidemiology of *Strongyloides stercoralis* on Mekong islands in southern Laos

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Keywords: *Strongyloides stercoralis*, Epidemiology, Baermann technique, Mekong island Laos

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Abstract

Strongyloides stercoralis is a neglected helminth infection potentially that can lead to systemic infection in immunocompromised individuals. In Lao People's Democratic Republic (Lao PDR, Laos), information on *S. stercoralis* infection is scarce. We assessed *S. stercoralis* infection and associated risk factors and symptoms on the Mekong islands in Southern Laos. Baermann and Kato-Katz techniques were performed on two stool samples from each individual to detect *S. stercoralis* larvae and concomitant helminth infections. Among 729 individuals, 41.0% were infected with *S. stercoralis*. Men were at higher risk than women (OR 1.97, 95% CI 1.45-2.67). Urticaria and body itching was associated with *S. stercoralis* infection (OR 2.4, 95% CI 1.42-4.05). Infection with *Opisthorchis viverrini* (72.2%), *Schistosoma mekongi* (12.8%), and hookworm (56.1%) were very common. Few infections with *Trichuris trichiura* (3.3%), *Ascaris lumbricoides* (0.3%) and *Taenia* spp. (0.3%) were detected. The majority of helminth infections were of light intensity, with prevalences of 80.4%, 92.9%, 64.5%, 100% and 100%, for *O. viverrini*, hookworm, *S. mekongi*, *T. trichiura* and *A. lumbricoides*, respectively. Nevertheless, heavy infection intensities were observed for *O. viverrini* (1.0%), *S. mekongi* (14.0%) and hookworm (2.9%). *S. stercoralis* is highly endemic on the islands of Khong district, Champasack province, Southern Laos. The national helminth control programme should take action to control this helminth infection.

4.1. Introduction

Strongyloides stercoralis is one of the most neglected soil-transmitted helminthiases (STH) (Olsen et al., 2009). It is transmitted through unprotected contact with soil and is endemic in tropical and temperate regions (Schär et al., 2013). Today, an estimated 30–100 million people are infected worldwide (Bethony et al., 2006).

The life cycle of *S. stercoralis* is complex. Humans acquire the infection through direct skin contact with infective third stage larvae (L3). Chronic infection occurs through repeated endogenous autoinfection that may last for several decades (Beckeret al.,2013). Infection among immunocompromised patients may lead to hyperinfection syndrome and may be fatal if not treated adequately (Becker et al., 2013; Siddiqui and Berk, 2001).

In Lao Peoples' Democratic Republic (Lao PDR, Laos,) information on *S. stercoralis* infection is scarce. The diagnostic techniques used in the country, i.e. direct smears and the Kato-Katz technique (Katz et al., 1972) have a very low sensitivity (Requena-Mendez et al., 2013). Therefore, hospital laboratory diagnosis might miss *S. stercoralis* infection, leading to underestimated prevalence rates. In 1996, the prevalence of *S. stercoralis* in Laos was estimated at 19% in Thakek and Hinboun district, Khammouane province, in the middle of the country by using agars plate culture method (Vannachone et al.,1998). In many parts of the country, e.g. on the Mekong islands in Champasack province, or Saravane province, water supply and sanitation facilities are absent (Sayasone et al., 2007). The rural populations' life style and farming activities favour transmission (e.g. intense skin contact with soil). Other helminthiasis such as STH, food-borne trematodiasis (FBT) and schistosomiasis mekongi are highly prevalent (Forrer et al., 2012; Rim et al., 2003; Sayasone et al., 2007, 2011).

We aimed to assess *S. stercoralis* infection and the risk of infection among the populations on the Mekong islands of Khong district, where other helminthiases have been reported previously. We conducted a cross-sectional study on three islands in Khong district, Champasack province, in Southern Laos.

4.2. Materials and methods

4.2.1. Ethics statement

The study was approved by the Lao National Ethics Committee for Health Research (NECHR), Ministry of Health, Laos. All procedures were explained to provincial, district and village authorities and their approval was obtained. Study participants were informed on study procedures, benefits and risks of the study as well as their rights to withdraw at any time. Before enrolment written informed consent was obtained from all study participants and parents or legal guardians of children below the age of 15 years. In addition a written assent was obtained from children and adolescent (<18 years). Participants were informed about the examinations. All infections diagnosed were treated according to the Lao national treatment guidelines (MOH, 2004). Those infected with *S. stercoralis* were treated with a single 200mg/kg dose of ivermectin free of charge (Satoh and Kokaze, 2004; Suputtamongkol et al.,2011).

4.2.2. Study area and population

The study was conducted in March 2011 on three islands, i.e. Donlong, Donthan and Donlieng island located in the Mekong River in Khong district, Champasack province, southern Laos. Donlong island composes of four villages, namely Haulong, Longsong, Longkang and Hanglong village whereas Donthan and Donlieng islands compose of one village each namely Donthan and Donlieng village, respectively. Donlong, Donthan and Donlieng have a population of approximately 2174 (Haulong: 567; Longsong: 543; Longkang: 510 and Hanglong: 554), 586 and 137 inhabitants, respectively. The main occupation of villagers in these three islands is rice subsistence farming, vegetable plantation, and fishing activities in the Mekong. Additionally, in Donlong a considerable number of farmers cultivate tobacco.

The study islands represent typical islands of the Khong districts. In the study villages the Provincial Health Office reported very low proportion of households with latrines. Twenty to thirty households were chosen from the households list of the head of the village, using a simple random sampling procedure. All household members aged 2 years or older were invited to participate in the study.

4.2.3. Field procedures and laboratory examinations

A household and an individual questionnaire were administered. The household questionnaire was addressed to the head of household. The following information was collected: having and using latrine at home, wearing shoes (slippers), and socioeconomic conditions by using household assets including electric devices, engines, agricultural land and livestock owner, etc.. The individual questionnaire collected information on demographic data, hygiene behaviour, history of illness including urticaria (skin itching), and consumption of antihelminthic drugs in the past two weeks.

Two stool samples were collected per study participants within a five day period. Each sample was examined by using Kato-Katz thick smears technique (Katz et al., 1972) and Baermann technique (Garcia and Burckner, 2001). Pre-labeled plastic 30ml stool containers (ID numbers, name, age and date of stool collection) were handed out to each participant. They were asked to provide a full container of stool. Each morning, filled

containers were collected and replaced with empty ones for stool collection on the following day. The stool samples were stored at ambient temperature and transferred to the laboratory of the Khong district Hospital within 2–3h post-collection where they were further processed. Kato-Katz and Baermann tests are described in detail elsewhere (Khieu et al., 2013a; Sayasone et al., 2011). In brief, approximately 5g of each stool sample was divided from each stool sample for performing Baermann test (Garcia and Burckner, 2001). The stool sample was placed on a gauze-lined mesh in a glass funnel equipped with a rubber tube and a clamp, and covered with de-chlorinated tap-water. After 2h, the water (approx. 50ml) was centrifuged and the sediment was examined under a microscope for S. stercoralis larvae (L1-stage). A single Kato-Katz thick smear (Katz et al., 1972) was prepared for each stool sample and examined within 1h of preparation. Helminth eggs were counted and recorded separately to obtain species-specific infection intensity estimates.

4.2.4. Data management and analysis

Questionnaire and stool data were double entered in EpiData version 3.1 (EpiData Association; Odense, Denmark) and validated. Statistical analyses were performed in STATA version 10 (StataCorp.; College Station, USA). Only participants with complete questionnaire and stool examination were analyzed. The intensity of helminth egg counts was expressed as eggs per gram of stool (EPG) obtained from Kato-Katz examination. Intensity of helminthic infections was classified as light, moderate and heavy infection (Sayasone et al., 2009; Upatham et al., 1984; WHO, 2002). An univariate logistic regression analysis was carried out to associate potential risk factors with S. stercoralis infection status for which matched odds ratio (OR) and its 95% confidence interval (CI) and p-value were calculated. The variables with $p<0.2$ in the univariate analysis were included in the multivariate logistic regression analysis. Socioeconomic status (SES) conditions in the household were calculated according to an asset-based method such as electric devices, engines, agricultural land and livestock owner, indicator data were defined by principal component analysis (PCA). SES conditions in the household were categorized into five wealth quintiles (i) most poor; (ii) very poor; (iii) poor; (iv) less poor; and (v) least poor according to their cumulative standardized asset scores. Details of this widely used approach have been presented elsewhere (Sayasone et al., 2011). A “smoothed” age

prevalence curve was used to present the infection prevalence by mean age and sex of participants.

4.3. Results

4.3.1. Study population

In total, 729 individuals had complete data records (Fig. 4.1). They originated from 247 households on the three islands: 347 (47.6%) and 382 (52.4%) individuals from Donlong and Donthan/Donlieng islands, respectively; 45.7% (333) were male; all were ethnic Laoloum. Age ranged from 2 to 95 years with a median age of 30.6 years. Among the participants, illiterate, and primary and secondary school graduate were 7.0%, 60.8% and 29.6%, respectively (Table 4.1). Only 2.6% had a technical/university level training. They lived in Donthan and Donlieng villages. The main occupation of the villagers was farming (61.9%) such as rice, tobacco, and vegetable farming while only a few were government employees (2.5%). The socio-economic status on Donlong was significantly lower than on the other two islands (p=0.032).

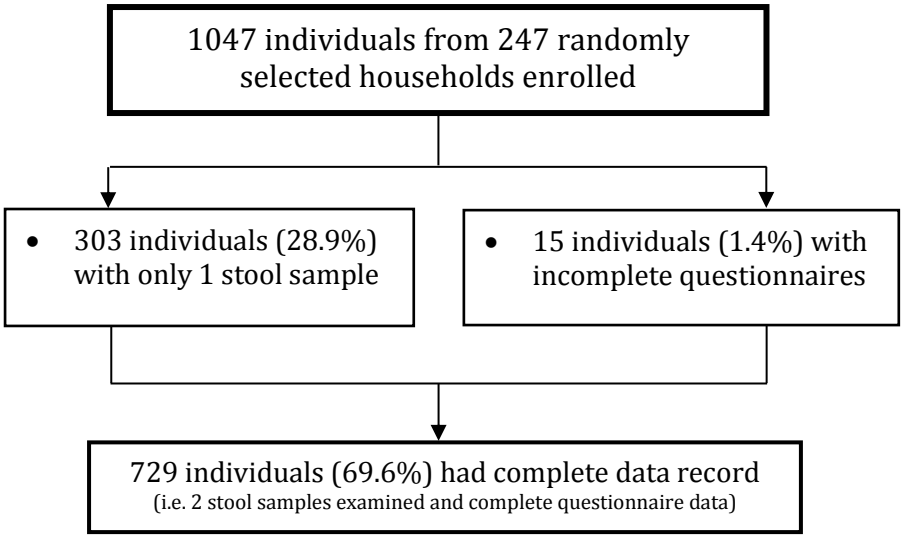

Figure 4.1: Study diagram

Table 4.1: Demographic characteristic of the participants

Characteristic	Overall	Locality		x^2	*p*-value
	n (%)	Donlong n (%)	Donthan/Donlieng n (%)		
N	729	347 (47.6)	382 (52.4)		
Age (years)					
Mean (range)	30.6 (2-95)	28.1 (2-81)	32.8 (2-95)		
Sex					
Female	396 (54.3)	187 (53.9)	209 (54.7)		
Male	333 (45.7)	160 (46.1)	173 (45.3)	0.049	0.824
Educational level					
Illiterate	51 (7.0)	27 (7.8)	24 (6.3)		
Primary school	443 (60.8)	231 (66.6)	212 (55.5)		
High school	216 (29.6)	89 (25.7)	127 (33.3)		
Technical school/University	19 (2.6)	0	19 (5.0)	25.053	<0.001
Occupation					
Farmer	451 (61.9)	216 (62.3)	235 (61.5)		
Student	212 (29.1)	101 (29.1)	111 (29.0)		
Child	48 (6.6)	29 (8.4)	19 (5.0)		
Government employee	18 (2.5)	1 (0.3)	17 (4.5)	15.934	0.001
Socio-economic status					
Most poor	146 (20.0)	55 (15.9)	91 (23.8)		
Very poor	147 (20.2)	69 (19.9)	78 (20.4)		
Poor	145 (19.8)	82 (23.6)	63 (16.5)		
Less poor	149 (20.4)	73 (21.0)	76 (19.9)		
Least poor	142 (19.5)	68 (19.6)	74 (19.4)	10.575	0.032

4.3.2. *Strongyloides stercoralis* infection and co-infections

The overall *S. stercoralis* infection prevalence was 41.0% (Table 4.2). The infection rate did not differ between the islands (Donlong 44.1% vs. Donthan/Donlieng 38.2%, p=0.107). The highest infection rate was observed with *O. viverrini* (72.2%), followed by hookworm (56.1%) and *S. mekongi* (12.8%). *T. trichiura* (3.3%), *A. lumbricoides* (0.3%) and *Taenia* spp. (0.3%). Infection prevalence of *O. viverrini* (76.1% vs. 68.6%, p=0.024) and *S. mekongi* (25.6% vs. 1.0%, p<0.001) was significantly higher on Donlong than on the other two islands. In contrast, hookworm infection prevalence was significantly higher on Donthan/Donlieng islands (63.9% vs. 47.6%, p<0.001).

Among the 729 individuals, only 11.1% were free of helminth infection. In 65.3% of the study participants two or more helminth infections were diagnosed. Multiple helminth

infections were significantly less frequent on Donthan and Donlieng than on Donlong (p=0.001, Table 4.2).

Table 4.2: Prevalence of helminth infections among villagers in the islands of Khong district, Champasack province (n=729)

Infections	Overall n=729 (%)	Donlong n=347 (%)	Donthan/Donlieng n =382 (%)	x^2	*p*-value
Nematodes					
Strongyloides stercoralis	299 (41.0)	153 (44.1)	146 (38.2)	2.59	0.107
Ascaris lumbricoides	2 (0.3)	0	2 (0.5)	1.82	0.177
Trichuris trichiura	24 (3.3)	12 (3.5)	12 (3.1)	0.05	0.811
Hookworm	409 (56.1)	165 (47.6)	244 (63.9)	19.67	< 0.001
Trematodes					
Opisthorchis viverrini	526 (72.2)	264 (76.1)	262 (68.6)	5.08	0.024
Schistosoma mekongi	93 (12.8)	89 (25.6)	4 (1.0)	98.87	< 0.001
Cestodes					
Taenia spp.	2 (0.3)	1 (0.3)	1 (0.3)	0.004	0.946
Multiparasitism					
Non infection.	81 (11.1)	31 (8.9)	50 (13.1)		
Single infection	172 (23.6)	84 (24.2)	88 (23.0)		
Double infection	276 (37.9)	121 (34.9)	155 (40.6)		
Triple infection	169 (23.2)	86 (24.8)	83 (21.7)		
Quartile infection.	31 (4.3)	25 (7.2)	6 (1.6)	18.8	0.001

The infection intensity of the diagnosed intestinal parasitic infections is given in Table 4.3. Most infections were of light intensity, e.g. 80.4% of *O. viverrini* infections. However, heavy infection intensities were found in patients with *S. mekongi* (14.0%), hookworm (2.9%) and *O. viverrini* (1.0%) infections.

Table 4.3: Intensity of helminth infections among villagers in the islands of Khong district, Champasack province (n=729)

Parasites	**Light** n (%)	**Moderate** n (%)	**Heavy** n (%)
Trematodes			
Opisthorchis viverrini	423 (80.4)	98 (18.6)	5 (1.0)
Schistosoma mekongi	60 (64.5)	20 (21.5)	13 (14.0)
Nematodes			
Ascaris lumbricoides	2 (100)	0	0
Trichuris trichiura	24 (100)	0	0
Hookworm	380 (92.9)	17 (4.2)	12 (2.9)

4.3.3. Risk factors associated with *S. stercoralis* infection

The results of the risk analyses for a *S. stercoralis* infection are presented in Table 4.4. The most important risk factor was sex. Male study participants had a significantly higher risk for a *S. stercoralis* infection than female participants taking into account the age of the study participants (adjusted OR 1.97, 95% CI 1.45–2.67).

S. stercoralis infection was diagnosed in participants of all ages. Children of the age group ≤5 years had the lowest infection prevalence (33.3%). However, in none of the older age groups the infection risk increased significantly. Interestingly, the age infection prevalence was distinctly different between male and female study participants (Fig. 4.2). In male participants, the infection prevalence reached peaked at 60% in the age group between 20 and 30 years, and remained at around 50% in the older age groups. In female participants the infection reached a plateau of 38% in 10 year olds, remained constant up to 40 years and dropped thereafter.

Table 4.4: Association among *Strongyloides stercoralis* infection and risk factors in the islands of Khong district, Champasack province

Characteristics	Positive, n (%)	Crude OR (95% CI)	*p*-value	Adjusted OR (95%, CI)	*p*-value
Age group (years)					
≤ 5	17 (33.3)	1.00			
6-15	82 (40.8)	1.37 (0.72-2.63)			
16-25	44 (43.6)	1.54 (0.76-3.11)			
26-35	44 (45.4)	1.66 (0.81-3.36)			
36-45	39 (43.3)	1.52 (0.74-3.12)			
≥ 46	73 (38.6)	1.25 (0.65-2.41)	0.708	na	na
Sex					
Female	134 (33.8)	1.00		1.00	
Male	165 (49.6)	1.92 (1.42-2.58)	<0.001	1.97 (1.45-2.67)	<0.001
Occupation					
Farmer	189 (41.9)	1.00			
Student	86 (40.6)	0.94 (0.67-1.31)			
Government employee	8 (44.4)	1.10 (0.42-2.86)			
Child	16 (33.3)	0.69 (0.36-1.29)	0.693	na	na
Educational level					
Illiterate	17 (33.3)	1.00			
Primary school	177 (40.0)	1.33 (0.72-2.46)			
High school	97 (44.9)	1.63 (0.86-3.09)			
Technical school, University	8 (42.1)	1.45 (0.49-4.29)	0.418	na	na
Having latrine at home					
No	194 (42.2)	1.00			
Yes	105 (39.0)	0.87 (0.64-1.19)	0.405	na	na
Habit of defecation					
Latrine	105 (39.5)	1.00			
Bush	156 (41.7)	1.09 (0.79-1.51)			
Rice field	38 (42.7)	1.14 (0.70-1.85)	0.802	na	na
Last defecation					
Latrine	107 (39.2)	1.00			
Bush	153 (41.8)	1.11 (0.8-1.53)			
Rice field	39 (43.3)	1.18 (0.73-1.92)	0.715	na	na
Wearing slippers (shoes)					
Yes	249 (41.8)	1.00			
No	50 (37.6)	1.19 (0.8-1.75)	0.375	na	na
Worked in rice field last year					
No	91 (38.2)	1.00			
Yes	208 (42.4)	1.18 (0.8-1.62)	0.288	na	na
Treated with antihelminth drugs in past 6 months					
No	264 (42.1)	1.00			
Yes	33 (34.0)	0.7 (0.45-1.11)			
Don't remember	2 (40.0)	0.91 (0.15-5.52)	0.314	na	na
Socio-economic status					
Most poor	69 (47.3)	1.00			
Very poor	52 (35.4)	0.61 (0.38-0.97)			
Poor	64 (44.1)	0.88 (0.55-1.39)			
Less poor	56 (37.6)	0.67 (0.42-1.06)			
Least poor	58 (40.9)	0.77 (0.48-1.22)	0.231	na	na
Study villages					
Donthan/Donlieng	146 (38.2)	1.00			
Donlong	153 (44.1)	1.27 (0.94-1.71)	0.107	na	na

na: not applicable

In our analyses, none of the socio-economic risk factors such as socio-economic status, occupation and level of education was associated with *S. stercoralis* infection. Hygiene behaviours such as wearing shoes (slippers), having and using a latrine and having been treated with antihelminthic drugs in the past six months were not significantly associated with *S. stercoralis* infection.

During interview, participants were asked to report symptoms from the last two weeks. Urticaria and/or body itching during the previous two weeks was the only reported symptom significantly associated with *S. stercoralis* infection. Having urticarial and/or experiencing itching was strongly associated with an *S. stercoralis* infection (adjusted OR 2.40, 95% CI 1.42–4.05, p=0.001).

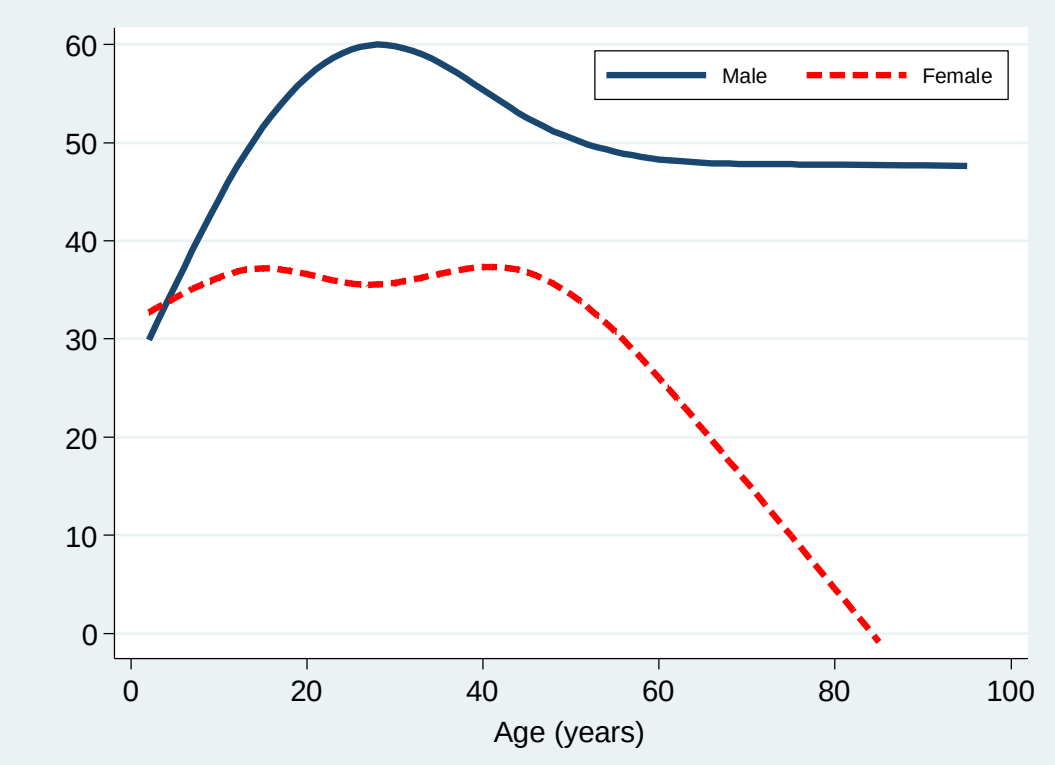

Figure 4.2: Age prevalence distribution by sex of *Strongyloides stercoralis* infection in villagers from Southern Laos

4.4. Discussion

S. stercoralis is one of the most neglected tropical diseases (Olsen et al., 2009). In resource poor countries with tropical climate, conditions are favourable for transmitting the parasite. Hence, *S. stercoralis* is most probably under reported in these settings (Schär et al., 2013). In Southeast Asia, a relative small number of studies document *S. stercoralis* infection. However, recent work in Cambodia reported very high infection rates of 25% in Kandal and Takeo provinces (Khieu et al., 2013a; Khieu et al., 2014b) and almost 50% in the most Northern Preah Vihear province (Khieu et al., 2014a). Furthermore, low socioeconomic status and low hygienic living conditions of the rural population were strongly associated with *S. stercoralis* infections.

Given the similar socioeconomic and environmental living conditions of the rural population in Laos, we aimed to document the level of *S. stercoralis* infection rates and risk factors in Southern Laos. We used a rigorous diagnostic approach conducting a Baermann test on two stool samples from each participant. We found a very high *S. stercoralis* infection prevalence of 41.0%. Of the risk factors examined only sex was significantly associated with *S. stercoralis*. Furthermore, reported urticaria (body itching) was significantly associated with the infection.

In Laos, only a very few studies of *S. stercoralis* have been conducted using an adequate diagnostic approach. Most data on *S. stercoralis* infection stem from studies examining other soil-transmitted helminthes and/or food-borne trematodes andreported prevalence rates below 20% (Paboriboune et al., 2014; Sayasone et al., 2011). Given the inadequate diagnostic techniques these studies used, their findings most like underestimate the true infection burden in the country. Therefore, more attention should be paid to *S. stercoralis* in Laos by incorporating sensitive diagnostic approaches in helminth surveillance activities.

In our study, we used the Baermann method on two stool samples per enrolled participant. The infection prevalence was comparable to recent reports from Cambodia (Khieu et al., 2013a, 2014a,b), but substantially higher than infection prevalences reported from neighbouring China (Steinmann et al., 2007, 2008) and Thailand (Jongsuksuntigul et al., 2003; Sithithaworn et al., 2003). Our diagnostic procedures could have been improved by examining more stool samples per person and by adding additional diagnostic techniques.

For example, in a study of Cambodian children three stool samples were examined per child with a combination of Baermann technique and Koga agar plate. Taking this approach as 'gold' standard, our examination of two samples with the Baermann technique results in a sensitivity of approximately 70%; in combination with the Koga agar plate method a 93% sensitivity could have been reached (Khieu et al., 2013a). However, the substantial material costs and time investments required to conduct the Koga-Agar plate culture must be taken into account when planning a field investigation. In our study, these factors did not allow for this method to be added to the diagnostic study procedures.

We identified gender as the most important risk factor in our study area. Boys and men had almost twice the risk for a *S. stercoralis* infection than did girls and women. This finding is in agreement with earlier reports from Cambodia (Khieu et al., 2014a,b) and Laos (Vannachone et al., 1998). It is most probably the gender specific daily activities of boys (recreational) and men (agricultural) that increase the exposure to contaminated soil, and hence lead to higher infection rates.

A striking finding of our study was the high infection rate among young children. One third (33.3%) of the children under 6 years of age were infected with *S. stercoralis*. Given the fact that these children have few daily activities outside the household, the transmission of *S. stercoralis* must take place at home. A similar observation was reported in Cambodia (Khieu et al., 2014a). In addition, in Cambodian households dogs were examined for intestinal infection and tested positive for Stronyloides larvae (Schär et al., 2014). We hypothesise that humans and dogs in the same household share the Strongyloides parasites and are responsible for contaminating the soil. However, further genetic studies on human and dog derived Strongyloides parasites are required in order to draw conclusions about anthropo-zoonotic transmission. In this context it is most interesting to note, that in the same Cambodian households, the dog hookworm *Ancylostoma ceylanicum* was found as a predominant hookworm species in humans (Inpankaew et al., 2014) indicating zoonotic transmission from dogs to humans. Given that fact that hookworm and *S. stercoralis* have the same transmission route a similar human–dog transmission pattern of the latter parasite seems likely.

In our study, we did not find any association between *S. stercoralis* infection and risk factors related to socio-economic status, access to sanitation facilities and hygiene behaviour of the population. These results were most surprising, as earlier studies identified clear an association between the parasite and low economic status and absence of sanitation facilities. For example, Cambodian schoolchildren had an almost five fold increased risk for a *S. stercoralis* infection when no latrine was present at home (Khieu et al., 2013a). In addition, attributable risk analysis showed that 70% of *S. stercoralis* infections could be averted if adequate sanitation were present (Khieu et al., 2013a).

Recent developments in our study area might have led to the absence of these associations. We selected the villages precisely because the Provincial Health Office reported low numbers of households with latrines in the island villages of the Khong district. However, during our investigations, we found that more than 40% of the households had a latrine. Indeed, in the last year, a number of health related intervention were undertaken in the Khong district, including general health promotion activities, and latrine construction and mass-deworming campaigns. Though the new developments account for the absence of the expected associations, people however remained infected with *S. stercoralis*.

Although *S. stercoralis* infection is highly prevalent in many settings its clinical significance is not understood. Long-lasting infection may contribute to chronic gastro-intestinal and skin morbidity. In our study, *S. stercoralis* infection was associated with reports of urticarial and/or itching in the previous weeks. A Cambodian study reported a very similar result. There, urticarial with intensive itching on all body parts was reported by patients. The symptoms were resolved after ivermectin treatment (Khieuet al., 2013b). However in this report, abdominal pain was also associated with *S. stercoralis* infection.

In our study, *O. viverrini* was the most frequent helminth infection (72.2%), followed by hookworm (56.1%) and *S. stercoralis* (41.0%) infections. In addition, a considerable *S. mekongi* infection prevalence was detected on Donlong island (25.6%). Therefore, multiparasitism was very common. However, the clinical consequences of concurrent helminth infections are unknown. Recently, it was shown that co-infection with *S. mekongi* aggravates *O. viverrini* related morbidity (Sayasone et al., 2012). However, information on

the contribution of *S. stercoralis* to the overall morbidity of individuals infected with multiple helminth species is unknown and will require further in-depth studies.

In conclusion, *S. stercoralis* infection is highly endemic in the islands of the Khong district, Champasack province, southern Lao PDR. The results of this study and other *S. stercoralis* reports from the country should be noted by the national helminth control programme. County-wide assessments of *S. stercoralis* infection prevalence and related morbidity would be most useful to further push the agenda of an intensified integrated soil-transmitted helminth control intervention in which *S. stercoralis* is adequately addressed.

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4.6. References

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5. Transmission of *Opisthorchis viverrini*, *Schistosoma mekongi* and soil-transmitted helminthes on the Mekong Islands, Southern Lao PDR

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Abstract

Background: Prevalence of *Opisthorchis viverrini, Schistosoma mekongi* and soil-transmitted helminths (STH) remains high in Lao People's Democratic Republic (Lao PDR), despite control efforts including mass-drug administration, education and communication campaigns. New approaches are required to advance helminth control.

Methodology: An ecohealth study was conducted on two Mekong islands in Southern Laos. Demographic and behavioural data were collected by questionnaire. Human and animal reservoir stools were examined. *Bithynia* spp. and *Neotricula aperta* snails were examined using shedding. Fresh water fish were examined using digestion technique. Multivariate random-effects analysis was used to find risk factors associated with helminth infections.

Principal findings: Human infection rates with *O. viverrini,* hookworm, *S. mekongi, Trichuris trichiura*, *Ascaris lumbricoides* and *Taenia* spp. were 60.7%, 44.1%, 22.2%, 4.1%, 0.6% and 0.1%, respectively. Heavy intensity infections were 4.2%, 3.6% and 1.8% for *O. viverrini*, *S. mekongi* and hookworm, respectively*. O. viverrini* infection rate among dogs and cats were 25.0% and 53.1%, respectively. *S. mekongi* infection rates among dogs were 14.7%. Prevalence of *O. viverrini* and *S. mekongi* in snails was 0.3% and 0.01%, respectively. Overall prevalence of *O. viverrini* infection in fresh water fish was 26.9%, with the highest infection rates occurring in *Hampala dispa* (87.1%)*, Cyclocheilichthys apogon* (85.7%) and *Puntius brevis* (40.0%). Illiteracy and lower socioeconomic status increased the risk of *O. viverrini* infection, while those aged 10–16 years and possessing latrines at home were less likely to be infected. Household dogs and cats that consumed raw fish were significantly and positively associated with *O. viverrini* infection of the household members. For *S. mekongi*, children under 9 years old were exposed significantly to this infection, compared to older age groups.

Conclusions/Significance:

There is a pressing need to design and implement an integrated helminth control intervention on the Mekong Islands in southern Lao PDR. Given the highly dynamic transmission of *S. mekongi, O. viverrini*, STH and extended multiparasitism, annual mass-drug administration is warranted along with environmental modifications, health education and improved access to clean water and adequate sanitation to consolidate morbidity control and move towards elimination.

5.1. Introduction

Helminthiases are neglected tropical diseases (NTDs) of major public health concern in many low- and middle-income countries (LMIC) in the tropics and sub-tropics, including in Lao People's Democratic Republic (Lao PDR) (Rim et al., 2003; Sripa et al., 2010; Utzinger et al., 2010; WHO, 2002b). Liver flukes (*Opisthorchis viverrini),* blood flukes (*Schistosoma mekongi*) and soil-transmitted helminths (STH) such as round worm (*Ascaris lumbricoides*), whipworm (*Trichuris trichiura*) and two-hookworm species (*Ancylostoma duodenale, Necator americanus*) are among the most prevalent infections in Lao PDR. *O. viverrini* is endemic nationwide but is most prevalent in the central and southern parts of the country. It occurs in the lowlands, along the Mekong River, where fish are abundant and local inhabitants prefer to consume traditional dishes prepared with raw fish (Rim et al., 2003; Sayasone et al., 2011; Sripa et al., 2010; Xayaseng et al., 2013). *S. mekongi* is only endemic in two districts of the most southern province, Champasack, bordering Cambodia (Muth et al., 2010; Sayasone et al., 2012; Urbani et al., 2002; WHO, 1993). STH are highly prevalent in the northern part of the country and in the mountainous areas along Lao-Vietnamese border (Laymanivong et al., 2014; Rim et al., 2003).

Infections with these helminths negatively affect human health and wellbeing. For example, untreated or chronic infection with *O. viverrini* may lead to severe hepatobiliary morbidity including cholangiocarcinoma (CCA), a fatal bile duct cancer (Sripa, 2003; Sripa et al., 2011a). Chronic infection with *S. mekongi* may result in portal hypertension and is associated with peri-portal liver fibrosis (Dumurgier et al., 2006; Keang et al., 2007; Monchy et al., 2006; Richter et al., 2016). In Champasack province, *O. viverrini* and *S. mekongi* are co-endemic (Sayasone et al., 2011; Sayasone et al., 2012; Vonghachack et al., 2015), further increasing the risk of hepatobiliary morbidity. Finally, anaemia and undernourishment are associated with long-lasting STH infections (Matangila et al., 2014; Soares Magalhaes et al., 2013).

Helminths have complex life cycles; *O. viverrini*, for example, involves two aquatic intermediate hosts, namely freshwater snails (of the genus *Bithynia*) and freshwater fish (of the *Cyprinidae* family). Humans and other mammals are infected by eating raw or undercooked fish (Kaewkes, 2003). The life cycle of *S. mekongi* involves humans and other

mammals (such as dogs, cats, pigs and possibly rats) (Kitikoon et al., 1973; Strandgaard et al., 2001). The *Neotricula aperta* snail, which lives in the crevices of submerged rocks in the Mekong River, serves as intermediate host. The cercariae emerge from the infected snails during the daytime and lie under the water surface (Shimada et al., 2007; Urbani et al., 2002). Humans and animals are infected with this parasite via skin penetration when they come into contact with infested waters (WHO, 1993). Lao PDR adheres to the preventive chemotherapy control strategy promoted by WHO (WHO, 1995, 2002b, 2011). Over the last decade, considerable efforts were employed to implement this strategy through deworming programmes targeting school-children (Phommasack et al., 2008) and through mass-drug administration (MDA) alongside information, education and communication (IEC) campaigns in high risk provinces of the country (Phongluxa et al., 2015). Despite these efforts, the prevalence of helminth infections, including multiple infections, remains high in many places (Aye Soukhathammavong et al., 2015; Forrer et al., 2012; Rim et al., 2003; Sayasone et al., 2009b; Vonghachack et al., 2015; WHO, 2011). Given the complexity of the transmission cycle of helminth infections and the risky behaviour of humans in endemic communities, it may be necessary to adapt the control strategy to improve the effectiveness of interventions.

Ecohealth research is an emerging field of research studying human health in close connectivity with the ecosystem (Leung et al., 2012). It is increasingly conducted to strengthen the sustainability of infectious disease control programmes (Asakura et al., 2015; Furie and Balbus, 2012; Nguyen et al., 2014) and was widely introduced in Southeast Asia (SEA) by the Canadian International Development Research Centre (IDRC) in the late 2000s (Kingsley et al., 2015; Nguyen-Viet et al., 2015). Ecohealth has been defined as follows: i) "EcoHealth involves research and practice to promote sustainability of individuals, animals and biodiversity by linking complex interaction of ecosystem, socio-cultural and economic factors" and ii) "Ecohealth is a comprehensive approach to understanding health at its human, animal and environmental interface in a socio-ecological systems context". Here, we employ an ecohealth approach to determine the prevalence and risk factors of *O. viverrini*, *S. mekongi* and STH infections in humans in the ecological environment of Khong district, where potential animal reservoir and intermediate hosts, like molluscs and fish, live in close connectivity.

5.2. Materials and Methods

5.2.1. *Study area*

Khong district is an island district located at the Southern border of Champasack province, Lao PDR (**Figure 5.1a**). It has an estimated population of 100,000 people and comprises a few dozen islands in the Mekong River (geographical coordinates: 13.57°-14.14°N latitude and 105.44°-106.08°E longitude). The district is a known endemic area for *O. viverrini, S. mekongi* and STH. Done Khon and Done Som are among the biggest islands and are popular tourist destinations. Done Khon has about 260 households with a total population of 1,560 people, while Done Som has some 378 households with a total population of 2,344 people.

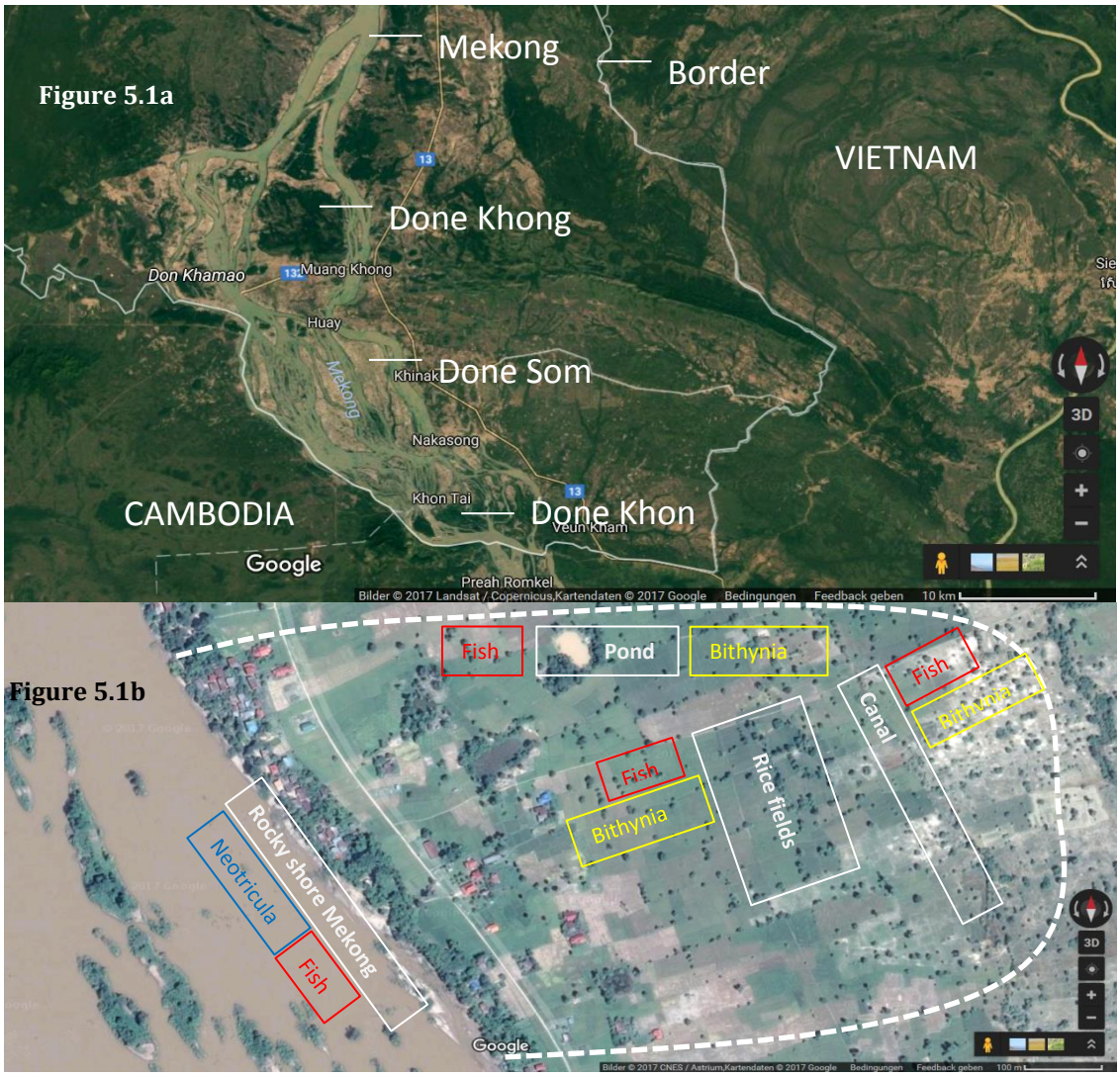

Figure 5.1: Study map: (Source: Google Map)

1a Khong district with main Mekong islands;

1b Selected western shore of Done Som with human settlements and ecological features.

5.2.2. *Study design and population surveyed*

Our cross-sectional study was carried out between October 2011 and August 2012 on Done Khon and Done Som islands. These study sites were selected based on a three-stage random sampling. First, we randomly selected two islands out of 10 known endemic islands for the targeted diseases. Second, two villages were selected on each island. For each village, 320 study participants were required based on our sample calculation using a formula of simple random sampling, e.g., $Z_{1-\alpha/2}^2 \times p(1-p)/d^2$ with a 30% proportion and 5% precision. Based on previous experiences, about 40% of all study participants (129 persons) failed to submit complete stool samples when they were asked to submit multiple stool samples (i.e., at least two). With this in mind, at least 904 study participants from both villages were required for this study. Finally, about 30 households in each village were randomly selected to meet the required sample size. All members of the selected households, aged two years and older and available on the survey day, were invited to participate in the study.

Potential animal reservoir hosts, i.e., dogs, cats, pigs and buffaloes, from selected households were also enrolled and examined for helminth infections. Due to the small numbers of these animals in the study villages (0.4 animals per household; from village record), we examined all of those present during the survey. Village health volunteers helped to identify the domestic animals and conduct follow-up examinations.

We collected intermediate hosts for *O. viverrini* (*Bithynia* spp. snails and *Cyprinoid* fish) and for *S. mekongi* (*Neotricula aperta* snails) from selected sites in the study villages and examined them for infection (**Figure 5.1b**).

Snails of the genus *Bithynia* spp. were collected with a scoop (Kitikoon et al., 1981) from water bodies near the study villages (e.g., ponds, canals, and rice fields). From each water body, 5–10 sites with an area of 1x1 meter were identified as collecting points. All *Bithynia* snails collected from each site were counted, recorded and examined separately. *Cyprinoid* freshwater fish were captured from the same selected water bodies as well as from the Mekong using a fishing net. Each captured fish was measured for length and weight and were examined at the field station for the presence of *O. viverrini* metacercariae.

N. aperta snails (Davis et al., 1976) live in the rocky area of the Mekong River. We identified 10 sites along the Mekong River, where water was frequently used by study villagers for their daily needs. Submerged stones were dredged and snails were hand-picked from them (Kitikoon et al., 1981). At each site, *N. aperta* snails were collected for 20 minutes by five malacologists. All collected snails were counted, placed in a plastic bag and carried to the field station for examination.

5.2.3. *Field procedures and laboratory examinations*

In each village, a house, school or temple was identified as a field study station. Two questionnaires were administered to all participating households. A household questionnaire was administered to the heads of households for collecting data on household characteristics (e.g., building type, toilette and water supply), asset ownership (e.g., farm engine, boat, car, motorbike, electricity, television, bicycle, telephone and agriculture land) and animal ownership (e.g., buffalo, cow, goat and pig). An individual questionnaire was used to interview all household members to collect demographic data (e.g., age, sex, educational attainment and professional activities and behavioural risks (e.g., food consumption habits, water contact, animal raising and personal hygiene). Parents or legal guardians answered for children under 10 years of age.

Eligible study participants were invited to submit two stool samples over consecutive days for parasitological analysis. The first stool container (pre-labelled with participant's name, unique identity number, age and date of collection) was handed to the study participant on the registration day, along with a detailed explanation of stool collection. The second empty container was handed out after study participants returned the first filled container.

Two Kato-Katz (KK) thick smears (Katz et al., 1972a) were prepared from each stool sample (i.e. four smears per person) and examined under light microscopes by an experienced technician within one hour of sample preparation. Eggs were counted and recorded for each helminth species separately.

We collected faecal samples from potential domestic reservoir animals owed by study households, namely cats, dogs, pigs and water buffaloes. To collect fresh faecal samples (Enes et al., 2010) from small animals (cats, dogs and pigs), rectal enemas were performed

using Sodium Chloride (NaCl) solution and petroleum jelly lubricant. Faecal samples from water buffaloes were collected by rectal swab. All faecal samples were immediately preserved in a 10% formalin solution and transported to the National Institute of Public Health (NIOPH), Vientiane, for processing using the formalin ether concentration technique (FECT) (Ebrahim et al., 1997).

Bithynia spp. and *N. aperta* snails were examined for the presence of cercariae infection using the shedding test, previously described by *Sri-Aroon* and colleagues (Sri-Aroon et al., 2007). In summary, the fresh water snails were put into a transparent plastic container filled with Mekong water and exposed to artificial light. After two hours, the container was examined under a stereoscope for the presence of cercariae. The infected snails were identified, counted and recorded separately.

The species identification of captured *Cyprinoid* fish was performed based on guidelines available at FishBase website . Fish digestion was performed using the pepsin enzyme digestion technique (WHO, 1995). The residue was examined for the presence of *O. viverrini* metacercariae. The metacercariae were counted and recorded for each infected fish.

5.2.4. *Data management and analysis*

Information from questionnaires and data forms were double entered into EpiData, version 3.1 (EpiData Association; Odense, Denmark) and validated for their correctness and completeness. Statistical analyses were performed with STATA, version 13.1 (StataCorp., College Station, USA). Only study participants with at least two KK thick smear examinations and with complete questionnaires were retained in the final analysis. Participants were stratified into five age groups: (i) ≤9 years, (ii) 10–16 years, (iii) 17–36 years, (iv) 37–50 years, and (v) ≥51 years. Socioeconomic status (SES) of the household was calculated using an asset-based method. Indicator data were defined by principal component analysis (PCA). The procedure is widely used and details can be found elsewhere (Raso et al., 2005; Sayasone et al., 2011; Steinmann et al., 2007). SES conditions in the household were categorized into one of five wealth quintiles, namely (i) most poor, (ii) very poor, (iii) poor, (iv) less poor, and (v) least poor according to their cumulative

standardized asset scores. Details of this widely used approach have been presented elsewhere (Sayasone et al., 2011).

The intensity of helminth egg counts was expressed as eggs per gram of stool (EPG) obtained from Kato-Katz examination. Based on WHO recommendations, infection intensity was classified as light (*S. mekongi*: 1–100 EPG; *O. viverrini*: 1-999 EPG; *A. lumbricoides*: 1–4,999 EPG; *T. trichiura*: 1–999 EPG and hookworm: 1–1,999 EPG), moderate (*S. mekongi*: 101–400 EPG; *O. viverrini*: 1,000–9,999 EPG; *A. lumbricoides*: 5,000–49,999 EPG; *T. trichiura*: 1,000–9,999 EPG and hookworm: 2,000–3,999 EPG), and heavy (*S. mekongi*:≥400 EPG; *O. viverrini*: ≥10,000 EPG; *A. lumbricoides*:≥ 50,000EPG; *T. trichiura*: ≥ 10,000 EPG and hookworm ≥4,000 EPG), respectively (Maleewong et al., 1992b; Sayasone et al., 2009b; WHO, 1995).

Prevalence of parasitic infections was determined and stratified by age, sex and study area (Done Khon *versus* Done Som). Chi-square test was used to examine the association among categorical variables. The geometric mean for helminth egg counts was calculated for infected individuals. Univariate random-effects logistic regression analysis was used to associate *S. mekongi* and *O. viverrini* infections (outcome) with potential risk factors (predictors). The crude odds ratio (cOR), 95% confidence interval (95% CI) and *P*-value were calculated. Explanatory variables with a *P*-value of <15% were included in the stepwise multivariate random-effects logistic regression model. Adjusted odds ratio (aOR) was calculated. Smoothed age distribution of *S. mekongi*, *O. viverrini, T. trichiura* and hookworm infection by gender were established. Statistical significance was defined as yielding a *P*-value smaller than 0.05.

5.3. Results

5.3.1. Characteristics of the study participants

A total of 994 study participants were included in this final analysis (**Figure 5.2**). Of these, 475 (47.8%) were from Done Khon and 519 (52.2%) from Done Som. There were slightly more female than male participants (51.8% *vs* 48.2%). Age ranged from 2 to 88 years (median age 29.8 years). The schooling rates did not differ between the two study islands. Subsistent rice farming and fishing were the main professional activities (60.0%). Less than half of the study participants reported having access to a latrine at home (Done Khon

49.7%, Done Som 38.9%). People living in Done Som had a lower socioeconomic status than in Done Khon (Most poor, 25.8% *vs* 16.4%, respectively). The sociodemographic characteristics of study participants are summarized in **Table 5.1**.

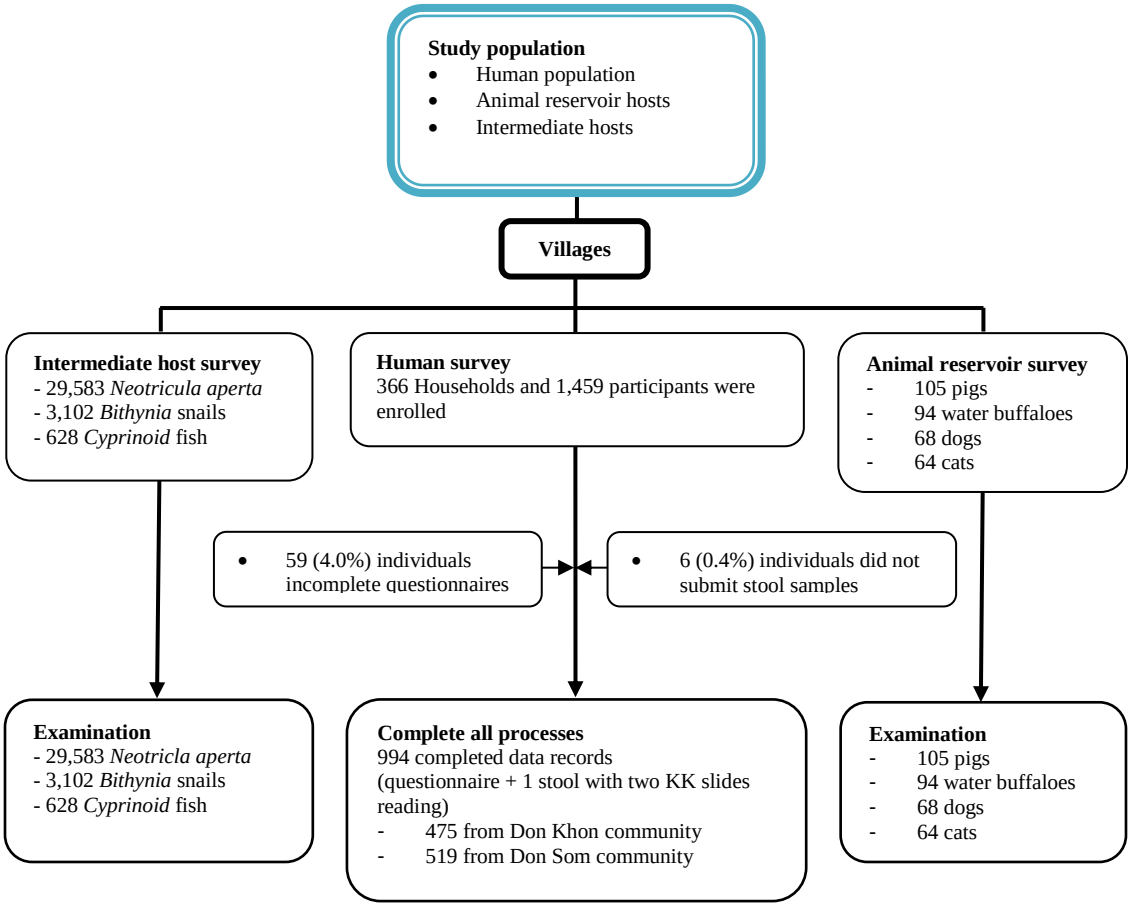

Figure 5.2: Study diagram

Table 5.1: Socio-demographic characteristics of study participants from two study islands (Done Khon and Done Som, Khong district (n=994)

Characteristics	Overall n (%)	Study area		x^2	*P*-value[a]
		Done Khon, n (%)	Done Som, n (%)		
Age (years)					
Mean (range)	29.8 (2-88)	30.0 (2-87)	29.6 (2-88)		
Age group					
≤9	216 (21.7)	99 (20.8)	117 (22.5)		
10-16	185 (18.6)	91 (19.2)	94 (18.1)		
17-36	204 (20.5)	102 (21.5)	102 (19.7)		
37-50	203 (20.4)	88 (18.5)	115 (22.2)		
≥ 51	186 (18.7)	95 (20.0)	91 (17.5)	3.3	0.511
Sex					
Male	479 (48.2)	212 (44.6)	267 (51.5)		
Female	515 (51.8)	263 (55.4)	252 (48.6)	4.6	0.032
Educational level					
Pre-schooler	108 (10.9)	52 (10.9)	56 (10.8)		
Illiteracy	97 (9.8)	59 (12.4)	38 (7.3)		
Primary school	538 (54.1)	237 (49.9)	301 (58.0)		
High school/above	251 (25.3)	127 (26.7)	124 (23.9)	10.4	0.015
Occupation					
Preschool child	108 (10.9)	52 (11.0)	56 (10.8)		
Student	290 (29.1)	137 (28.8)	153 (29.5)		
Farmer and fisher	596 (60.0)	286 (60.2)	310 (59.7)	0.05	0.975
Socioeconomic status					
Least poor	195 (19.6)	126 (26.5)	69 (13.3)		
Less poor	203 (20.4)	73 (15.4)	130 (25.1)		
Poor	192 (19.3)	107 (22.5)	85 (16.4)		
Very poor	192 (19.3)	91 (19.2)	101 (19.5)		
Most poor	212 (21.3)	78 (16.4)	134 (25.8)	48.6	<0.001
Latrine available					
No	556 (55.9)	239 (50.3)	317 (61.1)		
Yes	438 (44.1)	236 (49.7)	202 (38.9)	11.7	0.001
Opened defecation this year					
No	484 (48.7)	256 (53.9)	228 (43.9)		
Yes	510 (51.3)	219 (46.1)	291 (56.1)	9.9	0.002

P-value[a]: the comparison between Done Khone and Done Som island

5.3.2. Helminth infections in humans

Helminth infections were very frequent on the two islands. *O. viverrini*, hookworm, *S. mekongi*, and *T. trichiura* were found in 60.7%, 44.1%, 22.2% and 4.1% of the participants, respectively. Very few participants were infected with *A. lumbricoides* (0.6%) and *Taenia* spp. (0.1%). The prevalence of *O. viverrini* was almost two-times higher in Done Som compared to Done Khon (77.3% vs. 42.5%, *P*-value < 0.001). *S. mekongi* prevalence was similar on both islands (*P*-value = 0.329). Multi-parasitism was diagnosed in 40.5% of the study participants. Details of the helminth infections are given in **Table 5.2**.

Table 5.2: Prevalence of *Schistosoma mekongi, Opisthorchis viverrini,* soil-transmitted helminth and other intestinal helminth infections among study participants from two islands (Done Khon and Done Som) of Khong district (n=994)

Parasites	Positive, n (%) (n=994)	Done Khon, n (%) (n=475)	Done Som, n (%) (n=519)	x^2	*P*-value[a]
Trematodes					
Opisthorchis viverrini	603 (60.7)	202 (42.5)	401 (77.3)	125.4	<0.001
Schistosoma mekongi	221 (22.2)	112 (23.6)	109 (21.0)	0.9	0.329
Soil-transmitted helminth					
Hookworm	438 (44.1)	196 (41.3)	242 (46.6)	2.9	0.090
Trichuris trichiura	41 (4.1)	21 (4.4)	20 (3.9)	0.2	0.653
Ascaris lumbricoides	6 (0.6)	6 (1.3)	0	6.6	0.010
Cestodes					
Taenia spp.	1 (0.1)	1 (0.2)	0	1.1	0.296
Multiparasitism					
No infection	202 (20.3)	127 (26.7)	75 (14.5)		
Single species	379 (38.1)	197 (41.5)	182 (35.1)		
Multiple species	413 (40.5)	151 (31.8)	261 (40.5)	43.9	<0.001

P-value[a]: the comparison between Done Khone and Done Som island

Figure 5.3 displays the smoothed age prevalence of helminth infections by gender. *O. viverrini* infection appears to be acquired at a young age, with prevalence increasing gradually (**Figure 5.3a**). Hookworm infection is acquired at a very young age. For males, the prevalence peaked among adolescents aged 10 – 20 years and plateaued among older age groups. For females, prevalence peaked between 10–20 years old and again after 50 years old (**Figure 5.3b**). For males, two prevalence peaks were observed; the first among children under 10 years old and the second among adults between 40 and 50 years old. For

females, only one peak was seen among children under 10 years old. *T. trichiura* prevalence was distributed similarly among males and females independent of age (**Figure 5.3c**). *S. mekongi* prevalence was differently distributed among males and females (**Figure 5.3d**).

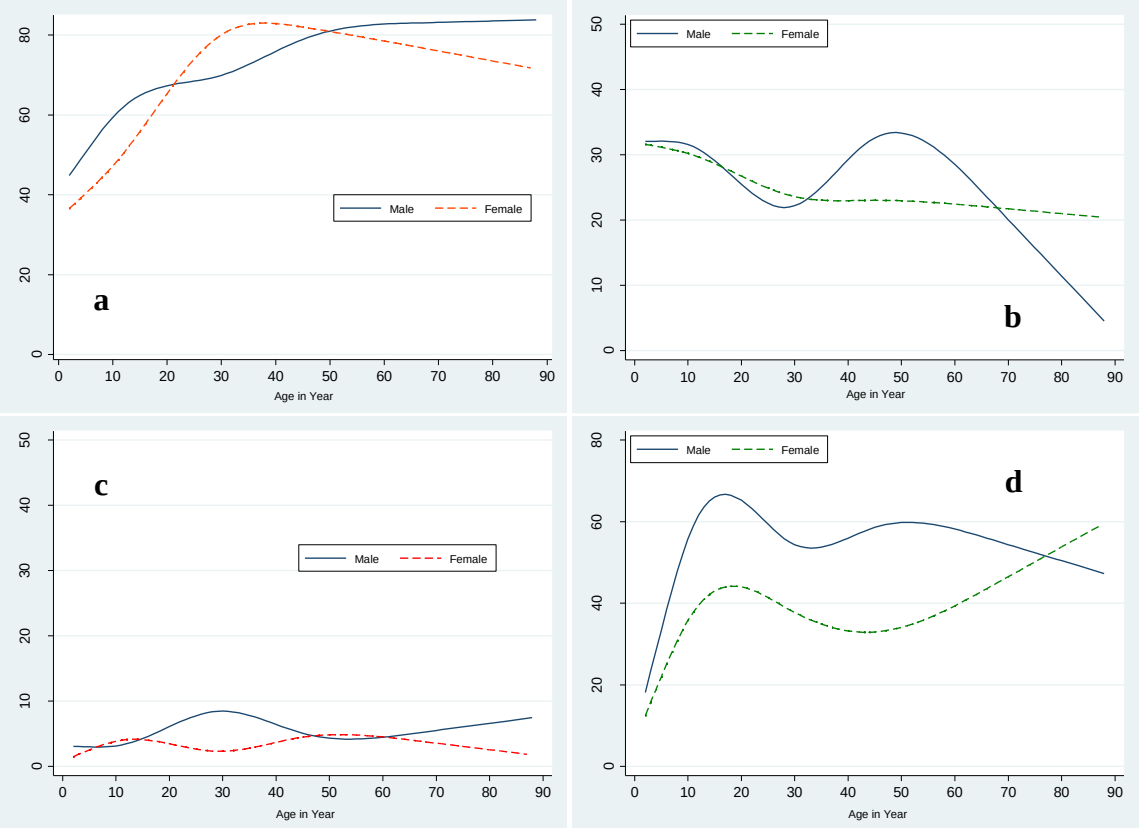

Figure 5.3: Age distribution of major helminth infections by gender on Done Khon and Done Som islands. The figures represent the smoothed age distribution of male (solid line) and female (dotted line) study participants for an infection with **a**: *Opisthorchis viverrini*, **b**: hookworm, **c**: *Trichuris trichiura* and **d**: *Schistosoma mekongi.*

Human helminth infection intensities are summarized in **Table 5.3**. Most helminth infections were categorized as light infections. Nevertheless, *O. viverrini, S. mekongi* and hookworm accounted for infections of heavy intensity in some cases (4.2%, 3.6% and 1.8%, respectively).

Table 5.3: Infection intensity of *Opisthorchis viverrini, Schistosoma mekongi* and soil-transmitted helminths among study participants from two islands (Done Khon and Done Som) of Khong district (n=994)

Infections	Light			Moderate			Heavy		
	Overall n (%)	Done Khon n (%)	Done Som n (%)	Overall n (%)	Done Khon n (%)	Done Som n (%)	Overall n (%)	Done Khon n (%)	Done Som n (%)
Opisthorchis viverrini	409 (67.8)	174 (86.1)	235 (58.6)	169 (28.0)	27 (13.4)	142 (35.4)	25 (4.2)	1 (0.5)	24 (6.0)
Schistosoma mekongi	187 (84.6)	100 (89.3)	87 (79.8)	26 (11.8)	10 (8.9)	16 (14.7)	8 (3.6)	2 (1.8)	6 (5.5)
Hookworm	420 (95.9)	191 (97.5)	229 (94.6)	10 (2.3)	2 (1.0)	8 (3.3)	8 (1.8)	3 (1.5)	5 (2.1)
Ascaris lumbricoides	5 (83.3)	5 (83.3)	0	1 (16.7)	1 (16.7)	0	0	0	0
Trichuris trichiura	41 (97.6)	22 (100.0)	19 (95.0)	1 (2.4)	0	1 (5.0)	0	0	0

5.3.3. Prevalence of helminth infections in animal reservoirs and intermediate hosts

Table 5.4 summarizes the results of infections in animals, snails and *Cyprinoid* fish. Analysis of animal faeces showed that overall prevalence of *O. viverrini* infection in cats, dogs and pigs was 53.1%, 25.0% and 0.9%, respectively, while only dogs (14.7%) were found to be infected with *S. mekongi*. Examination of intermediate host snails for *O. viverrini* (*Bithynia* spp.,) and for *S. mekongi* (*N. aperta*) detected infection rates of 0.3% and 0.01%, respectively (Table 5.4). A similar rate of *O. viverrini* infection was found in *Bithynia* spp. from Done Khon and Done Som (0.1% *vs.* 0.5%, *P*-value = 0.045), while only the *N. aperta* snails from Done Khon (0.02%) were found to be infected with *S. mekongi.*

Table 5.4: Prevalence of *Schistosoma mekongi* and *Opisthorchis viverrini* infections in animals on Done Khon and Done Som islands

Infections	No. exam	Overall, n (%)	Done Khon, n (%)		Done Som, n (%)		x^2	*P*-value[a]
			No. exam	No. positive	No. exam	No. positive		
Opisthorchis viverrini								
Dog	68	17 (25.0)	44	10 (22.7)	24	7 (29.2)	0.34	0.558
Cat	64	34 (53.1)	25	15 (60.0)	39	19 (48.7)	0.78	0.378
Pig	105	1 (0.9)	43	0	62	1 (1.6)	0.70	0.403
Water buffalo	94	0	32	0	62	0	na	na
Intermediate snails								
Bithynia spp.	3,102	9 (0.3)	1,719	2 (0.1)	1,383	7 (0.5)	4.03	0.045
Minute intestinal fluke (MIF)								
Dog	68	3 (4.4)	44	3 (6.8)	24	0	1.71	0.191
Cat	64	18 (28.1)	25	5 (20.0)	39	13 (33.3)	1.33	0.247
Large trematode eggs								
Water buffaloes	94	18 (19.1)	32	9 (28.1)	62	9 (14.5)	2.52	0.112
Pig	105	4 (3.8)	43	2 (4.6)	62	2 (3.2)	0.14	0.708
Schistosoma mekongi								
Dog	68	10 (14.7)	44	7 (16.0)	24	3 (13.0)	0.14	0.704
Cat	64	0	25	0	39	0	na	
Pig	105	0	43	0	62	0	na	
Water buffalo	94	0	32	0	62	0	na	
Intermediate snails								
Neotricula aperta	29,583	4 (0.01)	16,342	4 (0.02)	13,241	0	3.24	0.072

P-value[a]: the comparison between Done Khone and Done Som island

na: not applicable

Table 5.5 displays the prevalence of *O. viverrini* infection in the *Cyprinoid* fish collected from habitats in Done Khon and Done Som islands. In total, 628 fish representing 21 species were digested and examined. Of these, 622 represented 19 species of *Cyprinoid* fish, five fish were from the Osphronemidiae family and one fish from the Anabantidae family. Only Cyprinoidae fish species were infected with *O. viverrini*, with an overall prevalence of 26.9% and an average of 228.7 metacercariae per fish. The highest infection intensity was seen in *Cyclocheilichthys apogon,* with an average of 168.7 metacercariae per infected fish. Only one fish of the *Anabas testudineus* from Anabantidae family was examined. It was found positive for minute intestinal fluke metacercariae.

5.3.4. Risk factor analysis for *O. viverrini* and *S. mekongi* infections in human

Table 5.6 shows the association between risk factors of *S. mekongi* and *O. viverrini* infections. The stepwise multivariate analysis showed that illiteracy (illiteracy vs. preschool children: aOR = 6.0, 95% CI: 3.3-11.0, *P* = 0.028) and lower socioeconomic status were associated with an increased risk of being infected with *O. viverrini* (less poor *vs* least poor: aOR = 3.1, 95% CI: 1.7-7.5, *P* = 0.013), while school children in the age group 10–16 years (aOR = 0.1, 95% CI: < 0.1-0.4, *P* = 0.003) and those with a latrine at home (aOR = 0.2, 95% CI: 0.1-0.4), *P* = 0.001) were more likely to be protected against the infection. Furthermore, having household dogs and cats that eat raw fish was significantly and positively associated with *O. viverrini* infection of the household members (aOR = 1.9, 95% CI: 1.2-3.1, *P* = 0.007). The age group was the only factor significantly associated with *S. mekongi* infection. Children in the age group ≤ 9 years old were significantly exposed to this infection compared to older age groups (age group 10–16: aOR = 0.5, 95% CI: 0.2-0.9, *P* = 0.047, age group 17–36: aOR = 0.2, 95% CI: <0.1-0.8, *P* = 0.022; age group 37–50: aOR = 0.2, 95% CI: <0.1-0.8, *P* = 0.021 and age group ≥ 51: aOR = 0.2, 95% CI: <0.1-0.8, *P* = 0.024). The model revealed that age group (10–16 year: aOR = 1.7, 95% CI: 1.1-2.7, *P* = 0.015), educational level (illiteracy: aOR = 7.4, 95% CI: 3.2-17.3, *P* < 0.001, and primary school: aOR = 4.8, 95% CI: 2.0-11.3, *P* < 0.001) and raising pigs at home (aOR = 1.3, 95% CI: 1.1-1.7, *P* = 0.047) were significant risk factors for STH infection, while being a women (aOR = 0.4, 95% CI: 0.3=0.6, *P* < 0.001) or having a latrine at home (aOR = 0.6, 95% CI: 0.4-0.8, *P* < 0.001) were protective factors.

Table 5.5: Prevalence of *Opisthorchis viverrini* and minute intestinal flukes (MIF) metacercariae in *Cyprinoid* fish from Done Khon and Done Som islands

Scientific name Species	Lao name	No. exam.	No. of fish infected *O. viverrini* positive (%)	No. of *O. viverrini* metacercariae Mean, SD (range)	No. of fish infected MIF positive (%)	No. of MIF metacercariae Mean, SD (range)	Weight (gram) Mean, SD (range)
Morulius chrysophekadion	Pa phea	1	1 (100.0)	2.0, na	0	0	138 (na)
Hampala dispa	Pa soud	101	88 (87.1)	112.1, ±188.0 (3-1,468)	9 (8.9)	8.6, ±10.6 (1-48)	11.4, ±11.1 (1.9-66.4)
Cyclocheilichthys apogon	Pa dok-ngew	21	18 (85.7)	168.7, ±283.9 (2-984)	5 (23.8)	6.4, ±4.4 (2-12)	7.1, ±4.6 (1.5-20.1)
Puntius brevis	Pa khao-mon	100	40 (40.0)	120.2, ±322.2 (1-1,940)	22 (22.2)	10.7, ±10.9 (1-48)	8.5, ±9.3 (1.1-39.1)
Henicorhynchus lineatus	Pa soi	14	3 (21.4)	31, ±37.3 (7-74)	0	0	11.8, ±5.3 (3.7-23)
Barbonymus gonionotus	Pa pak-khao	16	2 (13.0)	10, ±7.1 (5-15)	0	0	38.2, ±25.5 (3.9-84.9)
Barbonymus altus	Pa wien-fai	17	2 (11.8)	2.5, ±0.7 (2-3)	0	0	21.5, ±7.5 (2.9-36.1)
Poropuntius deauratus	Pa chad	163	10 (6.1)	21.6, ±43.6 (1-142)	16 (9.8)	9.3, ±12.6 (1-42)	14.3, ±23.4 (1.4-148.6)
Puntioplites falcifer	Pa sa-khang	34	2 (6.0)	6.0, ±5.7 (2-10)	1 (2.9)	2.0, na	19.2, ±8.8 (3.3-38.7)
Scaphognathops bandanensis	Pa pieng	40	2 (5.0)	1.5, ±0.7 (1-2)	2 (5.0)	7.5, ±7.8 (2-13)	33.1, ±14.9 (6.2-68.7)
Albulichthys albuloides	Pa ta-sai	69	1 (1.5)	1, na	22 (31.9)	19.7, ±34.9 (1-132)	14.4, ±5.3 (4.9-27.9)
Opsarius koratensis	Pa sew-oua	16	0	0	1 (6.3)	2.0, na	6.3, ±5.3 (1.9-14.6)
Paralaubuca typus	Pa tab	5	0	0	1 (20.0)	2.0, na	8.5, ±5.1 (5.1-17.3)
Mystacoleucus atridorsalis	Pa lang-khon	9	0	0	0	0	9.1, ±14.1 (1.4-36.9)
Cyclocheilichthys enoplus	Pa choox	5	0	0	0	0	30.7, ±11.9 (18.6-48.6)
Luciosoma bleekeri	Pa mak-vai	4	0	0	0	0	26.1, ±3.9 (23.7-31.9)
Osteochilus melanopleurus	Pa nok-khao	3	0	0	0	0	8.8, ±9.7 (2.8-20)
Raiamas guttatus	Pa sa-nak	3	0	0	0	0	38.6, ±29.8 (10-69.4)
Probarbus labeamajor	Pa oearn	1	0	0	0	0	45.9 (na)
Trichogaster trichopterus[1]	Pa ka-deuth	5	0	0	0	0	4.6, ±2.4 (2.3-7.8)
Anabas testudineus[2]	Pa kheng	1	0	0	1 (100)	3.0, na	9.7 (na)
Total		**628**	**169 (26.9)**	**106.9 ±228.7 (1-1,940)**	**12.7**	**11.9, ±20.7 (1-132)**	**15.0, ±17.4 (1.1-148.6)**

Belongs to the [1]Osphronemidae and [2] Anabantidae family; na: not appropriate; SD: standard deviation; No: number

Table 5.6: Stepwise multivariate logistic regression (backward elimination) analyses the association between underlying risk factors and *S. mekongi*, *O. viverrini* and STH infections among study participants on both islands (Done Khon and Done Som islands (n=994)

Characteristics	*O. viverrini*				*S. mekongi*				Soil-transmitted helminth			
	Crude OR (95% CI)	*P*-value	Adjusted OR (95% CI)	*P*-value	Crude OR (95% CI)	*P*-value	Adjusted OR (95% CI)	*P*-value	Crude OR (95% CI)	*P*-value	Adjusted OR (95% CI)	*P*-value
≤9	1		1		1		1		1		1	
10-16	1.6 (1.1-2.4)	0.022	0.1 (< 0.1-0.4)	0.003	0.6 (0.4-1.1)	0.075	0.5 (0.2-0.9)	0.047	2.7 (1.8-4.0)	< 0.001	1.7 (1.1-2.7)	0.015
17-36	3.3 (2.2-4.9)	< 0.001	NS	NS	0.5 (0.3-0.8)	0.005	0.2 (< 0.1-0.8)	0.022	1.9 (1.3-2.9)	0.001	NS	NS
37-50	4.3 (2.8-6.4)	< 0.001	NS	NS	0.5 (0.4-0.9)	0.006	0.2 (< 0.1-0.8)	0.021	2.2 (1.5-3.2)	< 0.001	NS	NS
≥ 51	4.2 (2.7-6.4)	< 0.001	NS	NS	0.5 (0.4-0.9)	0.011	0.2 (< 0.1-0.8)	0.024	2.4 (1.6-3.6)	< 0.001	NS	NS
Sex												
Male/Female	1/1.1 (0.8-1.5)	0.579	NA	NA	1/0.8 (0.6-1.2)	0.401	NA	NA	1/0.5 (0.4-0.6)	< 0.001	1/0.4 (0.3-0.6)	< 0.001
Educational level												
Preschooler	1		1		1		1		1		1	
Illiteracy	6.0 (3.3-11.0)	< 0.001	9.4 (1.3-68.9)	0.028	0.6 (0.3-1.2)	0.131	NS	NS	4.0 (2.2-7.2)	< 0.001	7.4 (3.2-17.3)	< 0.001
Primary school	4.0 (2.5-6.2)	< 0.001	NS	NS	0.7 (0.3-1.2)	0.140	NS	NS	2.9 (1.8-4.6)	< 0.001	4.8 (2.0-11.3)	< 0.001
High school/above	2.7 (1.7-4.3)	< 0.001	NS	NS	0.6 (0.4-1.1)	0.101	NS	NS	2.8 (1.7-4.6)	< 0.001	NS	NS
Occupation												
Preschool child	1		1		1		1		1		1	
Student	1.2 (0.8-1.8)	0.377	NA	NA	1.5 (1.0-1.9)	0.034	NS	NS	1.9 (1.2-2.8)	0.003	NS	NS
Farmer	3.1 (2.1-4.6)	< 0.001	NS	NS	2.0 (1.0-2.6)	0.017	NS	NS	1.8 (1.3-2.7)	0.002	NS	NS
Socio-economic status												
Least poor	1		1		1		1		1		1	
Less poor	2.4 (1.3-4.7)	0.007	6.5 (1.2-37.5)	0.037	1.5 (0.9-2.3)	0.321	NA	NA	1.5 (1.0-2.2)	0.041	NS	NS
Poor	2.2 (1.1-4.2)	0.018	NS	NS	1.9 (1.1-2.9)	0.082	NS	NS	1.2 (0.8-1.8)	0.340	NA	NA
Very poor	1.5 (0.8-2.9)	0.213	NS	NS	0.9 (0.5-1.6)	0.816	NA	NA	1.2 (0.8-1.8)	0.395	NA	NA
Most poor	3.7 (2.0-7.0)	< 0.001	NS	NS	0.9 (0.6-1.6)	0.753	NA	NA	1.3 (0.9-1.9)	0.209	NA	NA
Latrine available												
No/Yes	1/0.4 (0.3-0.7)	< 0.001	1/0.2 (0.1-0.4)	0.001	1/0.8 (0.6-1.1)	0.148	NA	NA	1/0.6 (0.5-0.8)	< 0.001	1/0.6 (0.4-0.8)	< 0.001
Has ever heard about diseases												
No/Yes	1/1.5 (0.9-2.3)	0.090	NS	NS	1/0.8 (0.6-1.1)	0.154	NA	NA	NA	NA	NA	NA
Known about transmission route									NA	NA	NA	NA
No/Yes	1/1.8 (1.0-3.2)	0.041	NS	NS	1/0.8 (0.5-1.5)	0.660	NA	NA	NA	NA	NA	NA
Open defecation this year												
No/Yes	1/1.8 (1.4-2.3)	< 0.001	NS	NS	1/1.1 (0.8-1.5)	0.482	NA	NA	1/1.6 (1.2-2.1)	< 0.001	NS	NS
Water contact for fishing/farming												
No/Yes	1/1.5 (1.0-2.1)	0.038	NS	NS	1/0.9 (0.6-1.4)	0.730	NA	NA	1/1.6 (1.2-2.3)	< 0.005	NS	NS
Eating raw/undercooked fish												
No/Yes	1/4.3 (2.6-6.9)	< 0.001	NS	NS	1/0.6 (0.4-0.8)	0.004	NS	NS	1/0.8(0.5-3.2)	0.872	NS	NS
Raising cats at home												
No/Yes	1/1.0 (0.8-1.3)	0.959	NA	NA	1/0.8 (0.6-1.2)	0.542	NA	NA	1/1.2 (0.9-1.6)	0.094	NS	NS
Raising dogs at home												
No/Yes	1/0.9 (0.7-1.2)	0.397	NA	NA	1/0.7 (0.4-1.4)	0.343	NA	NA	1/1.2 (0.9-1.5)	0.132	NS	NS
Raising pigs at home												
No/Yes	1.1 (0.9-1.5)	0.398	NA	NA	1.2 (0.9-1.6)	0.336	NA	NA	1/1.2 (0.9-1.6)	0.132	1/1.3 (1.1-1.7)	0.047
Raising buffaloes at home												
No/Yes	1/1.1 (0.9-1.5)	0.394	NA	NA	1/1.3 (0.9-1.8)	0.133	NS	NS	1/1.1 (0.8-1.2)	0.845	NA	NA
Observed dog/cat eat raw/undercooked fish												
No/Yes	1/1.3 (0.9-1.6)	0.059	1/1.9 (1.2-3.1)	0.007	NA	NA	NA	Na	1/1.3 (0.8-1.7)	0.169	NA	NA

NA: not appropriate for analysis (all variables with *P*-value ≥ 15% and are removed by model); NS: not significant (all variables with *P*-value < 15%, but are not significant after adjusted analysis

5.4. Discussion

The Khong district, with its dozens of islands in the Mekong, has a distinct ecological setting (Figure 1). Human settlements line the island shores, while the rest of the island is used for agricultural activities, particularly rice farming. The Mekong River as well as the diverse water bodies on the islands represent a rich ecosystem for fish and mollusc populations. On two Mekong islands, highly endemic for multiple species of helminth infections, we studied the transmission of *O. viverrini, S. mekongi* and STH using an ecohealth approach (Kingsley et al., 2015; Leung et al., 2012) to better assess the relation of human infection status to environmentally present reservoir and intermediate hosts. Heavy infections and multi-parasitism were prevalent among the human population and age-gender distributions revealed parasite-specific patterns. Examination of potential animal reservoir hosts from the study participants' households (cats, dogs, pigs and buffaloes) yielded ten different helminth species, with many of them having zoonotic capacity. Infection rates of intermediate snail hosts *Bithynia* sp. and *N. aperta* were low but reflect on-going transmission. In addition, infection rates of locally caught Cyprinoid fish with *O. viverrini* and minute intestinal fluke (MIF) metacercariae were very high, pointing to a high risk of infection when they are consumed raw or undercooked.

In this study, we document high infection rates of *O. viverrini, S. mekongi* and selected species of STH, namely hookworm infections. The high infection rates are a surprise given that MDA campaigns were conducted annually between 2008 and 2013 (WHO, 2011), in which praziquantel (40mg/kg BW single dose) and albendazole (400mg single dose) were provided to the entire population (older than four years). In addition, biannual deworming (with mebendazole) takes place in all Lao primary schools (Phommasack et al., 2008). Local health authorities confirmed that all Mekong islands were targeted, but we could not find coherent information on the number of treatment rounds conducted on our study islands. Nevertheless, our results indicate that the impact of the intervention is insufficient.

The Ministry of Health's objective is to eliminate *S. mekongi* as a public health problem in Lao PDR by 2016. On our study islands, *S. mekongi* cannot be considered eliminated given the high infection rates. Our data indicate that *S. mekongi* infection in dogs may fuel the transmission by constantly infecting *Neotricula* populations in the Mekong. Of similar

importance are cats and dogs for the transmission of *O. viverrini*. Hence, animal reservoirs in households should also be a target of integrated parasite control on the Mekong islands, and throughout Lao PDR.

Several factors might account for the persisting high *O. viverrini* infection rates among humans on the Mekong islands. One such factor is the high infection prevalence among *Cyprinoid* fish. More than 80 species of the *Cyprinidae* family and at least 13 species of other families can serve as a secondary intermediate host (WHO, 1995). In our study, *O. viverrini* metacercariae were identified in 11 *Cyprinoid* fish species, while some had particularly high *O. viverrini* metacercariae infection rates, e.g. in 87.1% of *Hampala dispa*. All the *Cyprinoid* species in which we detected an infection are known to be good *O. viverrini* transmitting species (Manivong et al., 2009; Rim et al., 2008b; Rim et al., 2013; Sayasone et al., 2007). They were identified in all water bodies examined in this study. Fish are mostly likely infected while small and living in rice fields, canals and ponds. The metacercariae remain alive as the fish grow and move into the Mekong.

Cyprinoid fish accumulate the metacercariae over a long time. Low infection rates in *Bithynia* snails may be sufficient for transmission (Chai et al., 2005a). We found a low infection rate of 0.3% in *Bithynia* sp. snails. Other studies have detected infection rates between 0.3–8.3% (Kiatsopit et al., 2012). But infection rates may vary considerably, depending on sampling locality and season (Kiatsopit et al., 2014; Kiatsopit et al., 2012). It is important to note that even low infection prevalence rates are sufficient for maintaining transmission.

We observed low *S. mekongi* infection rates in *N. aperta* (0.02%) compared to other reports. The presence of infected molluscs gives evidence that *S. mekongi* transmission is currently on-going. Therefore, abandoning control activities would inevitably lead to an increase in infection rates among humans. There are many more *S. mekongi* endemic Mekong islands, which might display a different *N. aperta* population distribution and infection pattern (Muth et al., 2010; Urbani et al., 2002).

A major finding from our study is the dramatically high helminth infection rates among domestic cats, dogs, pigs and buffaloes. Ten different parasite species were detected in these animal hosts residing in the households of our study participants. By using FECT, we

could distinguish *O. viverrini* eggs in dogs and cats from other small trematode eggs. Our results showed higher rates than Aunpromma et al (2012) found in neighbouring Thailand, where 0.37% and 35.5% of the dogs and cats were infected, respectively (Aunpromma et al., 2012). The infection rate among dogs, in particular, was 20 times higher than that found in the study of Aunpromma et al (2012). Through observation and from interviewing animal owners in both communities, it appears that most of the dogs and cats were free-roaming and usually accompanied their owners to the rice field where they caught and ate fish directly from the canals or rice fields. Moreover, raw and undercooked fish were often fed to these animals. These phenomena, in combination with the high infection rates of dogs and cats, likely maintain the transmission of *O. viverrini* and other fish-borne trematode infections in the communities.

We did not find any *S. mekongi* eggs in pigs or water buffaloes. Only dogs were diagnosed with *S. mekongi* in this study, which is consistent with other study findings (Kitikoon et al., 1975; Strandgaard et al., 2001; Urbani et al., 2002).. However, Strandgaard and colleague reported the finding of *S. mekongi* eggs in pigs in 2001 (Strandgaard et al., 2001). However, they are not of importance for transmission on our study islands. On other Mekong islands where these animals are more free-roaming, their infection status could be higher and, thus, their contribution to transmission of greater importance.

The results of our risk factor analysis for *O. viverrini* infection differed from many previous studies (Forrer et al., 2012; Sayasone et al., 2011; Sayasone et al., 2007). More than half of our risk factors dropped out after multivariate analysis, whereas the initial univariate analysis showed significant associations between infection and age group, occupation, socioeconomic status, latrine availability, history of open defecation this year, and eating raw and/or undercooked fish (**Table** 6). The association between *O. viverrini* and socioeconomic status was not clear for our study population. The study area was geographically very small. Therefore, the variation in socioeconomic status and living conditions might not have varied enough to results in risk differentiation. Furthermore, control activities such as the annual treatments between 2008 and 2013, have had an impact on infection status, which in turn might have blurred important associations. For example, eating raw/undercooked fish was not significantly associated with *O. viverrini* infection, although deeply rooted habits of eating raw or improperly cooked fish is a well-

known factor in sustaining helminth infections in humans and difficult to control (Chai et al., 2005a; Forrer et al., 2012; Phongluxa et al., 2013).

In our multivariable analysis, we did not find any association between *S. mekongi* infection and risk factors, except for age. Children under nine years old had a higher risk of infection than older study participants. This result is likely due to MDA over the years having reduced infection rates among older villagers. Therefore, controls targeting lower age groups could further contribute to eliminating *S. mekongi* on the Mekong islands.

Our study suffers from some limitations. Our diagnostic procedure most likely underestimated the true infection burden. Although examining a duplicate Kato-Katz thick smear per faecal sample has a considerably higher sensitivity than a single smear, the egg detection rate remains far below that of a multiple stool sample diagnostic procedure (Sayasone et al., 2011; Vonghachack et al., 2015). Furthermore, the Kato-Katz technique cannot differentiate small trematode eggs (Lovis et al., 2012). It is therefore possible that some of the infections in humans were counted as *O. viverrini* infections instead of MIF.

5.5. Conclusion

We conclude that human intestinal helminth infections, namely *O. viverrini, S. mekongi* and hookworms are still highly endemic on the Mekong islands in Khong district. The low prevalence of *O. viverrini* and *S. mekongi* infection in intermediate snail hosts point at on-going transmission. Animal reservoir hosts, particularly cats and dogs, have high *O. viverrini* infection rates, while only dogs are infected with *S. mekongi*. An appropriate integrated control approach involving interventions targeting human behaviour, animal reservoirs, and environmental modification might improve the effectiveness of interventions and lead to the elimination of infections.

5.6. List of abbreviations

95% CI	95% confidence interval
A. duodenale	*Ancylostoma duodenale*
A. lumbricoides	*Ascaris lumbricoides*
aOR	Adjusted Odds Ratio
BW	Body weight
CCA	Cholangiocarcinoma
cOR	Crude Odds Ratio
EPG	Eggs per gram of stool
FECT	Formalin Ether Concentration Technique
IEC	Information, Education and Communication
KAPP	Knowledge, Attitude, Practice and Perception
Lao PDR	Lao People's Democratic Republic
LMIC	Low and Middle Income Countries
MDA	Mass Drug Administration
MIF	Minute Intestinal Flukes
N. americanus	*Necator americanus*
N. aperta	*Neotricula aperta*
NaCl	Sodium Chloride
NIOPH	National Institute of Public Health
NTDs	Neglected Tropical Diseases
O. viverrini	*Opisthorchis viverrini*
PCA	Principle Component Analysis
S. mekongi	*Schistosoma mekongi*
S. stercoralis	*Strongyloides stercoralis*
STH	Soil Transmitted Helminth
T. trichiura	*Trichuris trichiura*
WHO	World Health Organization

5.7. Consent for Publication

A written, informed consent to share and disseminate data was obtained from all study participants before enrolment. For children aged below 18 years, the consent was obtained from their parent or legal guardian.

5.8. Trail registration number

Our findings presented here are from a cross-sectional study, therefore, it has not been registered.

5.9. Availability of data and materials

All datasets analysed during the current study are available from the corresponding author upon reasonable request.

5.10. Acknowledgement

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5.11. Conflict of interest

We declare that we have no conflict of interest.

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5.13. Contributors

YV, PO, KA and SS designed the study; SS, YV, SP, KT implemented the study; YV, PO and SS analyzed and interpreted the data; YV wrote the first draft of the manuscript; PO and SS revised the manuscript. All authors read and approved the final version of the manuscript.

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6. Comparison of novel and standard diagnostic tools for the detection of *Schistosoma mekongi* infection in Lao People's Democratic Republic and Cambodia

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Keywords: Cambodia, food-borne trematodes, Kato-Katz, Lao People's Democratic Republic, point-of-care circulating cathodic antigen, *Schistosoma mekongi*, serology, soil-transmitted helminths, UCP-LF CAA.

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Abstract

Background: Given the restricted distribution of *Schistosoma mekongi* in one province in Lao People's Democratic Republic (Lao PDR) and two provinces in Cambodia, together with progress of the national control programmes aimed at reducing morbidity and infection prevalence, the elimination of schistosomiasis mekongi seems feasible. However, sensitive diagnostic tools will be required to determine whether elimination has been achieved. We compared several standard and novel diagnostic tools in *S. mekongi*-endemic areas.

Methods: The prevalence and infection intensity of *S. mekongi* were evaluated in 377 study participants from four villages in the endemic areas in Lao PDR and Cambodia using Kato-Katz stool examination, antibody detection based on an enzyme-linked immunosorbent assay (ELISA) and schistosome circulating antigen detection by lateral-flow tests. Two highly sensitive test systems for the detection of cathodic and anodic circulating antigens (CCA, CAA) in urine and serum were utilized.

Results: Stool microscopy revealed an overall prevalence of *S. mekongi* of 6.4% (one case in Cambodia and 23 cases in Lao PDR), while that of *Opisthorchis viverrini*, hookworm, *Trichuris trichiura*, *Ascaris lumbricoides* and *Taenia* spp. were 50.4%, 28.1%, 3.5%, 0.3% and 1.9%, respectively. In the urine samples, the tests for CCA and CAA detected *S. mekongi* infections in 21.0% and 38.7% of the study participants, respectively. In the serum samples, the CAA assay revealed a prevalence of 32.4%, while a combination of the CAA assay in serum and in urine revealed a prevalence of 43.2%. There was a difference between the two study locations with a higher prevalence reached in the samples from Lao PDR.

Conclusions: The CCA, CAA and ELISA results showed a substantially higher prevalence estimates for *S. mekongi* compared to Kato-Katz thick smears. Active schistosomiasis mekongi in Lao PDR and Cambodia might thus have been considerably underestimated previously. Hence, sustained control efforts are still needed to break transmission of *S. mekongi*. The pivotal role of highly sensitive diagnostic assays in areas targeting elimination cannot be overemphasised.

6.1. Background

Human schistosomiasis is caused by any of six species of blood flukes, namely *Schistosoma mansoni*, *S. japonicum*, *S. haematobium*, *S. mekongi*, *S. intercalatum* and *S. guineensis* (Colley et al., 2014). The latter three species are not only in a clear minority but are also geographically restricted. *S. intercalatum* is endemic along part of Congo River and *S. guineensis* is found in lower Guinea on the African continent, while *S. mekongi* existsin limited areas near the border between Lao People's Democratic Republic (Lao PDR) and Cambodia. Transmission of *S. mekongi* is highly focal (Ohmae et al., 2004; Sinuon et al., 2007) with the overall distribution delineated by environmental variables suitable for the intermediate host snail *Neotricula aperta* (Attwood et al., 2008). The at-risk population is estimated at around 50,000 households comprising an estimated 150,000 people (Ohmae et al., 2004) (Figure 6.1). Infection and re-infection in the endemic areas sustain the severe, chronic consequences of schistosome infection with its various complications (Biays et al., 1999). Due to their high level of water contact, children are at the highest risk, which might result in retardation of growth and cognitive development.

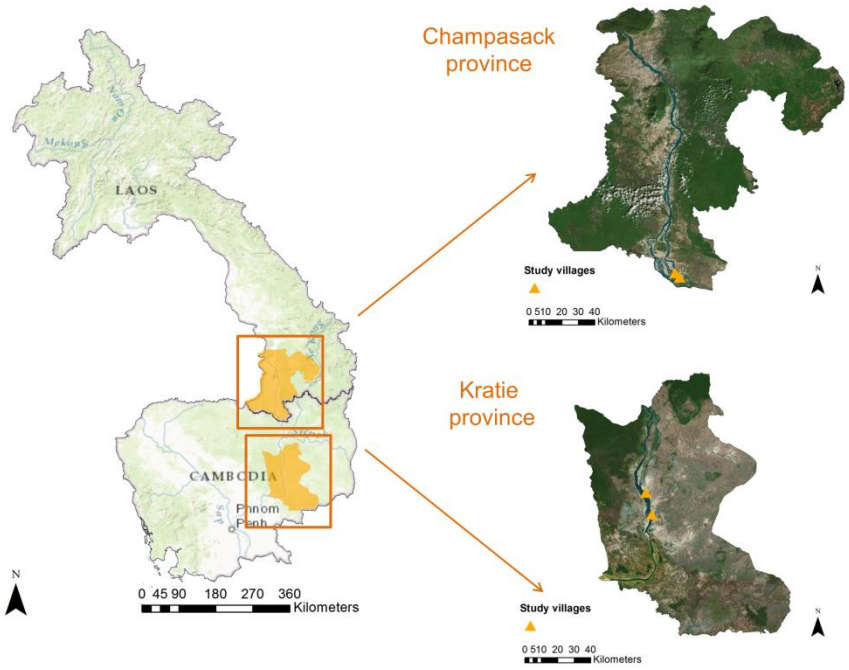

Figure 6.1: Lower course of the Mekong River at its crossing of the border between Lao PDR and Cambodia indicating the study area.

The World Health Organization (WHO) roadmap for elimination of neglected tropical diseases (NTDs) (WHO, 2012b) and the Regional Action Plan for NTDs in the Western Pacific Region for 2012-2016 issued by the WHO's Regional Western Pacific Region Office (WPRO) (Region, Regional action plan for neglected tropical diseases in the Western Pacific (2012-2016). Webdocument, accessed on 25.12.2016 (http://www.wpro.who.int/mvp/documents/ntd_rap_2012_2016/en/)) recommend targeting schistosomiasis mekongi for elimination. Delineation of infection occurrence based on valid documentation is a necessary step to reach this goal and success depends crucially on the availability of highly sensitive diagnostic techniques providing non-equivocal prevalence values in remaining endemic pockets. In mass deworming campaigns, schistosomiasis is treated with oral single-dose praziquantel (40 mg/kg body weight) since the early 1980s when this drug was introduced (Davis and Wegner, 1979). The current approach in communities affected by *S. mekongi* consists of preventive chemotherapy targeting at-risk populations (e.g. the entire population of villages along the Mekong) without prior diagnosis, complemented with the distribution of information, education and communication (IEC) packages and improvement of water, sanitation and hygiene (WASH) whenever resources allow (WHO, 2006). The next stage now being considered is the elimination of this infection as a public health problem. Given its restricted distribution, eradication of *S. mekongi* might be envisaged. However, the preventive chemotherapy programmes implemented in endemic areas in Cambodia and Lao PDR make individuals harbouring mainly light-intensity infections likely to be missed by the standard and widely used Kato-Katz thick smear technique (Zhu et al., 2014) resulting in imprecise assessment of the impact of preventive chemotherapy and other interventions. The solution lies in modifying the methodology applied according to the prevailing diagnostic need (Bergquist et al., 2009b), which obliges assays to be more sensitive and specific when priorities shift from control of morbidity to interruption of transmission followed by surveillance (Bergquist et al., 2009b; Utzinger et al., 2015).

Apart from egg deposition, schistosome worms excrete (regurgitate) a number of different antigens into the host's blood. The circulating anodic antigen (CAA) and its cathodic counterpart (CCA), described by Deelder and colleagues as early as in 1976 (Deelder et al., 1976a), are the most well-studied ones. Their detection in serum and urine has been

followed up with continuously improving techniques, e.g., by De Jonge et al. (de Jonge et al., 1990a), Van Lieshout et al. (van Lieshout et al., 2000), van Dam et al. (van Dam et al., 2004) and Corstjens et al. (Corstjens et al., 2008). Importantly, detection of the antigens in either blood or urine is evidence for an ongoing active infection as both antigens are subject to rapid renal clearance from the human circulation (Deelder et al., 1976b). On the other hand, stool examination is marred by the problem that schistosome eggs can be detected up to several weeks after cure (de Jonge et al., 1990b).

Diagnostic assays should preferably be applied in the field providing results at the point-of-care (POC) to allow appropriate test-and-treat approaches. Diagnostics utilizing the user-friendly, rapid-test platform based on lateral-flow (LF) immunochromatography are well suited for this type of test protocols (Fenton et al., 2009). As an alternative to egg detection in stool samples, a rapid POC assay for CCA detection in urine (POC-CCA) was developed for *S. mansoni* infection (van Dam et al., 2004). The POC-CCA assay is a visually read field assay, which takes about 20 min to perform and which does not require any equipment except the disposables provided with the kit. The colour intensity of the test line on the LF strip has a correlation to the number of eggs in the stool sample investigated (Mwinzi et al., 2015; Standley et al., 2010) and the read-out is at least as sensitive as duplicate Kato-Katz thick smears and considerably less laborious (Casacuberta et al., 2016; Coulibaly et al., 2013; Kittur et al., 2016). Although it was specifically developed for detection of *S. mansoni* infections, it has been shown to be suitable for other intestinal schistosomiasis-causing species such as *S. mekongi* and *S. japonicum* (van Dam et al., 2015a). In order to increase sensitivity and wider applicability to other schistosome species, another LF-based test that detects CAA specifically and is based on the luminescent up-converting phosphor (UCP) reporter technology has been developed (Corstjens et al., 2008). This test is referred to as UCP-LF CAA and includes different formats, depending on the matrix and sample volume used for testing (Corstjens et al., 2014). The UCP-LF CAA test provides an assay applicable for all known *Schistosoma* species (including veterinarian ones) and is assumed to allow detection down to the level of a single worm pair while maintaining 100% specificity (Corstjens et al., 2014). The CAA concentration is considered a good proxy for the number of worms present in the host (Corstjens et al., 2008). This approach, has been shown to work for *S. mansoni* (Corstjens et al., 2015) and *S. haematobium* infections (Knopp et al.,

2015; Stothard et al., 2009) as well as for infections by *S. japonicum* (van Dam et al., 2015b) and *S. mekongi* (van Dam et al., 2015a). In the People's Republic of China, the UCP-LF CAA assay demonstrated a *S. japonicum* prevalence of about 10 times higher than that estimated by triplicate Kato-Katz thick smears (van Dam et al., 2015b). However, unlike the POC-CCA, the current UCP-LF CAA assay format is still a laboratory-based assay due to the need of centrifugation steps, hence not yet convenient for POC test-and-treat approaches.

Realizing that verification of transmission interruption requires a high level of sensitivity, we aimed to evaluate the new diagnostic techniques and to compare their results to the standard tools (e.g. Kato-Katz). We used the UCP-LF CAA assay formats to validate the POC-CCA test results. The POC-CCA test was expected to have higher sensitivity than the stool examination. As an extra control for sensitivity, *Schistosoma* serology based on an enzyme-linked immunosorbent assay (ELISA) was included as all active infections indicated by the POC-CCA assay should test positive with this approach unless the infection was very acquired recently or cured a very long time ago. It has to be noted that a positive test for specific antibodies is not only assured during active infections but indicate also former infections as antibody titres normally persist for a long time. This study compared a set of available assays to get a handle on the real prevalence and intensity of *S. mekongi* infections in the endemic enclaves in Cambodia and Lao PDR as the results should indicate a negotiable way forward with regard to elimination of the disease.

6.2. Methods

6.2.1. Study design, area and population

A cross-sectional study was conducted between February and April 2016 in *S. mekongi*-endemic villages in Lao PDR and Cambodia. Repeated stool examinations for intestinal helminth infections were conducted with particular emphasis on *S. mekongi* infection. Furthermore, urine and serum samples were obtained from each study participant to be tested for *Schistosoma* infection by the POC-CCA, UCP-LF CAA and ELISA assays.

Four villages, two in each of the endemic districts of Lao PDR and Cambodia, respectively, were selected. The villages Som VenOok and Ban Yai VeunSom in Khong District, Champasack Province in southern Lao PDR were selected together with the villages Kbal

Chuor and Sre Khoeurn in Kratié Province in northern Cambodia (Figure 6.1). The main occupation of the villagers was farming and fishing. All household members older than 6 years were enrolled. They were invited to fill in a questionnaire pertaining to demographic details and risk factors for infection, information on hygiene, disease knowledge and anthelminthic drugs taken during the last 6 months.

In Lao PDR, about 200 individuals living in Som VenOok and Ban Yai VeunSom, situated on islands in the Mekong River, were approached about the study. The study households were randomly selected from a list of households of the two villages. In Cambodia, according to the 2008 census, the total population was 2,339 people (1,602 in Kbal Chuor and 737 in Sre Khoeurn). Between 120 and 130 individuals were randomly selected from 30 to 35 households in each village.

6.2.2. Sample collection and handling

Stool samples

Three stool samples were obtained from each participant during five consecutive days. Stool samples were subjected to examination by duplicate Kato-Katz thick smears (41.7 mg stool per smear) examined under a light microscope (Katz et al., 1972b) by an experienced technician within 1 hour after preparation on site in the study villages. Prior to microscopy, the thick smears were allowed to clear for 30 min after set-up. Eggs of all intestinal helminth species were counted and recorded for each species separately. The Kato-Katz thick smear examinations were performed directly in a convenient place in the study village (i.e. the village temple in Lao PDR; the village chief's house in Cambodia).

Serum and urine samples

Blood samples were obtained from each participant, i.e. 5 ml venous blood (taken with vacutainers without anticoagulant) for serodiagnosis of *S. mekongi* infection and for the UCP-LF CAA assay. Urine samples (i.e. 10 ml urine) were obtained for CCA/CAA examination. Blood and urine samples were stored in cool-boxes at around 4°C. In Cambodia, blood samples were centrifuged at Kratié Provincial Hospital a few hours after collection. Coagulated blood samples were centrifuged at 3,000 rpm for 5 min and the

upper part (serum) transferred to fresh tubes that were frozen and kept at -20°C immediately after spinning, while the urine samples were directly frozen at -20°C in the 15 ml-tubes they were collected in (van Dam et al., 2015b).

All samples were transferred frozen to a central national laboratory in Cambodia or Lao PDR and eventually shipped on dry ice to speciality laboratories at Swiss Tropical and Public Health Institute (Swiss TPH) in Basel, Switzerland and Leiden University Medical Center (LUMC) in Leiden, The Netherlands.

6.2.3. Laboratory procedures

Detection of *S. mekongi* antibodies

Schistosoma serology was performed by ELISA at Swiss TPH using *S. mansoni* adult worm extract (AWE) and *S. mansoni* soluble egg antigen (SEA). Both *S. mansoni* antigens show cross-reactivity with antibodies elicited by other *Schistosoma* spp. (*S. haematobium*, *S. mekongi* or *S. japonicum*). The combination of both serological tests exhibits a sensitivity of 94.5% for *S. mekongi* infections and a specificity of 96% and 92% for AWE and SEA, respectively (Ampah et al., 2016; Nickel et al., 2015).

AWE was prepared as described previously (Nickel et al., 2015). In brief, adult *S. mansoni* worms were homogenized in phosphate-buffered saline (PBS) of pH 7.2 containing 2-mM phenylmethylsulfonyl fluoride (***PMSF***). The extract was centrifuged at 80,000 x g for 3 hours at 4°C and the pellet further extracted with PBS containing 1% Nonidet P40. After overnight incubation at 4°C the suspension was centrifuged again in the same way. After the supernatant had been concentrated and centrifuged at 15,300 x g for 5 min at 4°C, it was stored in aliquots at -80°C until use. SEA was made from frozen *S. mansoni* eggs homogenized in PBS of pH 7.2 on ice and subsequently extracted for 3 hours at 4°C. The extract was centrifuged at 100,000 x g for 2 hours at 4°C and the supernatant was stored in aliquots at -80°C until use.

ELISA testing was carried out using Immulon 2HB plates (Thermo Labsystems; Beverly, MA, USA) coated with *S. mansoni* antigens in 0.05 M sodium carbonate buffer (pH 9.6) for 48 hours at 4°C. After washing with tap water containing 0.05% Tween 20, diluted sera (1:160

in PBS, pH 7.2, 0.05% Tween 20) were added to the plates that were incubated for 15 min at 37°C. After additional washing steps, horseradish peroxidase conjugated goat-anti-human-IgG from Kirkegaard & Perry Laboratories (KPL) (http://kem-en-tec-nordic.com/kpl/) was added. Plates were incubated for 15 min at 37°C, subsequently washed and o-phenylendiamine dihydrochloride (OPD) from Sigma (http://www.sigmaaldrich.com), diluted in 0.6-M sodium phosphate buffer of pH 5.0 supplemented with 0.03% H_2O_2, was added. The reaction was stopped with 8-M H_2SO_4 and the absorption read with a Thermo Scientific Multiscan FC reader (http://corporate.thermofisher.com) at 492 nm. The results of the ELISA tests were interpreted according to the cut-offs previously determined by receiver operating characteristic (ROC) analysis with sera from healthy Swiss blood donors, sera from *S. mansoni* infected patients and sera from patients with other parasitic infections as described before (Ampah et al., 2016; Nickel et al., 2015).

Detection of circulating schistosome antigens

This part of the study was carried out at LUMC. The POC-CCA test devices were obtained from Rapid Medical Diagnostics (Pretoria, South Africa) and tests were performed according to the manufacturer's description. The amount of urine analysed per strip was 30 µl applied by pipette. Test results were visually interpreted, including distinction of trace-signals (weak colouration of the test line).

The UCP-LF CAA assay for urine was performed with 2 ml urine (the UCAA2000 assay format) as described earlier (Corstjens et al., 2014). In short, 2 ml urine was extracted with 2-ml 4% (w/v) trichloroactetic acid (TCA). An Amicon centrifugal filtration device was used to concentrate the resulting clear supernatant (approximately 4 ml) to a final volume of 20-30 µl, of which 20 µl was analysed on UCP-LF CAA test strips using the wet-reagents format (Corstjens et al., 2014). CAA concentrations were determined from standard series spiked in a negative urine sample and treated similarly to the clinical urine samples. The quality control (QC) cut-off threshold for singlet testing using the UCAA2000 wet-assay is 0.1 pg CAA per ml urine and the lower limit of detection = 0.05 pg/ml for testing performed in triplicate. Samples generating test results with a concentration between 0.05 and 0.1 pg per

ml were counted as indecisive; samples with test results below 0.05 pg were considered CAA-negative (Corstjens et al., 2014).

The UCP-LF CAA assay for serum was performed with 0.5 ml serum (SCAA500) as described earlier (Corstjens et al., 2014). The procedure was the same as described above with the difference that 0.5 ml TCA serum supernatant was concentrated to a final volume of 20 µl and the QC cut-off threshold was 1 pg CAA per ml serum with the lower limit of detection = 0.5 pg/ml. Samples generating test results with a concentration between 0.5 and 1 pg per ml were counted as indecisive; samples with test results below 0.5 pg were considered CAA-negative (Corstjens et al., 2014).

Note that the ultrasensitive assay format, specifically the SCAA500 test, is considered to allow identification of the majority of all active infections (including single-worm ones) (Corstjens et al., 2014). In order to achieve the highest specificity, results were analysed considering the POC-CCA trace scores as well as the urine- and serum-CAA indecisive scores as negative. As this is a preliminary analysis, we decided to follow a conservative approach (Corstjens et al., 2014). Generally, samples generating test results in the indecisive category would ideally require retesting with a larger sample volume to verify the true infection status.

6.2.4. Statistical analysis

Demographic details of participants and their exposure to infection were obtained by questionnaire. Data were digitally collected using electronic tablets. The questionnaires and forms were developed in Commcare (http://www.commcarehq.org) format using the open data kit (ODK) programme (version 2.8) that was installed on the tablets for field data collection. Statistical analyses were performed in STATA version 13.1 (Stata Corp.; College Station, TX, USA). Only results from participants who had completed their questionnaires and stool examination were included in the final analysis.

The intensity of infection, expressed as eggs per gram of stool (EPG) obtained from Kato-Katz thick smear examinations were classified as light, moderate or heavy (Maleewong et al., 1992a; WHO, 2002a). The χ^2-test was used to examine the association of categorical

variables. The Spearman rank correlation test was used to correlate the results of the different diagnostic tests with each other. Spearman *r*- and *p*-values were reported. A *p*-value below 5% was considered statistically significant.

The *Schistosoma* ELISA assay (a marker of former or active infection) was composed of two separate assays, one based on AWE and the other based on SEA. For this study both these ELISAs were combined and an overall interpretation of both test results was applied. A result was interpreted as positive if at least one of the two ELISA tests was positive. A result was interpreted as inconclusive if both ELISA tests generated an inconclusive result. A result was considered negative if both ELISA were negative.

Combined reference

We compared the diagnostic performance of all our stool, urine- and serum-based diagnostic tests to a combined reference. This consisted of a combination of test results of the Kato-Katz test, the urine-CAA test and the CAA-serum assay, all assays with a very high specificity, in particular as we followed a conservative approach accepting a relatively high cut-off threshold for the CAA tests. The sensitivity, specificity, positive and negative predictive values were calculated for all diagnostic tests based on this composite measure. For these calculations, traces and indecisive tests results of CAA and ELISA were taken as negative results. The urine- and serum-CAA tests were also combined into a total CAA outcome, which was deemed as positive when at least one of the two tests produced a positive outcome. This approach of comparing assays to a combined reference is a widely recognized method for assessment of diagnostic tests in the absence of a highly sensitive and specific 'gold standard' method and has been recommended by the WHO/TDR Diagnostics Evaluation Expert Panel (Panel et al., 2010).

6.3. Results

6.3.1. Study population

Data records could be completed for a total of 377 persons and they were included in the analysis carried out as shown schematically in Figure 6.2. Of these, 196 (52.0%) were from Cambodia and 181 (48.0%) from Lao PDR. The age of the participants ranged from 6 to 79 years with a median of 25 years; slightly more females than males were enrolled (52.3% *versus* 47.8%). About half of the participants had finished primary school (53.3%); most of them were subsistence rice farmers and fishermen (61.3%). The social and demographic characteristics of study participants are summarised in Table 6.1.

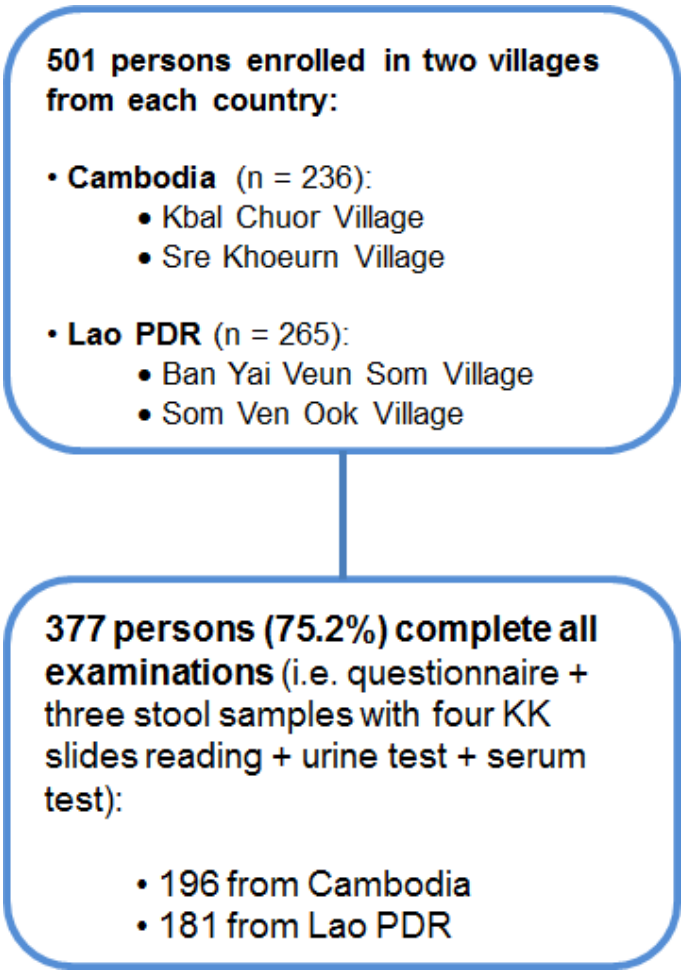

Figure 6.2: Study diagram

Table 6.1: Demographic characteristics of the study participants

Parameter	Overall n (%)	Cambodia n (%)	Lao PDR n (%)	χ^2 *	*P-value* *
Number of subjects	377 (100)	196 (52.0)	181 (48.0)		
Age (years)					
Median (IQR)※	25 (12-44)	14 (11-35)	35 (15-47)	NA	NA
Sex					
Male	180 (47.8)	101 (51.5)	79 (43.7)		
Female	197 (52.3)	95 (48.5)	102 (56.4)	2.3	0.126
Age group (years)					
≤ 9	40 (10.6)	25 (12.8)	15 (8.3)		
10-16	116 (30.8)	82 (41.8)	34 (18.8)		
17-36	92 (24.4)	43 (21.9)	49 (27.1)		
37-50	68 (18.0)	22 (11.2)	46 (25.4)		
≥ 51	61 (16.2)	24 (12.2)	37 (20.4)	33.5	<0.001
Educational level					
Illiterate	11 (2.9)	0	11 (6.1)		
Primary school	201 (53.3)	101 (51.5)	100 (55.3)		
Secondary school	134 (35.5)	95 (48.5)	39 (21.6)		
High school	23 (6.1)	0	23 (12.7)		
≥Technical school	8 (2.1)	0	8 (4.4)	64.9	<0.001
Occupation					
Farmer/fisherman	231 (61.3)	101 (51.5)	130 (71.8)		
Student	146 (38.7)	95 (48.5)	51 (28.2)	16.3	<0.001

(IQR)※: Inter quantile range; NA: Not applicable, * comparison between countries

6.3.2. Egg detection

The status of the participants according to helminth infection intensity categories is shown in Table 6.2. Overall, *S. mekongi* infection prevalence was 6.4% (24/377) with a much higher prevalence of 12.7% (23 positives) in Lao PDR, compared to 0.5% (one positive) in Cambodia. The overall results for prevalence of other helminth infections, such as *O. viverrini*, hookworm, *T. trichiura*, *A. lumbricoides* and *Taenia* spp. were 50.4%, 28.1%, 3.5%, 0.3% and 1.9%, respectively. Significantly higher prevalence rates were found for *O. viverrini* (90.1%), hookworm (50.8%) and *Taenia* (3.3%) in Lao PDR. Multiparasitism was observed in both countries with much higher frequency in Lao PDR than in Cambodia. Table 6.3 shows infection intensity categories recorded as EPGs. All infections were found

to be light in Cambodia, while a large number of the *O. viverrini* infections were of moderate intensity in Lao PDR; some heavy infections (4 out of 181) were also identified there.

Table 6.2: Prevalence of *S. mekongi, O. viverrini* and other helminth infections among all study participants according to Kato-Katz examination

Subject/Helminth species	Overall (%)	Cambodia (%)	Lao PDR (%)	χ^2 *	*P-value* *
Number of subjects	377 (100)	196 (52.0)	181 (48.0)		
Trematode					
Schistosoma mekongi	24 (6.4)	1 (0.5)	23 (12.7)	23.5	<0.001
Opisthorchis viverrini	190 (50.4)	27 (13.8)	163 (90.1)	219.0	<0.001
Nematode					
Hookworm	106 (28.1)	14 (7.1)	92 (50.8)	88.9	<0.001
Ascaris lumbricoides	1 (0.3)	0	1 (0.6)	1.1	0.297
Trichuris trichiura	13 (3.5)	7 (3.6)	6 (3.3)	0.02	0.892
Cestode					
Taenia spp.	7 (1.9)	1 (0.5)	6 (3.3)	4.1	0.044
Multiparasitism					
Non-infection	157 (41.6)	150 (76.5)	7 (3.9)		
Single infection	115 (30.5)	42 (21.4)	73 (40.3)		
Double infection	90 (23.9)	4 (2.0)	86 (47.5)		
Triple infection	14 (3.7)	0	14 (7.7)		
Quadruple infection	1 (0.3)	0	1 (0.6)	228.1	<0.001

* comparison between countries

Table 6.3: Intensity of helminth infections among the infected study participants according to Kato-Katz examination

Subject/Type of infection	Overall (%)	Cambodia (%)	Lao PDR (%)
Number of subjects	377	196	181
Schistosoma mekongi			
Light infection	24 (100)	1 (100)	23 (100)
Opisthorchis viverrini			
Light infection	116 (61.1)	27 (100)	89 (54.6)
Moderate infection	70 (36.8)	0	70 (42.9)
Heavy infection	4 (2.1)	0	4 (2.5)
Hookworm			
Light infection	104 (98.1)	14 (100)	90 (97.8)
Moderate infection	2 (1.9)	0	2 (2.2)
Ascaris lumbricoides			
Light infection	1 (100)	0	1 (100)
Trichuris trichiura			
Light infection	12 (100)	6 (100)	6 (100)

6.3.3. *Antigen detection*

In total, 377 urine and serum samples were tested for *S. mekongi* infection (Table 6.4). In the urine samples, the CCA- and CAA-based test formats detected *S. mekongi* infections in 21.0% and 38.7% of all subjects, respectively. Compared to Cambodia, both urine tests diagnosed a higher *S. mekongi* prevalence in Lao PDR: 23.8% *versus* 18.4% with respect to CCA, and 42.5% *versus* 35.2% with respect to CAA. In serum, the latter test format detected a 32.4% overall prevalence with a similar difference between the two countries as found with the urine samples, 26.0% for Cambodia *versus* 39.2% for Lao PDR.

Table 6.4: Diagnosis of *S. mekongi* infection using serum and urine samples (n=377)

Method/Type of sample	Overall (%)	Cambodia (%)	Lao PDR (%)	χ^2	*P-value*
Urine					
POC-CCA					
negative	174 (46.2)	97 (49.5)	77 (42.5)		
trace	124 (32.9)	63 (32.1)	61 (33.7)		
positive	79 (21.0)	36 (18.4)	43 (23.8)	2.4	0.308
UCAA					
negative	206 (54.6)	110 (56.1)	96 (53.0)		
indecisive range	25 (6.6)	17 (8.7)	8 (4.4)		
positive	146 (38.7)	69 (35.2)	77 (42.5)	4.0	0.133
Serum					
SCAA					
negative	240 (63.7)	133 (67.9)	107 (59.1)		
indecisive range	15 (4.0)	12 (6.1)	3 (1.7)		
positive	122 (32.4)	51 (26.0)	71 (39.2)	10.9	0.004
ELISA combined*					
negative	115 (30.5)	76 (38.8)	39 (21.6)		
equivocal	132 (35.0)	68 (34.7)	64 (35.4)		
positive	130 (34.5)	52 (26.5)	78 (43.1)	16.7	<0.001
Combined Reference**					
negative	203 (53.8)	121 (61.7)	82 (45.3)		
positive	174 (46.2)	75 (38.3)	99 (54.7)	10.2	0.001

AWE= adult worm antigen; SEA= soluble egg antigen; *either AWE or SEA positive; **at least one of the three tests (UCAA, SCAA, Kato-Katz) positive

6.3.4. Detection of *S. mekongi* antibodies

The combined results of the two ELISA tests were positive in 34.5% of study participants, with a more than 16% higher rate in Lao PDR than in Cambodia (43.1% *versus* 26.5%). For all the diagnostic tests performed, the positivity rates were statistically significantly higher in Lao PDR compared to Cambodia (Table 6.4).

6.3.5. Analysis of tests using the combined reference

We defined an active infection as an individual found positive for CAA (in urine or serum) or with a positive Kato-Katz thick smear. Table 6 shows the calculated sensitivity and specificity and the predictive values of the urine and serum tests in relation to this composite measure. The combined CAA tests had the highest calculated sensitivity (93.7%) followed by the urine- (83.9%) and serum-CAA (70.1%) test. The combined ELISA tests had a calculated sensitivity of 52.9% and a specificity of 81.3% against this combined reference. Triplicate Kato-Katz and single POC-CCA had a comparatively low sensitivity of 13.8% and 24.1%, respectively, and a negative predictive value of 57.5% and 55.7%, respectively.

Table 6.5: Diagnostic characteristics of the various tests to diagnose *S. mekongi* infection using a combined reference [a]

Method	**ELISA [b]**	**Kato-Katz**	**POC-CCA**	**CAA**	**CAA**	**CAA**
Target	**Antibodies**	**Parasite eggs**		**Circulating antigens**		
Sample	**Serum**	**Faeces**	**Urine**	**Urine**	**Serum**	**Serum+urine**
	(%)	(%)	(%)	(%)	(%)	(%)
Sensitivity	52.9	13.8	24.1	83.9	70.1	93.7
Specificity	81.3	100	81.8	100	100	100
PPV*	70.8	100	53.2	100	100	100
NPV**	66.8	57.5	55.7	87.9	79.6	94.9

*Positive predictive value; **Negative predictive value; [a] Infection-positive by either egg- or CAA-positivity (serum and urine combined, assuming 100% specificity of the CAA result). [b] For the ELISA, either AWE and/or SEA positive was considered positive;

6.3.6. Analysis with respect to age and sex

Table 6.6 shows the positivity rate of the different diagnostic tests in relation to sex and age-groups. In general, all tests showed a higher positivity rate in males. A peak of positivity can be observed for the CAA tests in age group 10-16 years. The ELISA results did not decrease with age to the same extent which could be explained by persistence of antibody titres for long time even after cured infections.

Table 6.6: Sex and age distribution of *S. mekongi* infection: results of various approaches

Method	ELISA	Kato-Katz	POC-CCA	CAA	CAA	CAA
Target	Antibodies	Parasite eggs		Circulating antigens		
Sample	Serum	Faeces	Urine	Urine	Serum	Serum+urine
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Sex						
Male	68 (37.8)	15 (8.3)	39 (21.7)	71 (39.4)	65 (36.1)	80 (44.4)
Female	62 (31.5)	9 (4.6)	40 (20.3)	75 (38.1)	57 (28.9)	83 (42.1)
Age group (years)						
≤ 9	3 (7.5)	1 (2.5)	6 (15.0)	8 (20.0)	6 (15.0)	9 (22.5)
10-16	44 (37.9)	2 (1.7)	25 (21.6)	56 (48.3)	53 (45.7)	64 (55.2)
17-36	36 (39.1)	10 (10.9)	23 (25.0)	39 (42.4)	27 (29.4)	42 (45.7)
37-50	25 (36.8)	6 (8.8)	9 (13.2)	22 (32.4)	19 (27.9)	24 (35.3)
≥ 51	22 (36.1)	5 (8.2)	16 (26.2)	21 (34.4)	17 (27.9)	24 (39.3)

6.3.7. Correlation analysis

Correlation analysis of the different diagnostic tests showed positive and statistically significant correlations between urine- and serum-CAA (r=0.64, p<0.001) and combined ELISA tests with serum-CAA (r=0.55, p<0.001) and urine-CAA (r=0.38, p<0.001). Furthermore, weakly positive but statistically significant correlations were detected between the infection intensity results of Kato-Katz and ELISA (r=0.14, p=0.005), POC-CCA (r=0.12, p=0.017), and urine (r=0.11, 0.005) and serum-CAA (r=0.17, p=0.001) (Figure 3). The correlation of the POC-CCA test results with the other tests were all weakly positive but statistically significant for urine-CAA (r=0.15, p=0.003) and serum-CAA (r=0.14, p=0.005). The correlation between the test results of POC-CCA and ELISA were weakly positive but not statistically significant (r=0.09, p=0.083).

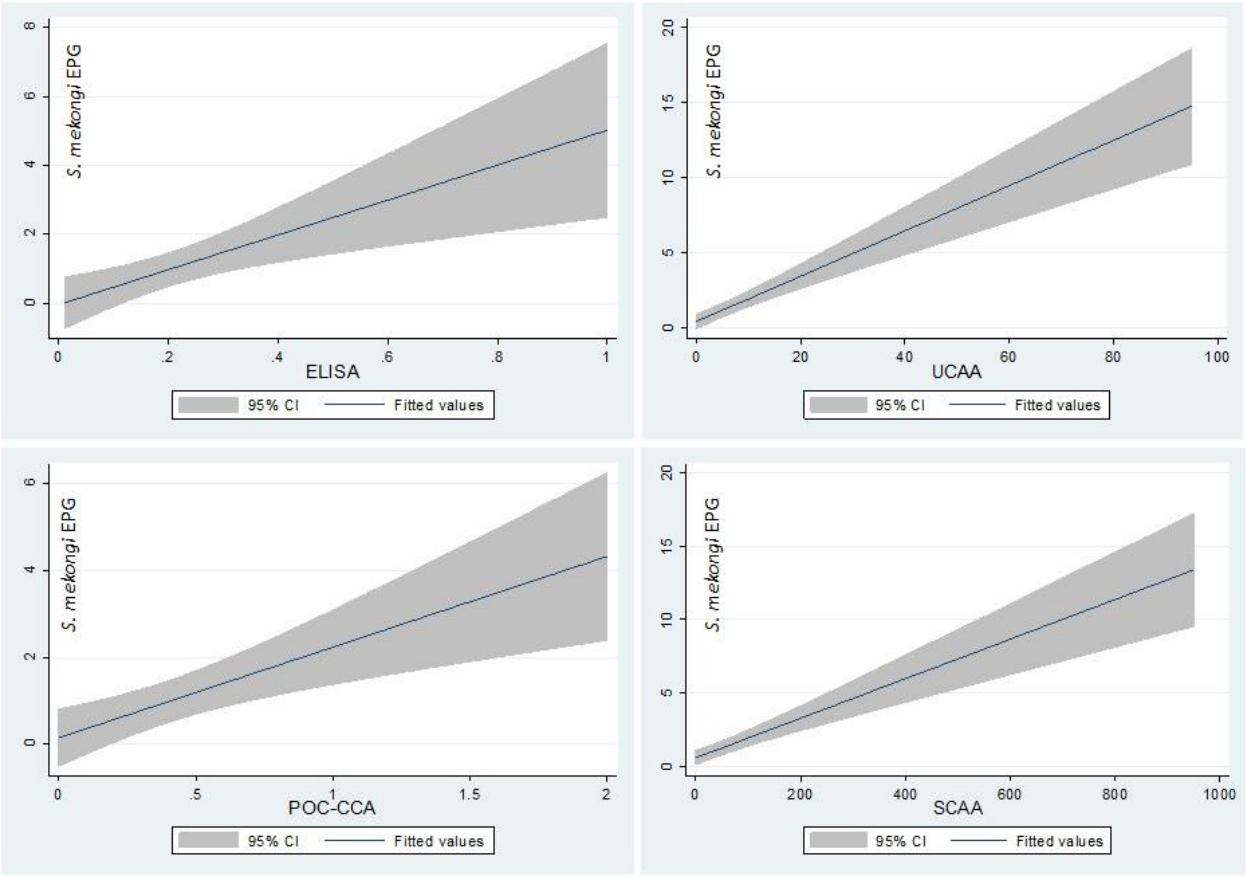

Figure 6.3: Correlation between combined ELISA (top left), POC-CCA (bottom left) and urine (top right) and serum CAA (bottom right) and infection intensity of *Schistosoma mekongi* (eggs per gram stool).

6.4. Discussion

The implementation of preventive chemotherapy has decreased schistosomiasis morbidity in endemic countries worldwide, including *S. mekongi* affected areas in Cambodia and Lao PDR (Keang et al., 2007; Muth et al., 2010). The current lower intensity of disease, however, is a compelling fact to recommend replacing stool examination using Kato-Katz with more sensitive diagnostic tools. Serology based on antibody detection is a helpful adjunct, but in order to determine cure and the level of worm burdens detection, assays based on the detection of circulating antigens are required. This approach has been successfully used for all major schistosome species showing that it is 10-20 times more sensitive than standard stool microscopy (Corstjens et al., 2008).

WHO recommends targeting schistosomiasis mekongi for elimination as the endemic areas are very limited and both stool examination according to Kato-Katz and antibody testing using ELISA serology indicate low intensity of disease after several rounds of preventive chemotherapy (WHO, 2012b). However, as has already been shown in the Peoples' Republic of China, highly sensitive tests for schistosome circulating antigens give considerably higher prevalence results than Kato-Katz (van Dam et al., 2015b). With the proof-of-principle of testing for excreted antigens in the urine shown for *S. mekongi* (van Dam et al., 2015a), it was now felt that a field study in the endemic areas in southern Lao PDR and northern Cambodia would be warranted to establish this approach. In contrast to antibody detection, the Kato-Katz stool examinations along with the tests for circulating schistosome antigens (POC-CCA and UCP-LF-CAA) are all indicators of active infections. Antibody titres can persist for very long time after cure and therefore serology is not suitable for assessing treatment outcomes or as single diagnostic approach for detection of active infections.

In the field, detection of active infection and cure are all highly important, particularly when moving from control of a disease to transmission interruption and elimination. It is equally important for the individual patient. While the better sensitivity of antigen detection compared to Kato-Katz is obvious, it is also clear that CAA detection (both in serum and urine) performs much better than CCA. These results are in agreement with previous reports for *S. japonicum* and *S. mekongi* (Van 't Wout et al., 1995; van Dam et al., 2015a; van Dam et al., 2015b).

The advantage of the POC-CCA test is that it is a standardized urine test applicable in the field without the need for any extra equipment (fulfilling all 'ASSURED' characteristics). It has been mainly and widely validated for *S. mansoni* detection, but shows limited use for the other schistosome species (Kittur et al., 2016). However specificity is limited to some extent, because CCA has epitopes common with certain human components (Lewis-X structures) that sometimes end up in the urine causing false positive reactions (Polman et al., 2000). The UCP-LF CAA test, on the other hand, is applicable for all schistosome species and for various human liquid samples, such as urine and serum, as well as potentially saliva (Corstjens et al., 2014). In contrast to the POC-CCA assay, the UCP-LF CAA test format is not yet commercially available nor is its current format applicable for POC application because

of a sample preparation procedure and the use of an UCP strip-reader. While the cost of the former is USD 1-1.5 per test, that of the latter, being a manual laboratory test, is at least 10-fold higher. However, as shown here, the UCP-LF CAA test does display a superior sensitivity by concentration of the clinical sample and may therefore detect single-worm infections (Corstjens et al., 2014). Still, as our results show that the POC-CCA assay is applicable for field diagnosis of *S. mekongi*, this assay should be the approach of choice for schistosomiasis diagnosis in Lao PDR and Cambodia with the current infrastructure.

We found a strong correlation of the test results of the urine and serum CAA tests and ELISA, while the correlations between the two CAA tests and the Kato-Katz and POC-CCA were weaker. These observations are consistent with previous studies in the People's Republic of China (van Dam et al., 2015b) and elsewhere (Knopp et al., 2015; Lamberton et al., 2014) and are largely a reflection of the different sensitivities of these diagnostic tests.

It should be mentioned that the results presented here are interpreted rather conservatively with respect to the cut-off threshold, leaving the POC-CCA trace scores and the UCP-LF CAA indecisive values as negatives. A more detailed comparison of the different assays using e.g., latent class analysis may shed a better insight in the actual status of trace and indecisive samples. Such additional analyses, incorporating also a quantitative analysis of the POC-CCA results using a gold strip reader, are being planned.

In agreement with previous evaluations of the various assays for circulating schistosome antigens in areas endemic for other schistosome species, we found that the POC-CCA is both more rapid and more sensitive than multiple Kato-Katz thick smears. In the present study the number of positives identified by POC-CCA was significantly higher than those found by Kato-Katz in both counties. These results are in accordance with published results which showed that POC-CCA prevalence was between 1.5- and up to 6-fold higher than Kato-Katz prevalence estimates in areas with low infection intensity (Kittur et al., 2016). The comparable cost levels per determination for POC-CCA and Kato-Katz (Sousa-Figueiredo et al., 2009; Worrell et al., 2015) should not prevent the application of the rapid test in national schistosomiasis control programmes. Furthermore, people are more likely to provide urine samples than any other type of sample, leading to higher compliance.

While eggs continue to be excreted by the host for a few weeks after cure, both CCA- and CAA-levels drop quickly, sometimes turning negative within 1 week after treatment (de Jonge et al., 1989; Lamberton et al., 2014), making this approach a promising tool to monitor drug efficacy. The sensitivity of CCA-based tests is not as high as what the UCP-LF CAA assay or what DNA-based detection methods can offer (Lodh et al., 2013; Obeng et al., 2008), while the ultrasensitive SCAA500 format of the UCP-LF CAA test surpasses PCR in sensitivity (Stothard et al., 2014; Wilson et al., 2006). As many different diagnostic assay systems are now available, planning to assess geographic areas potentially endemic for schistosomiasis, multiple diagnostic approaches should be compared taking into account modelling and statistical methods in combination with knowledge how biological systems operate (Knopp et al., 2015; Koukounari et al., 2013).

6.5. Conclusion

Where low egg counts are most common, such as in areas characterised by low endemicity slated for elimination, the sensitivity and specificity of diagnostic tests must be taken into account when deciding which approach to choose. CCA-based assays are already available for use in the field, but tests targeting CAA still need the laboratory due to some of the sample preparation steps. Although the latter approach is the most sensitive antigen test, it would still be useful to apply POC-CCA testing for screening. While the results presented here will be subjected to further analysis, it would be useful to start planning for wider testing including application of geographical information systems (GIS) to establish the real boundaries of the areas endemic for *S. mekongi*, prevalence and intensity of disease before moving on to transmission control and eventual elimination of the disease in Cambodia and Lao PDR.

6.6. Declarations

6.6.1. Ethics approval and consent to participate

The study was approved by the ethics committees in Lao PDR (070 NIOPH/NECHR, 4 December 2015) and Cambodia (394 NECHR, 10 November 2015). A written informed consent was obtained from all study participants. Helminth infections diagnosed during the study were treated according to the national treatment guidelines, i.e. praziquantel (single

oral 40mg/kg body weight) for *S. mekongi* and *O. viverrini* infection and albendazole (single oral dose 400 mg) or mebendazole (single oral dose 500 mg) for soil-transmitted helminth infections.

All parasitic infections diagnosed were treated with the standard treatment regimens recommended by the Ministry of Health in each country (MOH, 2004).

6.6.2. Consent for publication

Not applicable

6.6.3. Availability of data and material

Please contact author for data requests

6.6.4. Competing of interests

We declare that we have no conflict of interest.

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6.6.6. Author's contributions

SS, VK, RB, GJvD, BN, JU, SM and PO designed the study; YV, SS, VK and SM implemented the field work; GJvD, PTH, PL, BN and HM performed the diagnosis in urine and serum samples; YV, SS, VK, GJvG, PTH and PO performed the analysis; YV, RB, PO wrote the first draft and all other authors contributed to the writing; All authors read and approved the final manuscript.

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7. Improved latrines have a small short term impact the transmission of *Schistosoma mekongi, Opisthorchis viverrini* and other helminth infections on Mekong islands, Southern Lao PDR

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Abstract

Schistosoma mekongi and *Opisthorchis viverrini* are other helminth infections are highly endemic in Lao People's Democratic Republic (Lao PDR). In many rural parts sanitation is virtually absent. Improvements of water, sanitation and hygiene are essential to reduce helminth transmission. We assessed the short-term effect of improved latrines and their use on the transmission of *S. mekongi* and *O. viverrini* and other intestinal helminth infections on Mekong islands, in Southern Lao PDR.

We performed an experimental study in four villages where latrine construction intervention. We compared pre- (baseline) and post-intervention (follow-up) helminth infection rates with those from control villages. Household-based promoting latrine construction was conducted. Kato-Katz and Baermann techniques were used for assessing intestinal helminth infections. After baseline assessment a mass-drug administration was conducted for all diagnosed helminth infections.

The helminth infection rates at baseline for *S. mekongi*, *O. viverrini*, hookworm, *Ascaris lumbricoides*, *Trichuris trichiura*, *Enterobius vermicularis*, *Strongyloides stercoralis* and *Taenia* spp. were 16.7%, 76.1%, 56.3%, 0.4%, 2.8%, 0%, 41.0% and 0.4%, respectively. At follow-up they were reduced to 13.7%, 64.5%, 31.6%, 2.2%, 2.0%, 0.8%, 32.8% and 0.4%, respectively. Reduction in prevalence of four important helminthiasis were significantly observed (baseline vs follow-up, *P-value*), *S. mekongi* (28.6% vs 22.6%, *P-value*<0.001)*, O. viverrini* (79.5% vs 68.2%, *P-value*<0.001), Hookworm (48.8% vs 26.2%, *P-value*<0.001) and *S. stercoralis* (43.1% vs 31.1%, *P-value*<0.001) in the intervention villages. While *S. mekongi* infection in the control villages was increased (1.8% vs 2.6%, *P-value*=0.74) and other helminths were decreased *O. viverrini* (71.8% vs 59.9%, *P-value*=0.027), Hookworm (65.6% vs 38.3%, *P-value*<0.001) and *S. stercoralis* (38.3% vs 34.8%, *P-value*=0.001).

In conclusion, this study revealed that short term impact of the utilisation of the improved latrines had small impact on the transmission of *S. mekongi, O. viverrini* and other helminth infections on Mekong islands, Southern Lao PDR. Most probably are the latrine interventions effective only at on a longer-term basis.

7.1. Introduction

Globally, an estimated 2.5 billion people do not have access to adequate sanitation facilities (WHO/UNICEP, 2014). In developing regions almost half of the population does not have access to sanitary facilities and an estimated 1 billion people practice open defecation, exposing themselves and their communities to major health risks (WHO, 2014). Sanitation facilities and their proper utilisation play a key-role for adequate hygienic conditions in a community. Absence or non-utilisation of sanitation favours the transmission of a range of communicable diseases, including viral, bacterial and parasitic infections (Awoke and Muche, 2013; Clasen et al., 2014; Ziegelbauer et al., 2012) which lead a wide array of disease outcomes which representing large public health burden.

In rural parts of Lao People's Democratic Republic (Lao PDR) sanitation coverage is very low. Overall the coverage of improved sanitation is less than 50% with a large difference between urban and rural area; More than 80% in urban and 40% or less in rural areas (UNICEF, 2009). Helminth infections linked to open defecation are highly prevalent. For instance, on Mekong island in the southern province of Champasack (Khong district) the infection rates of liver fluke (*Opisthorchis viverrini*) exceed 60% in most communities (Forrer et al., 2012; Lovis et al., 2009; Sayasone et al., 2011). This parasite is responsible for the development of a fatal liver cancer (cholangiocarcinoma) (Sripa et al., 2011b). Furthermore, Mekong schistosomiasis (*Schistosoma mekongi*) is endemic in this setting leading to intestinal and hepatobiliary diseases (Sayasone et al., 2011; Urbani et al., 2002; Vonghachack et al., 2015). In addition, soil-transmitted helminthiasis (STH) such as *Ascaris lumbricoides*, *Trichuris trichiura,* hookworm and *Strongyloides stercoralis* are highly endemic (Sayasone et al., 2011; Vonghachack et al., 2015). Although treatment is available re-infection rates are high as the main transmission route through open defecation is not altered (Jia et al., 2012; Sayasone et al., 2007).

Given the high helminth infection rates and the low coverage of latrines we hypothesise that improved sanitation has an immediate impact on the transmission of intestinal helminth infections. Hence, the objective of this study was to assess the impact of improved sanitation and its use on the re-infection rates of intestinal helminth infections in highly

endemic areas. We conducted an intervention study in two villages where after a base-line assessment of helminth infection and a mass-drug administration (MDA) with anti-helminthic drug latrines were constructed. The impact of the change was evaluated one year after the latrines were present in the villages. The results were compared with two control villages where no latrines were built.

7.2. Materials and methods

7.2.1. Ethical Considerations

Approval to conduct the study was granted by the Lao National Ethics Committee for Health Research (NECHR), Ministry of Health, Lao PDR. A letter describing the study, its purpose, methods, potential risks, benefits of participation, and the protection of confidentiality was given to all eligible participants. All study participants and parents or legal guardians of children below the age of 15 years consented and all children assented to take part in the study. All infections diagnosed were treated with a single dose of albendazole (400mg) and praziquantel (40mg/kg) according to the Lao national treatment guidelines (MoH., 2004). Those infected with *S. stercoralis* were treated with a single 200µg/kg dose of ivermectin free of charge (Satoh and Kokaze, 2004; Suputtamongkol et al., 2011).

7.2.2. Study area and population

The study was carried out in March 2011 to January 2013 on three islands, i.e. Donlong, Donthan and Donlieng island located in the Mekong River in Khong district, Champasack province, southern Laos. Donlong island composes of four villages, namely Haulong, Longsong, Longkang and Hanglong village which were the intervention village group whereas Donthan and Donlieng islands compose of one village each namely Donthan and Donlieng village, respectively which were the control village group. Details of village population were described elsewhere (Vonghachack et al., 2015). The study islands represent typical Mekong islands of the Khong district. The study villages were selected according to the report of Provincial Health Office as very low proportion of households with latrines. Twenty to thirty households were chosen randomly from the households list of the head of the village, using a simple random sampling procedure. All household members aged 2 years or older were invited to participate in the study.

7.2.3. Intervention

An experimental pre-test and post-test with one control group was used to assess the effects of latrine in the study villages on preventing of helminth infections particularly *O. viverrini* and *S. mekongi*. Household-based promoting latrine construction was conducted. There were three different phases of the study as described below and illustrated in Figure 7.1.

First, a cross-sectional baseline survey was carried out in March 2011 to assess intestinal parasitic infections, and people's knowledge, attitudes, perception and behaviour (KAPB) about latrine, personal hygiene and raw food consumption in both intervention and control villages. After the cross-sectional study, all inhabitants aged 4 years and above from the intervention and control villages were offered treatment as mass drug administration approach (MDA), free of charge, with praziquantel (single 40 mg/kg oral dose) and albendazole (single 400 mg oral dose) (MoH., 2004). It was called first MDA.

Second, in the intervention villages each household committed to construct a latrine. Within a period of 9 months more than 300 were constructed. The project subsidized the lining of the pit and the slab while the rest of the construction was conducted by the household members. After the latrine construction, all individuals living in both settings (control and intervention areas) received another full MDA, free of charge, with praziquantel and albendazole. Those infected with *S. stercoralis* were treated with a single 200μg/kg dose of ivermectin free of charge in this phase (Satoh and Kokaze, 2004; Suputtamongkol et al., 2011).

Finally, a follow-up survey was carried out 12 months later after the second MDA, using the identical survey methodology as at base-line.

7.2.4. Field and laboratory procedures

Within the baseline and follow up surveys, parasitological methods were used the same process. Two stool samples were collected per study participants within a five day period. Each sample was examined by using Kato-Katz thick smears technique (Katz et al., 1972a) and Baermann technique. Kato-Katz and Baermann tests are described in detail elsewhere (Khieu et al., 2013; Sayasone et al., 2011). In brief, a single Kato-Katz thick smear was prepared for each stool sample and examined within 1h of preparation. Approximately 5g of each stool sample was divided from each stool sample for performing Baermann test (Garcia and Bruckner, 2001). The stool sample was placed on a gauze-lined mesh in a glass funnel equipped with a rubber tube and a clamp, and covered with de-chlorinated tap-water. After 2h, the water (approx. 50ml) was centrifuged and the sediment was examined under a microscope for *S. stercoralis* larvae (L1-stage). Helminth eggs were counted and recorded for each species separately to obtain species-specific infection intensity estimates.

7.2.5. Risk factors assessment by questionnaire

The questionnaire was used to collect information at the individual level, e.g. personal perception and behaviour about latrines and their construction, the use of toilets in daily life, and personal hygiene. Raw and insufficiently cooked food consumption behaviour (fish, pork, beef, and vegetable) and daily life activities were also investigated. The questions on socio-economic status (SES) were assessed at the household level. Head of the family who could be either husband or wife were asked to provide the information on household assets including electric devices, engines (motorcycle, truck, engine boat,…), agricultural land and livestock ownership, construction material of house and latrines.

7.2.6. Data management and analysis

Data were double-entered into EpiData (version 3.1) and validated (Epidata Association; Odense, Denmark). Discrepancies were cross-checked against the data sheets. Study participants were subdivided into five age groups, namely (i) ≤9 years, (ii) 10–16 years, (iii) 17–35 years, (iv) 35–50 years, and (v) ≥55 years. The principal component analysis (PCA) was used to define the indicator. SES conditions in the household were categorized into five

wealth quintiles as (i) most poor, (ii) very poor, (iii) poor, (iv) less poor and (v) least poor according to their cumulative standardized asset scores. Details of this widely used approach have been presented elsewhere (Sayasone et al., 2011). The intensity of helminth egg counts was expressed as eggs per gram of stool (EPG). The Infection intensity was categorized into intensity groups based on WHO recommendations into light (*S. mekongi*: 1-100 EPG, *O. viverrini*: 1-999 EPG, *A. lumbricoides*: 1-4,999 EPG, *T. trichiura*: 1-999 EPG and hookworm: 1-1,999 EPG), moderate (*S. mekongi*: 101-400 EPG, *O. viverrini*: 1,000-9,999 EPG, *A. lumbricoides*: 5,000-49,999 EPG, *T. trichiura*: 1,000-9,999 EPG and hookworm: 2,000-3,999 EPG), and heavy infection intensities (*S. mekongi*:≥400 EPG, *O. viverrini*: ≥10,000 EPG, *A. lumbricoides*: ≥ 50,000EPG, *T. trichiura*: ≥ 10,000 EPG and hookworm ≥4,000 EPG), respectively (Maleewong et al., 1992b; Sayasone et al., 2009b; WHO, 1995, 2002c).

All statistical analyses were conducted in STATA version 13.1 (Stata Corporation; College Station, TX, USA). Only those individuals with a complete data records, i.e. who had two KK thick smear readings, at least one Baermann test result, and a complete questionnaire were included in the final analyses. Chi-square test was used to assess any differences in prevalence rates between intervention and control villages. Multiple logistic regression was used to assess the association intervention (intervention *versus* control group), surveys (follow-up *versus* base-line survey) and their interaction factor (interventions * surveys) on helminth infections and risk factors observations (outcome). Odds ratio (OR), its 95% confidence interval (95% CI) and the *p*-value were retained. A *p*-value below 5% was considered statistically significant.

7.3. Results

7.3.1. Demographic characteristics of participants

Complete parasitological and questionnaires data were obtained for 510 individuals from 1,128 enrolled individuals in 247 households (Fig. 7.1). Of these, 283 and 227 individuals belonged to intervention and control villages, respectively. 52.9% were female and age ranged from 2-95 years which 21.8% and 24.3% were 37-50 and over 50 years old,

respectively. Farming and fishing were the main occupation (66.5%) while 24.5% were primary and high school students.

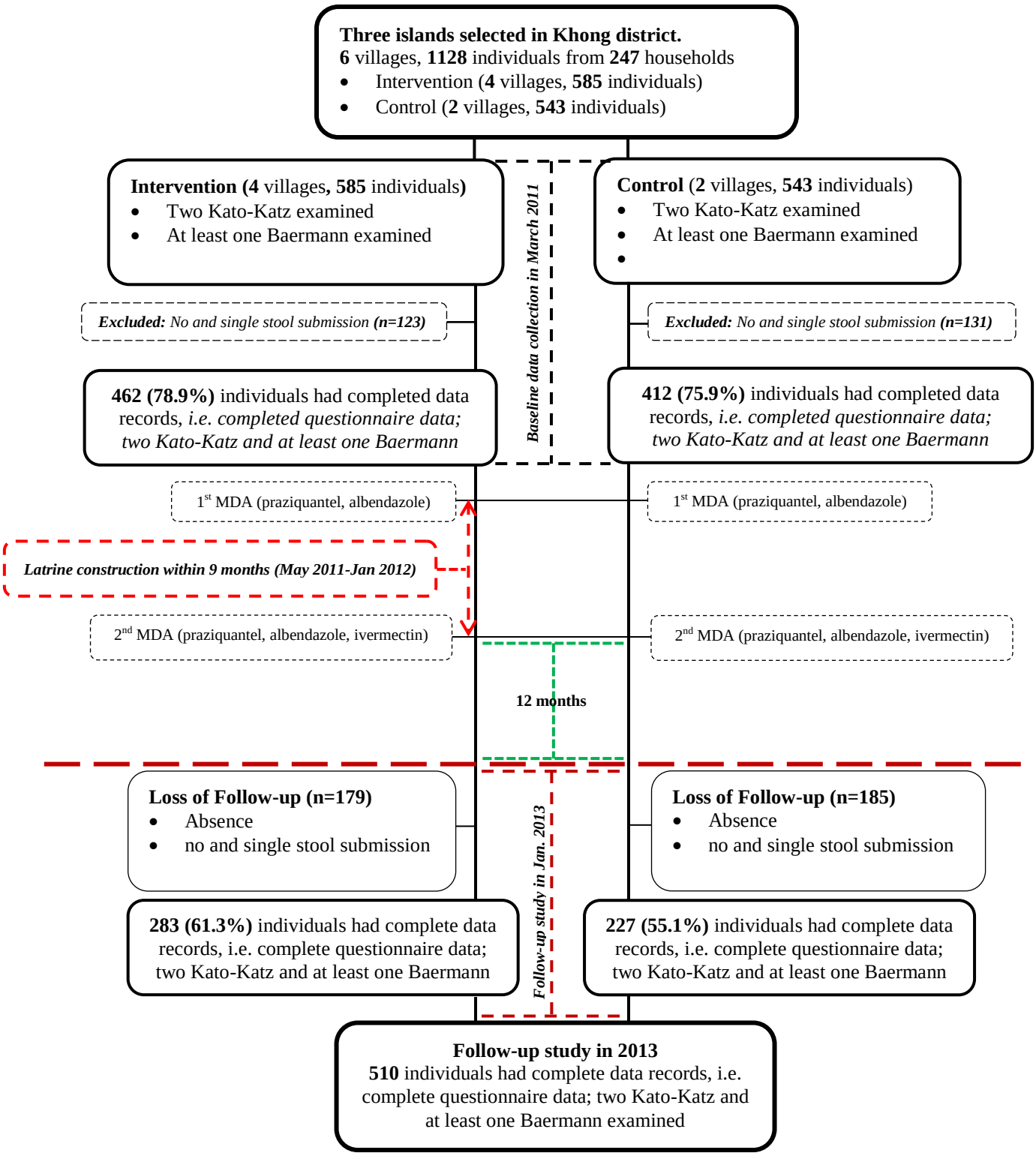

Figure 7.1: Study diagram

There was no statistically significant difference of sex (*P-value*=0.614) between participants of the intervention versus control group. In the control villages, the study participants were statistically significantly older than in the intervention group (*P-value*<0.001); They had a higher degree of education (*P-value*<0.001); And, they were more farmer/fisherman (73.6% vs 60.8%, *P-value*<0.001). The socio-economic status of the intervention group was similar to the control group (*P-value*=0.820) (Table 7.1).

Table 7.1: Baseline Characteristics of participants in study islands (n=510)

Characteristic	Overall n (%)	Study areas		x^2	*P-value*
		Intervention n (%)	Control n (%)		
N	510	283	227		
Sex					
Female	270 (52.9)	147 (51.9)	123 (54.2)		
Male	240 (47.1)	136 (48.1)	104 (45.8)	0.3	0.614
Age group (years)					
≤9	85 (16.7)	59 (20.8)	26 (11.5)		
10-16	98 (19.2)	62 (21.9)	36 (15.9)		
17-36	92 (18.0)	39 (13.8)	53 (23.3)		
37-50	111 (21.8)	69 (24.4)	42 (18.5)		
≥51	124 (24.3)	54 (19.1)	70 (30.8)	24.6	<0.001
Age (years)					
Mean (range)	32.9 (2-95)	29.9 (2-81)	36.7 (2-95)		
Educational level					
Pre-schooler	46 (9.0)	36 (12.7)	10 (4.4)		
Illiterate	37 (7.2)	23 (8.1)	14 (6.2)		
Primary school	286 (56.1)	167 (59.0)	119 (52.4)		
High school and above	141 (27.7)	57 (20.1)	84 (37.0)	24.3	<0.001
Occupation					
Preschool Child	46 (9.0)	36 (12.7)	10 (4.4)		
Farmer/fisherman	339 (66.5)	172 (60.8)	167 (73.6)		
Primary school student	93 (18.2)	62 (21.9)	31 (13.7)		
High school student	32 (6.3)	13 (4.6)	19 (8.4)	20.3	<0.001
Socio-economic status					
Most poor	28 (20.3)	14 (19.2)	14 (21.5)		
Very poor	28 (20.3)	13 (17.8)	15 (23.1)		
Poor	27 (19.6)	16 (21.9)	11 (16.9)		
Less poor	28 (20.3)	14 (19.2)	14 (21.5)		
Least poor	27 (19.6)	16 (21.9)	11 (16.9)	1.5	0.820

7.3.2. Helminth infections

The overall helminth infections during the baseline survey were as follows: *S. mekongi*, *O. viverrini*, hookworm, *A. lumbricoides*, *T. trichiura*, *E. vermicularis*, *S. stercoralis* and *Taenia* spp. were found in infection rates of 16.7%, 76.1%, 56.3%, 0.4%, 2.8%, 0%, 41.0% and 0.4%, respectively while the follow-up study was 13.7%, 64.5%, 31.6%, 2.2%, 2.0%, 0.8%, 32.8% and 0.4%, respectively.

Table 7.2 summarized the helminth infections in the study villages before (baseline) and after (follow-up) the latrine construction in the intervention villages. At baseline and at follow-up, *S. mekongi* infection was significantly more prevalent in intervention than in control villages (28.6% *vs* 1.8%, *P-value*<0.001 and 22.6% *vs* 2.6%, *P-value*<0.001, respectively). In contrast, hookworm infection was more prevalent in the control than in the intervention villages (48.8% *vs* 65.6%, *P-value*<0.001 and 26.2% *vs* 38.3%, *P-value* =0.003, respectively). *O. viverrini* infection was highly prevalent in intervention and control village (> 50%).

After the latrine program, *S. mekongi* infection decreased in the intervention villages (28.6% *vs* 22.6%, *P-value*<0.001), which was statistical significantly lower compared to the baseline survey (baseline *vs* follow-up, *P-value*). In contrast, *S. mekongi* infection was increased (1.8% *vs* 2.6%) in the control villages. For *O. viverrini* infection, the prevalence rate was decreased in both intervention and control villages (79.5% *vs* 68.2%, *P-value* <0.001 and 71.8% *vs* 59.9%, *P-value*=0.027, respectively). Other two helminth infections included hookworm and *S. stercoralis* were also decreased (48.8% *vs* 26.2%, *P-value*<0.001 and 43.1% *vs* 31.1%, *P-value*<0.001, respectively). Regarding the multi-parasitic infections, in the intervention villages the number of non-infected and single infection persons increased (8.8% *vs* 20.9% and 33.6% *vs* 45.6%, *P-value*<0.001, respectively). In contrast, the number of double and triple infected persons decreased (46.3% *vs* 26.2% and 10.6% *vs* 6.4%, *P-value*<0.001, respectively). The quadruple infections increased. In the control villages the same pattern was observed. The *P-value* in this paragraph was separately calculated from Table 7.2 purposing to compare within the same village group during the baseline versus follow-up.

The intensity of helminth infections intensities before and after intervention is provided in Table 7.3. Most helminth infection intensities were light in the study area. Heavy infections were observed for *S. mekongi*, *O. viverrini* and hookworm before and after the intervention. However, the heavy infection intensity of *S. mekongi* decreased during the intervention (from 13.6% to 6.3%). In the control villages, only light infection intensities were found for *S. mekongi* infection while other helminth infections had light, moderate and heavy infection intensities, including *O. viverrini* and hookworm.

Table 7.2: *S. mekongi, O. viverrini* and other helminth infections among participants in intervention (N=283) and control villages (N=227)

Parasites	Baseline, n (%)		x^2	*P-value*	Follow-up, n (%)		x^2	*P-value*
	Intervention	Control			Intervention	Control		
Trematodes								
Schistosoma mekongi	81 (28.6)	4 (1.8)	65.4	<0.001	64 (22.6)	6 (2.6)	42.4	<0.001
Opisthorchis viverrini	225 (79.5)	163 (71.8)	4.1	0.043	193 (68.2)	136 (59.9)	3.8	0.052
Nematodes								
Hookworm	138 (48.8)	149 (65.6)	14.8	<0.001	74 (26.2)	87 (38.3)	8.6	0.003
Ascaris lumbricoides	1 (0.3)	1 (0.4)	0.02	0.876	7 (2.5)	4 (1.8)	0.3	0.583
Trichuris trichiura	10 (3.5)	4 (1.8)	1.5	0.224	3 (1.1)	7 (3.1)	2.7	0.101
Enterobius vermicularis	0	0	na	na	1 (0.4)	3 (1.3)	1.5	0.218
Strongyloides stercoralis	122 (43.1)	87 (38.3)	1.2	0.275	88 (31.1)	79 (34.8)	0.8	0.375
Cestodes								
Taenia spp.	0	2 (0.9)	2.5	0.114	1 (0.4)	1 (0.4)	0.02	0.876
Multiparasitism								
Non infection	25 (8.8)	34 (15.0)			59 (20.9)	60 (26.4)		
Single infection	95 (33.6)	71 (31.3)			129 (45.6)	98 (43.2)		
Double infection	131 (46.3)	114 (50.2)			74 (26.2)	62 (27.3)		
Triple infection	30 (10.6)	8 (3.5)			18 (6.4)	6 (2.6)		
Quartile infection	2 (0.7)	0	14.8	0.005	3 (1.1)	1 (0.4)	6.2	0.183

Table 7.3: Intensity of helminth infections among infected participants from study islands (n=510)

Infections	Baseline, n (%)		Follow-up, n (%)	
	Intervention	Control	Intervention	Control
Schistosoma mekongi				
Light infection	48 (59.3)	4 (100)	39 (60.9)	6 (100)
Moderate infection	22 (27.7)	0	21 (32.8)	0
Heavy infection	11 (13.6)	0	4 (6.3)	0
Means of EPG (Std. Dev.)*	66.1 (229.9)	0.7 (5.7)	44.8 (272.2)	0.6 (3.9)
Opisthorchis viverrini				
Light infection	173 (76.9)	131 (80.4)	150 (77.7)	126 (92.7)
Moderate infection	48 (21.3)	31 (19.0)	39 (20.2)	9 (6.6)
Heavy infection	4 (1.8)	1 (0.6)	4 (2.1)	1 (0.7)
Means of EPG (Std. Dev.)*	852.1 (1919.9)	585.7 (1523.1)	743.9 (2276.6)	259.9 (1067.5)
Hookworm				
Light infection	130 (94.2)	135 (90.6)	71 (95.9)	83 (95.4)
Moderate infection	3 (2.2)	7 (4.7)	1 (1.3)	2 (2.3)
Heavy infection	4 (1.8)	1 (0.6)	4 (2.1)	1 (0.7)
Means of EPG (Std. Dev.)*	330.5 (1145.4)	521.3 (1328.1)	113.5 (435.2)	179.7 (734.8)
Ascaris lumbricoides				
Light infection	1 (100)	1 (100)	7 (100)	4 (100)
Moderate infection	0	0	0	0
Heavy infection	4 (1.8)	1 (0.6)	4 (2.1)	1 (0.7)
Means of EPG (Std. Dev.)*	0.17 (2.9)	9.6 (144.9)	15.9 (143.3)	2.9 (23.9)
Trichuris trichiura				
Light infection	15 (100)	7 (100)	8 (100)	7 (100)
Moderate infection	0	0	0	0
Heavy infection	4 (1.8)	1 (0.6)	4 (2.1)	1 (0.7)
Means of EPG (Std. Dev.)*	3.8 (19.4)	2.4 (16.6)	2.9 (22.2)	1.5 (9.8)

7.3.3. KAPP for latrine, personal hygiene and food consumption of the participants

At baseline and at follow-up an interview was conducted with the study participants with questions on latrine availability in the household and their use, on knowledge, attitude, perception and practices related to latrine use, and on personal hygiene and food consumption behaviour (Table 7.4). At baseline, the latrine availability was 34.3% in the intervention and 44.9% in the control villages. During the intervention all study households of the intervention villages constructed a latrine constructed reaching a 100% coverage. In the control villages the coverage remained unchanged. Similarly, the latrine utilisation increased in the intervention villages from 33.9% to 90.8% while in the control villages the latrine utilisation remained similar (44.5% *vs* 48.9%). Interestingly open defecation remained frequent in the intervention villages also after the intervention (31.5% *vs* 35.7%). In the control village two third of the study participants declared to openly defecate at follow-up (65.2%). A considerable number of study participants (15.9%) in the intervention and control villages reported that the water availability in the latrine was a problem.

The study participants displayed risk behaviour for helminth infections. In all study villages contact with the Mekong River was very frequent. More than 95% reported to have daily contact with river water. Furthermore, the consumption of raw vegetables was reported in all villages in high frequencies. The consumption of fish dishes, such as Lab/Koi, was very frequent. A considerable portion of the participants reported that the fish used for these dishes was raw or insufficiently cooked (Table 7.4). Finally, exposure to soil was very high as more than two-third of study participants reported to have worked in the rice fields during the year.

Table 7.4: Knowledge, attitude, practices and perceptions on latrines among participants from study islands (n=510)

Description	Baseline, n (%)		x^2	*P-value*	Follow-up, n (%)		x^2	*P-value*
	Intervention	Control			Intervention	Control		
Latrine available								
Yes	97 (34.3)	102 (44.9)			283 (100.0)	102 (44.9)		
No	186 (65.7)	125 (55.1)	6.01	0.014	0	125 (55.1)	206.4	<0.001
After latrine construction, will/did you still do open defecation?								
Yes	89 (31.5)	148 (65.2)			101 (35.7)	53 (23.4)		
No	194 (68.5)	79 (34.8)	4.1	0.043	182 (64.3)	174 (76.6)	43.9	<0.001
Will/did water be problem for using latrine								
Yes	12 (5.2)	20 (8.8)			45 (15.9)	7 (3.1)		
No	271 (95.8)	207 (91.2)	0.5	0.493	238 (84.1)	220 (96.9)	5.7	0.017
Can latrine prevent parasitic diseases								
Yes	236 (83.4)	67 (29.5)			110 (38.9)	187 (82.4)		
Do not know	47 (16.6)	160 (70.5)	0.09	0.762	173 (61.1)	40 (17.6)	4.9	0.027
Usually (Your daily) defecation								
Latrine	96 (33.9)	111 (48.9)			257 (90.8)	101 (44.5)		
Rice field/brush	187 (66.1)	116 (51.1)	5.9	0.015	26 (9.2)	126 (55.5)	110.1	<0.001
Last defecation, washing hands with soap								
Yes	48 (17.0)	41 (18.1)			59 (20.9)	25 (11.0)		
No	235 (83.0)	186 (81.9)	3.6	0.057	224 (79.1)	202 (89.0)	0.6	0.431
Before meal, washing hands with soap								
Yes	10 (3.5)	26 (11.5)			32 (11.3)	5 (2.2)		
No	273 (96.5)	201 (88.5)	0.8	0.377	251 (88.7)	222 (97.8)	0.003	0.959
Eating Lab/Koi beef within past 7 days								
Yes	29 (10.3)	39 (17.2)			72 (25.4)	7 (3.1)		

No	254 (89.8)	188 (82.8)	9.9	0.002	211 (74.6)	220 (96.9)	5.05	0.025
How Lab/Koi beef prepared								
Cooked	23 (79.3)	30 (76.9)			56 (77.8)	2 (28.6)		
Insufficient cooked	1 (3.5)	4 (10.3)			11 (15.3)	3 (42.9)		
Raw	5 (17.2)	5 (12.8)	10.3	0.006	5 (6.9)	2 (28.6)	1.4	0.486
Eating Lab/Koi fish within past 7 days								
Yes	158 (55.8)	181 (79.7)			205 (72.4)	131 (57.7)		
No	125 (44.2)	46 (20.3)	0.2	0.670	78 (27.6)	96 (42.3)	3.6	0.056
How Lab/Koi fish prepared								
Cooked	76 (48.1)	92 (50.8)			102 (49.7)	45 (34.4)		
Insufficient cooked	65 (41.1)	37 (20.4)			77 (37.6)	26 (19.9)		
Raw	17 (10.7)	52 (28.7)	46.6	<0.001	26 (12.7)	60 (45.8)	21.8	<0.001
Eating raw vegetables within past 7 days								
Yes	258 (91.2)	220 (96.9)			253 (89.4)	221 (97.4)		
No	25 (8.8)	7 (3.1)	8.5	0.004	30 (10.6)	6 (2.6)	10.6	0.001
Taking a bath in Mekong river								
Yes	269 (95.1)	225 (99.1)			264 (93.3)	224 (98.7)		
No	14 (4.9)	2 (0.9)	5.1	0.023	19 (6.7)	3 (1.3)	10.9	0.001
Wearing slippers								
Yes	239 (84.5)	197 (86.8)			255 (90.1)	185 (81.5)		
No	44 (15.6)	30 (13.2)	0.8	0.376	28 (9.9)	42 (18.5)	1.4	0.240
Worked in rice field last year								
Yes	183 (64.7)	159 (70.0)			203 (71.7)	157 (69.2)		
No	100 (35.3)	68 (30.0)	1.1	0.284	80 (28.3)	70 (30.8)	0.2	0.676

7.3.4. Impact of the intervention

We used the intervention status (intervention *versus* control group) and the survey time point (follow-up *versus* baseline survey) and their interaction factor (intervention groups * survey groups) as predictors for the outcome in helminth infections and risk factors (Table 7.5).

Among the five helminth infections, only *T. trichiura* was statistically significantly associated (OR 0.2, 95% CI 0.03-1.0, *P-value*=0.050) with the interaction term indicating that the intervention reduced the re-infection significantly by taking into account the changes in the two survey and the difference between the intervention groups. The benefit was small: In the intervention group *T. trichiura* decreased from 3.5% to 1.1% while it increased in the control group from 1.8% to 3.1%. For the remaining four helminth species the interaction term was not associated with the infection.

At follow-up the infection was statistically significantly reduced for *O. viverrini* (OR 0.6, 95% CI 0.4-0.9, *P-value*=0.008) and hookworm (OR 0.3, 95% CI 0.2-0.5, *P-value*<0.001) indicating that the treatment at baseline had some effects. The infection remained in the intervention villages significantly more frequent for *O. viverrini* (OR 1.5, 95% CI 1.0-2.3, *P-value*=0.040) and less frequent for hookworm (OR 0.5, 0.3-0.7, *P-value*<0.001). However, *O. viverrini* dropped equally in the intervention and the control villagers (Fig. 7.2). *S. mekongi* was significantly more frequent in the intervention villages. No associations were observed for *S. stercoralis*.

The intervention had its immediate impact on the latrine use. The interaction term was significantly associated with open defecation and daily defecation in latrine practices. The results show that the open defecation was significantly reduced (OR 0.2, 95% CI 0.1-0.3, *P-value*<0.001) (Fig. 7.3) while the daily defecation in latrine practice was significantly increased (OR 16.1, 95% CI 8.9-29.4, *P-value*<0.001)(Fig. 7.4). Furthermore, we observed a significant impact of the intervention on the anti-helminthic treatment (in precedent 6 months). Study participants in the intervention villages had a significantly higher change to get treated with antihelminthic drugs (OR 5.6, 95% CI 1.9-18.0, *P-value*=0.002).

Habits of hand washing with soap and the habit of eating raw food study (raw fish and beef dishes and raw vegetable) were not associated with the interaction term. However raw beef (OR 6.5, 95% CI 2.8-14.9, *P-value*<0.001) and raw fish dishes (OR 2.9, 95% CI 1.9-4.4, *P-value* <0.001) was significantly more frequently reported in the follow-up compared to baseline survey.

The frequency of taking bath in the Mekong, wearing slippers and having worked in the rice field was not associated with the interaction term. However, in the intervention villages the study participants used significantly less frequent the Mekong river for a bathing (OR 0.3, 95% 0.1-0.9, *P-value*=0.035).

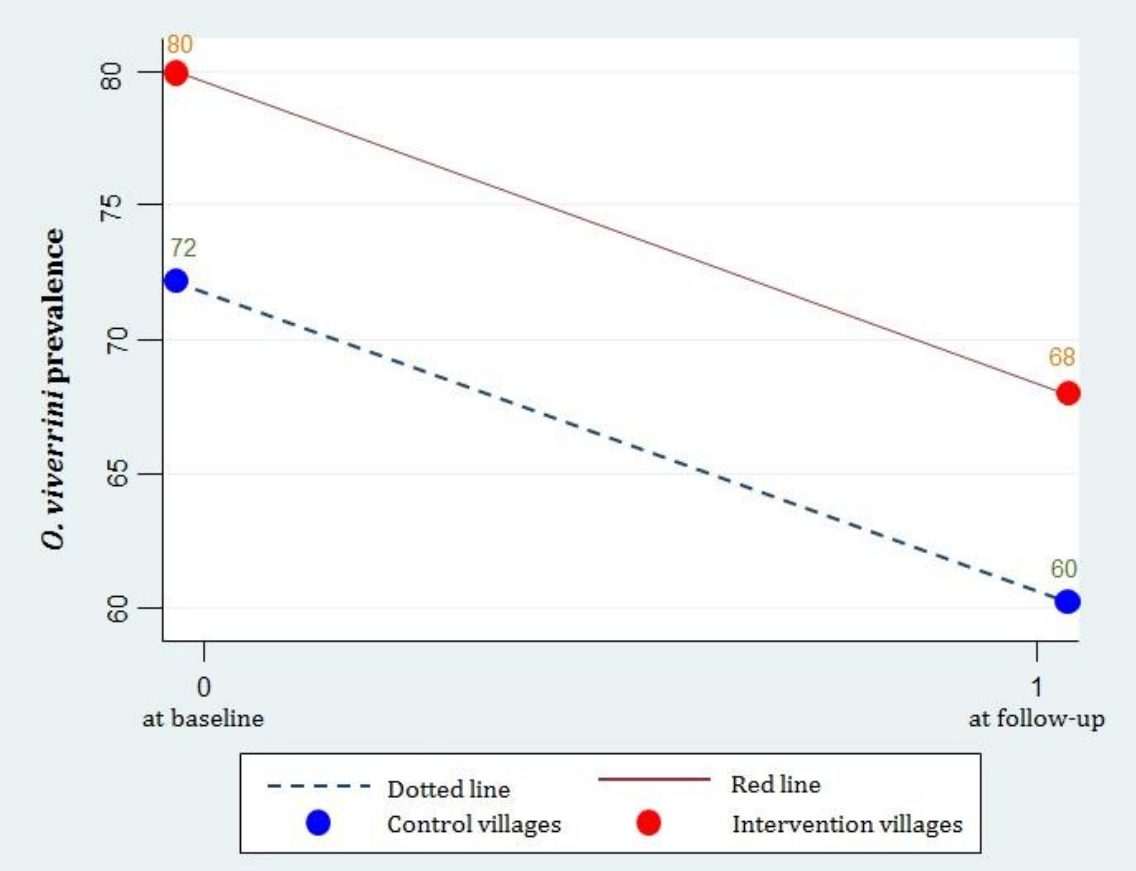

Figure 7.2: Comparison between control and intervention villages at baseline and follow-up. There was no effect yet for *O. viverrini* prevalence by reducing in both intervention and control villagers after latrine construction.

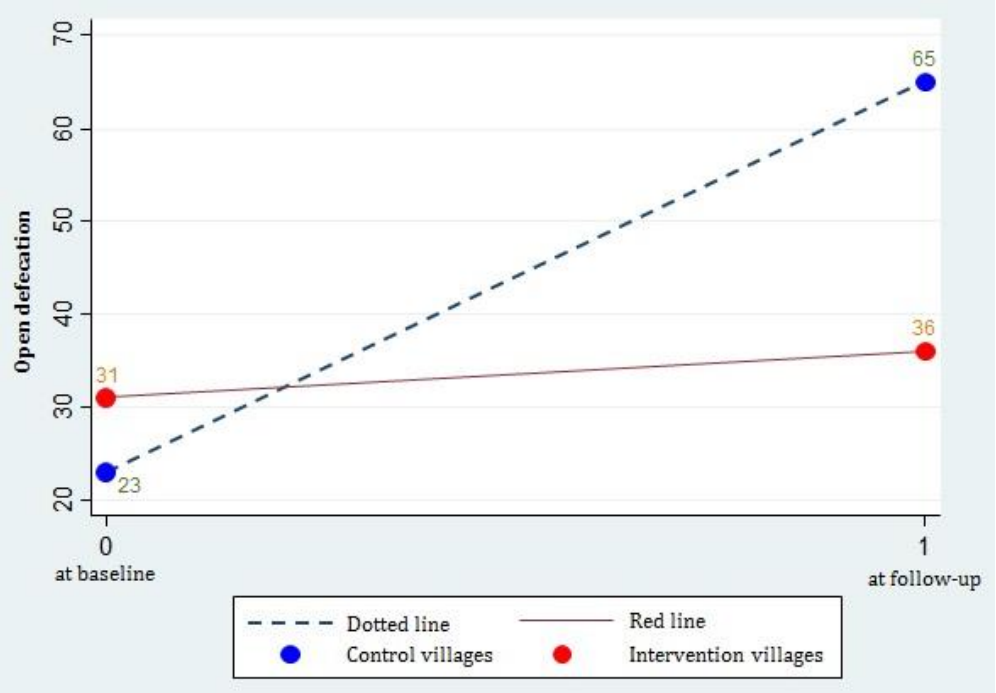

Figure 7.3: Comparison between control and intervention villages at baseline and follow-up. Open defecation behavior was reduced among the intervention villagers.

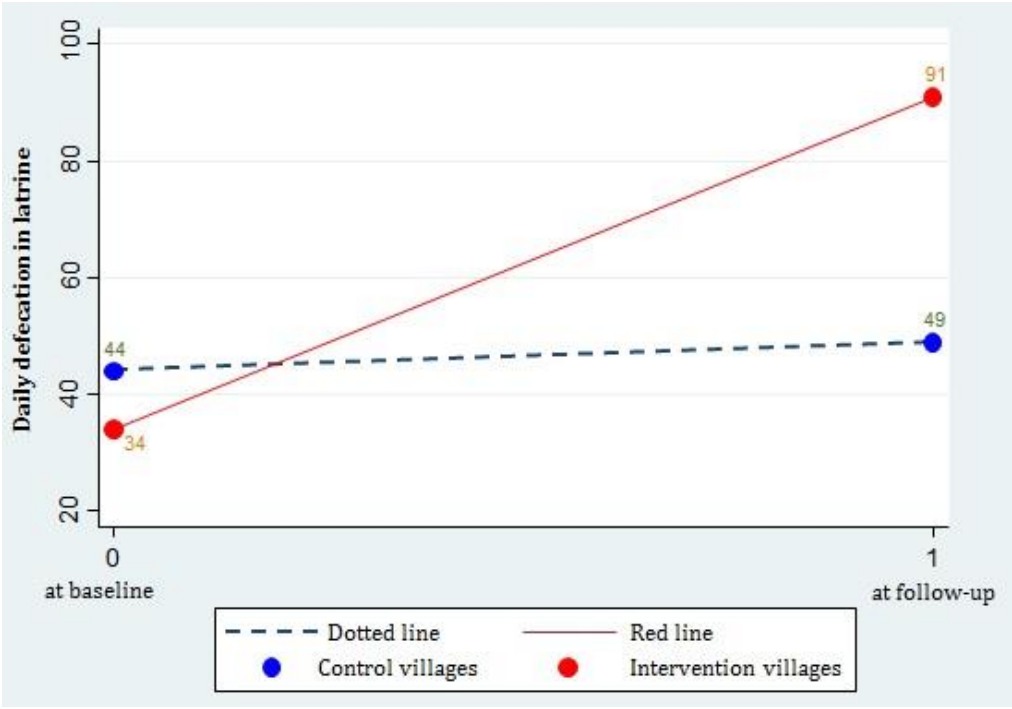

Figure 7.4: Comparison between control and intervention villages at baseline and follow-up. Daily defecation in latrine practices was increased among the intervention villagers.

Table 7.5: Impact of intervention on helminth infection and risk factors: Comparison of surveys, intervention groups and interaction factor

	Follow-up *versus* baseline survey			Intervention *versus* control group			Interaction: Surveys * groups		
	OR	**95% CI**	***p*-value**	**OR**	**95% CI**	***p*-value**	**OR**	**95% CI**	***p*-value**
Helminth infections									
Schistosoma mekongi	1.5	0.4-5.3	0.525	22.4	8.0-62.1	< 0.001	0.5	0.1-1.8	0.283
Opisthorchis viverrini	0.6	0.4-0.9	0.008	1.5	1.0-2.3	0.044	0.9	0.5-1.6	0.831
Hookworm	0.3	0.2-0.5	< 0.001	0.5	0.3-0.7	< 0.001	1.1	0.7-1.9	0.614
Trichuris trichiura	1.8	0.5-6.1	0.366	2.0	0.6-6.4	0.233	0.2	0.03-1.0	0.050
Strongyloides stercoralis	0.9	0.6-1.3	0.436	1.2	0.9-1.7	0.275	0.7	0.4-1.2	0.163
Risk factors									
Open defecation	6.2	4.1-9.3	< 0.001	1.5	1.0-2.2	0.043	0.2	0.1-0.3	< 0.001
Daily defecation in latrine	1.2	0.8-1.7	0.347	0.6	0.4-0.9	0.015	16.1	8.9-29.4	< 0.001
Anti-helminthic treatment in last 6 months	0.1	0.04-0.3	< 0.001	0.5	0.3-0.9	0.012	5.8	1.9-18.0	0.002
Water problem in latrine	3.0	1.3-7.3	0.014	1.4	0.5-3.6	0.495	1.4	0.5-4.2	0.544
Hand washing after defecation	1.8	1.0-3.0	0.035	1.7	1.0-2.8	0.058	0.7	0.7-1.4	0.354
Hand washing with soap before meal	5.7	2.2-15.2	< 0.001	1.6	0.5-4.8	0.381	0.6	0.2-2.1	0.421
Eating raw beef dish (lab)	6.5	2.8-14.9	< 0.001	3.5	1.5-8.4	0.003	0.5	0.2-1.2	0.108
Eating raw fish dish	2.9	1.9-4.4	< 0.001	0.9	0.7-1.2	0.670	0.7	0.4-1.2	0.240
Eating raw vegetables last 7 days	0.9	0.3-2.6	0.779	0.3	0.1-0.7	0.006	1.0	0.3-3.3	0.946
Taking bath in Mekong	1.5	0.2-9.1	0.665	0.3	0.1-0.9	0.035	0.5	0.1-3.3	0.457
Wearing slippers	1.5	0.9-2.5	0.125	1.2	0.8-2.0	0.376	1.1	0.5-2.3	0.748
Worked in rice field past year	1.0	0.7-1.6	0.838	0.8	0.6-1-2	0.284	1.3	0.8-2.3	0.296

7.4. Discussion

A large number of studies have been undertaken in order to investigate the effects of sanitation upon health such as constructing of ventilated improved pit latrines, provision of clean drinking water, and hygiene education which affected to helminth and protozoa infection decreasing (Gelaye et al., 2014; Graham and Polizzotto, 2013; Pruss et al., 2002; Strunz et al., 2014; WHO, 2014; Wolf et al., 2014). Strunz and colleages conducted a systematic review of WASH on infection with STH and showed that WASH access and practices are generally related to reduced odds of STH infection. Use of treated water was associated with lower odds of STH infection (odds ratio [OR] 0.46, 95% CI 0.36–0.60). Access to sanitation was associated with decreased likelihood of infection with any STH (OR 0.66, 95% CI 0.57–0.76), *T. trichiura* (OR 0.61, 95% CI 0.50–0.74), and *A. lumbricoides* (OR 0.62, 95% CI 0.44–0.88), but not with hookworm infection (OR 0.80, 95% CI 0.61–1.06) (Strunz et al., 2014).

We compared intestinal helminth infection rates before and after intervention consisting of a latrine construction and utilisation campaign in selected intervention villages. The intervention villages were compared with control villages where no latrine construction and utilisation campaign was conducted. In all villages a MDA against all endemic parasitic infections were performed twice, in particularly against *S. mekongi, O. viverrini* (using praziquantel), STH using albendazole), including *S. stercoralis* (by using Ivermectin). After the intervention an excellent latrine coverage of 100% was reached in the intervention villages while in the control villages the latrine coverage remained unchanged. The follow-up surveys were conducted 12 months after the latrine intervention was completed and the second MDA was performed. We found that in all study villages the infection rates of *S. mekongi*, *O. viverrini*, hookworm and *S. stercoralis* were significantly lower compared to the initial infection prevalence, regardless whether the villages belonged to intervention or control group. We attribute the reduction the MDA in all study villages. Given the considerable infection rates assessed at follow-up indicate that the transmission of these helminth species is on-going in all study villages and has led to the observed infection rates.

There is a difference in re-infection rates between intervention and control villages. However the differences are marginal. Obviously the latrines did not avoid a complete re-infection of the studied helminthiasis particularly *S. mekongi*, *O. viverrini*, hookworm and *S.*

stercoralis. The reason might be the continuing of open defecation of villagers even having latrine at home which was still high 35.7% in the intervention villages (Table 7.3). Regarding to human side of intervention villages, one of the reason why latrines were not totally in use was that the daily life of farmer or fisherman which have to go for work in early morning then they did defecation in the rice field or some islands nearby their work places. The explanation above was from our observation and interviewing some villagers but was not included in the questionnaires. Despite, other factors should be also considered to be the reasons of re-infection such as the infection of intermediate and animal reservoir hosts, personal hygiene, raw food consumption behaviour, etc. according to the life cycle of each parasite which can be the potential sources for the transmission and distribution.

Other reasons for the high infection rates might be that main risk factors for infection persist. For *S. mekongi*, given the villagers daily life relate to water contact in Mekong River which almost more than 90% such as bathing, clothes washing, fishing, etc. and *O. viverrini* infection relates to raw fish consumption behaviour which was deep cultural practice in this area. Those main factors support these trematodes parasite transmission and their re-infection in the studies areas.

Hookworm and *S. stercoralis* are transmitted by their larval form burrowing through the skin of the foot as someone squats to defecate on an area of soil previously used for defecation by others or whenever bare foot working in the rice field (Ericsson et al., 2001; Hotez et al., 2004). The KAPP showed that people in both control and intervention villages were practice bare foot more than 80% which allowed for those parasites re-infection.

Regarding to *S. mekongi*, it is important to note that the nearest and similar environment neighbouring islands for the intervention island were already done MDA with praziquantel by local health authority in a few months before our study implementation therefor we had chosen other islands to be the control villages. That was why the quite big different was observed in terms of the infection rate between intervention and control villages from both baseline and follow-up study (28.6% vs 1.8% and 22.6% vs 2.6%, respectively). It was might not good control group selection for this intervention. However, it was not affect to other helminthic infection.

Although this study did not collect data on the community mobilization specifically, the small providing of the project such as the pit and the slab then let the household members conducted the latrines by themselves. This such activity in our study demonstrated that community mobilization can be an effective, low-cost way to increase latrine ownership which many solid and nice latrines were built in the intervention villages.

7.5. Conclusions

In conclusion, this study revealed a marginal short term impact the transmission of *S. mekongi, O. viverrini* and other helminth infections on Mekong islands, Southern Lao PDR. Hookworm and *S. stercoralis* were showed clearly reducing of the re-infection. The period of latrine intervention in this study could not stop the parasite re-infection. Therefore, long-term latrine intervention with eco-health combination approaches might be more effect to reduce all gastro-intestinal helminthiasis prevalence.

7.6. Acknowledgements

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8. Discussion

8.1. General discussion

This PhD thesis addresses to test for appropriate and novel control approaches for *O. viverrini* and other helminth infections, particularly *S. stercoralis*, *S. mekongi* in co-endemic areas in Southern Lao PDR. Our first work of this thesis was to determine and test the efficacy of latrine promotion in reducing the infection rate of *O. viverrini, S. mekongi* and other helminthiasis. Moreover, the sensitive diagnosis of *S. stercoralis* infection by using Baermann technique was also tested during the baseline survey of the latrine promotion programme. Therefore, more accurate result of *S. stercoralis* infection was presented and published as the title: "Epidemiology of *Strongyloides stercoralis* on Mekong islands in southern Laos". And the result of latrine efficacy has been obtained and preparing to publish as the title: "Improved latrines have a small short term impact on the transmission of *Schistosoma mekongi, Opisthorchis viverrini* and other helminth infections on Mekong islands, Southern Lao PDR".

We conducted the eco-health approach to determine the prevalence and risk factors of *O. viverrini*, *S. mekongi* and STH infections in humans in the ecological environment of Khong district, where potential animal reservoir and intermediate hosts, like molluscs and fish, live in close connectivity, but it is important to note that the obtained data in this PhD thesis was only its baseline data which could not assess the impact of this approach yet.

Furthermore, two highly sensitive test systems for the detection of cathodic and anodic circulating antigens (CCA, CAA) of *S. mekongi* in urine and serum were compared and evaluated which their results showed a substantially higher prevalence estimates for *S. mekongi* compared to KK thick smears.

8.2. Discussion on study findings

8.2.1. Epidemiology of *Strongyloides stercoralis* on Mekong islands in southern Laos

Study findings presented in this PhD thesis suggest that *S. stercoralis* infection prevalence was very high of 41.0% on Mekong islands. Concerning to the risk factors analysis, only sex was significantly associated with *S. stercoralis*. Furthermore, reported urticaria (body itching) was significantly associated with the infection. From our knowledge, there were

small numbers of *S. stercoralis* studies in Southeast Asia. In Lao PDR, only a very few studies of *S. stercoralis* have been conducted using an adequate diagnostic approach. Most data on *S. stercoralis* infection stem from studies examining other soil-transmitted helminthes and/or food-borne trematodes and reported prevalence rates below 20% (Paboriboune et al., 2014; Sayasone et al., 2011). Given the inadequate diagnostic techniques these studies used, their findings most like underestimate the true infection burden in the country. Therefore, more attention should be paid to *S. stercoralis* in Laos by incorporating sensitive diagnostic approaches in helminth surveillance activities. Recently, *S. stercoralis* study in Cambodia reported high infection rates of 25% in Kandal and Takeo provinces (Khieu et al., 2013a; Khieu et al., 2014b) and almost 50% in the most Northern Preah Vihear province (Khieu et al., 2014a). Furthermore, low socioeconomic status and low hygienic living conditions of the rural population were strongly associated with *S. stercoralis* infections.

In our study, we used the Baermann method on two stool samples per enrolled participant. The infection prevalence was comparable to recent reports from Cambodia (Khieu et al., 2013a, 2014a,b), but substantially higher than infection prevalences reported from neighbouring China (Steinmann et al., 2007, 2008) and Thailand (Jongsuksuntigul et al., 2003; Sithithaworn et al., 2003). Our diagnostic procedures could have been improved by examining more stool samples per person and by adding additional diagnostic techniques. For example, in a study of Cambodian children three stool samples were examined per child with a combination of Baermann technique and Koga agar plate. Taking this approach as 'gold' standard, our examination of two samples with the Baermann technique results in a sensitivity of approximately 70%; in combination with the Koga agar plate method a 93% sensitivity could have been reached (Khieu et al., 2013a). However, the substantial material costs and time investments required to conduct the Koga-Agar plate culture must be taken into account when planning a field investigation.

We identified gender as the most important risk factor in our study area. Boys and men had almost twice the risk for a *S. stercoralis* infection than did girls and women. This finding is in agreement with earlier reports from Cambodia (Khieu et al., 2014a,b) and Laos (Vannachone et al., 1998). It is most probably the gender specific daily activities of boys (recreational) and men (agricultural) that increase the exposure to contaminated soil, and hence lead to higher infection rates. Furthermore, we observed high infection rate among young children in this study which one third (33.3%) of the children under 6 years of age

were infected with *S. stercoralis*. Given the fact that these children have few daily activities outside the household, the transmission of *S. stercoralis* must take place at home. A similar observation was reported in Cambodia (Khieu et al., 2014a). In addition, in Cambodian households dogs were examined for intestinal infection and tested positive for Stronyloides larvae (Schär et al., 2014). We hypothesise that humans and dogs in the same household share the Strongyloides parasites and are responsible for contaminating the soil. However, further genetic studies on human and dog derived Strongyloides parasites are required in order to draw conclusions about anthropo-zoonotic transmission. The association between *S. stercoralis* infection and risk factors related to socio-economic status, access to sanitation facilities and hygiene behaviour of the population were not found in our study which were most surprising. As earlier studies in Cambodia, schoolchildren had an almost five fold increased risk for a *S. stercoralis* infection when no latrine was present at home and presenting of adequate sanitation showed that 70% of *S. stercoralis* infections could be averted (Khieu et al., 2013a). Recent developments in our study area might have led to the absence of these associations. We selected the villages precisely because the Provincial Health Office reported low numbers of households with latrines in the island villages of the Khong district. However, during our investigations, we found that more than 40% of the households had a latrine. Indeed, in the last year, a number of health related intervention were undertaken in the Khong district, including general health promotion activities, and latrine construction and mass-deworming campaigns. Though the new developments account for the absence of the expected associations, people however remained infected with *S. stercoralis*.

Although *S. stercoralis* infection is highly prevalent in many settings its clinical significance is not understood. Long-lasting infection may contribute to chronic gastro-intestinal and skin morbidity. In our study, *S. stercoralis* infection was associated with reports of urticarial and/or itching in the previous weeks. A Cambodian study reported a very similar result. There, urticarial with intensive itching on all body parts was reported by patients. The symptoms were resolved after ivermectin treatment (Khieuet al., 2013b). However in this report, abdominal pain was also associated with *S. stercoralis* infection.

Multiparasitism was very common in our study, *O. viverrini* was the most frequent helminth infection (72.2%), followed by hookworm (56.1%) and *S. stercoralis* (41.0%) infections. In addition, a considerable *S. mekongi* infection prevalence was detected on Donlong island

(25.6%). However, the clinical consequences of concurrent helminth infections are unknown. Recently, it was shown that co-infection with *S. mekongi* aggravates *O. viverrini* related morbidity (Sayasone et al., 2012). However, information on the contribution of *S. stercoralis* to the overall morbidity of individuals infected with multiple helminth species is unknown and will require further in-depth studies.

8.2.2. Transmission of *Opisthorchis viverrini*, *Schistosoma mekongi* and soil-transmitted helminthes on the Mekong Islands, Southern Lao PDR

Presented data in this PhD thesis was obtained from the baseline survey of our eco-health intervention against mainly *O. viverrini* and *S. mekongi* infection in the setting areas of their co-infection in Khong district with its dozens of islands in the Mekong, has a distinct ecological setting. Human settlements line the island shores, while the rest of the island is used for agricultural activities, particularly rice farming. Daily life of the people in the islands deeply related to Mekong River such as fishing, washing, bathing, etc. The Mekong River as well as the diverse water bodies on the islands represents a rich ecosystem for fish and mollusc populations. On two Mekong islands, highly endemic for multiple species of helminth infections, we studied the transmission of *O. viverrini, S. mekongi* and STH using an ecohealth approach (Kingsley et al., 2015; Leung et al., 2012) to better assess the relation of human infection status to environmentally present reservoir and intermediate hosts.

In this study, we document high infection rates of *O. viverrini, S. mekongi* and selected species of STH, namely hookworm infections. The high infection rates are a surprise given that MDA campaigns were conducted annually between 2008 and 2013 (WHO, 2011), in which praziquantel (40mg/kg BW single dose) and albendazole (400mg single dose) were provided to the entire population (older than four years). In addition, biannual deworming (with mebendazole) takes place in all Lao primary schools (Phommasack et al., 2008). Local health authorities confirmed that all Mekong islands were targeted, but we could not find coherent information on the number of treatment rounds conducted on our study islands. Nevertheless, our results indicate that the impact of the intervention is insufficient.

Regarding to the result of *S. mekongi* infection in our study islands, the goal of Ministry of Health to eliminate *S. mekongi* as a public health problem in Lao PDR by 2016 cannot be achieved cause of the high infection rates was still found. Furthermore, the study indicates

that *S. mekongi* infection in dogs may fuel the transmission by constantly infecting *Neotricula* populations in the Mekong. Of similar importance are cats and dogs for the transmission of *O. viverrini*. Hence, animal reservoirs in households should also be a target of integrated parasite control on the Mekong islands, and throughout Lao PDR. Several factors might account for the persisting high *O. viverrini* infection rates among humans on the Mekong islands. One such factor is the high infection prevalence among *Cyprinoid* fish. Regarding to our knowledge, more than 80 species of the *Cyprinidae* family and at least 13 species of other families can serve as a secondary intermediate host (WHO, 1995). In our study, *O. viverrini* metacercariae were identified in 11 *Cyprinoid* fish species, while some had particularly high *O. viverrini* metacercariae infection rates, e.g. in 87.1% of *Hampala dispa*. All the *Cyprinoid* species in which we detected an infection are known to be good *O. viverrini* transmitting species and many types of them are the main and common raw/fermented fish dishes among the villagers in the southern part of Lao PDR (Manivong et al., 2009; Rim et al., 2008b; Rim et al., 2013; Sayasone et al., 2007; Xayaseng et al., 2013). They were identified in all water bodies examined in this study. Fish are mostly likely infected while small and living in rice fields, canals and ponds. The metacercariae remain alive as the fish grow and move into the Mekong till they are caught and eaten by definitive hosts. *Cyprinoid* fish accumulate the metacercariae over a long time. For first intermediate host analysis, we found a low infection rate of 0.3% in *Bithynia* sp. snails. Other studies have detected infection rates between 0.3–8.3% (Kiatsopit et al., 2012). But infection rates may vary considerably, depending on sampling locality and season (Kiatsopit et al., 2014; Kiatsopit et al., 2012). It is important to note that even low infection prevalence rates in *Bithynia* snails but are sufficient for maintaining transmission(Chai et al., 2005a).

We also observed low *S. mekongi* infection rates in *N. aperta* (0.02%) compared to other reports. The presence of infected molluscs gives evidence that *S. mekongi* transmission is currently on-going. Therefore, abandoning control activities would inevitably lead to an increase in infection rates among humans. There are many more *S. mekongi* endemic Mekong islands, which might display a different *N. aperta* population distribution and infection pattern (Muth et al., 2010; Urbani et al., 2002).

A major finding from our study is the dramatically high helminth infection rates among domestic cats, dogs, pigs and buffaloes. By using FECT, we could distinguish *O. viverrini* eggs in dogs and cats from other small trematode eggs. Our results showed higher rates than

Aunpromma et al (2012) found in neighbouring Thailand, where 0.37% and 35.5% of the dogs and cats were infected, respectively (Aunpromma et al., 2012). In our study, the infection rate among dogs was 20 times higher than that found in the study of Aunpromma et al (2012). Through observation and from interviewing animal owners in both communities, it appears that most of the dogs and cats were free-roaming and usually accompanied their owners to the rice field where they caught and ate fish directly from the canals or rice fields. Moreover, raw and undercooked fish were often fed to these animals. These phenomena, in combination with the high infection rates of dogs and cats, likely maintain the transmission of *O. viverrini* and other fish-borne trematode infections in the communities.

Only dogs were diagnosed with *S. mekongi* in this study, which is consistent with other study findings (Strandgaard et al., 2001; Urbani et al., 2002). We did not find any *S. mekongi* eggs in pigs or water buffaloes. However, Strandgaard and colleague reported infected domestic pig with *S. mekongi* in Had Xay Khoun village of Khong district which was suggested that the rout of infection may occur through cercariae-infested drinking water during feeding (Strandgaard et al., 2001). On other Mekong islands where these animals (dog and pig) are more free-roaming, their infection status could be higher and, thus, their contribution to transmission of greater importance. However, pigs are not of importance for transmission on our study islands, except dogs.

The results of our risk factor analysis for *O. viverrini* infection differed from many previous studies (Forrer et al., 2012; Sayasone et al., 2011; Sayasone et al., 2007). More than half of our risk factors dropped out after multivariate analysis, whereas the initial univariate analysis showed significant associations between infection and age group, occupation, socioeconomic status, latrine availability, history of open defecation this year, and eating raw and/or undercooked fish (Table 6). The association between *O. viverrini* and socioeconomic status was not clear for our study population. The study area was geographically very small. Therefore, the variation in socioeconomic status and living conditions might not have varied enough to results in risk differentiation. Furthermore, control activities such as the annual treatments between 2008 and 2013, have had an impact on infection status, which in turn might have blurred important associations. For example, eating raw/undercooked fish was not significantly associated with *O. viverrini* infection, although deeply rooted habits of eating raw or improperly cooked fish is a well-

known factor in sustaining helminth infections in humans and difficult to control (Chai et al., 2005a; Forrer et al., 2012; Phongluxa et al., 2013).

In our multivariable analysis, we did not find any association between *S. mekongi* infection and risk factors, except for age. Children under nine years old had a higher risk of infection than older study participants. This result is likely due to MDA over the years having reduced infection rates among older villagers. Therefore, controls targeting lower age groups could further contribute to eliminating *S. mekongi* on the Mekong islands.

Our study suffers from some limitations. Our diagnostic procedure most likely underestimated the true infection burden. Although examining a duplicate Kato-Katz thick smear per faecal sample has a considerably higher sensitivity than a single smear, the egg detection rate remains far below that of a multiple stool sample diagnostic procedure (Sayasone et al., 2011; Vonghachack et al., 2015). Furthermore, the Kato-Katz technique cannot differentiate small trematode eggs (Lovis et al., 2012). It is therefore possible that some of the infections in humans were counted as *O. viverrini* infections instead of MIF.

8.2.3. Comparison of novel and standard diagnostic tools for the detection of *Schistosoma mekongi* infection in Lao People's Democratic Republic and Cambodia

The implementation of preventive chemotherapy has decreased schistosomiasis morbidity in endemic countries worldwide, including *S. mekongi* affected areas in Cambodia and Lao PDR (Keang et al., 2007; Muth et al., 2010). The current lower intensity of disease, however, is a compelling fact to recommend replacing stool examination using Kato-Katz with more sensitive diagnostic tools. Serology based on antibody detection is a helpful adjunct, but in order to determine cure and the level of worm burdens detection, assays based on the detection of circulating antigens are required. This approach has been successfully used for all major schistosome species showing that it is 10-20 times more sensitive than standard stool microscopy (Corstjens et al., 2008).

WHO recommends targeting schistosomiasis mekongi for elimination as the endemic areas are very limited and both stool examination according to Kato-Katz and antibody testing using ELISA serology indicate low intensity of disease after several rounds of preventive chemotherapy (WHO, 2012b). However, as has already been shown in the Peoples' Republic of China, highly sensitive tests for schistosome circulating antigens give considerably higher prevalence results than Kato-Katz (van Dam et al., 2015b). With the proof-of-principle of testing for excreted antigens in the urine shown for *S. mekongi* (van Dam et al., 2015a), it was now felt that a field study in the endemic areas in southern Lao PDR and northern Cambodia would be warranted to establish this approach. In contrast to antibody detection, the Kato-Katz stool examinations along with the tests for circulating schistosome antigens (POC-CCA and UCP-LF-CAA) are all indicators of active infections. Antibody titres can persist for very long time after cure and therefore serology is not suitable for assessing treatment outcomes or as single diagnostic approach for detection of active infections.

In the field, detection of active infection and cure are all highly important, particularly when moving from control of a disease to transmission interruption and elimination. It is equally important for the individual patient. While the better sensitivity of antigen detection compared to Kato-Katz is obvious, it is also clear that CAA detection (both in serum and urine) performs much better than CCA. These results are in agreement with previous reports for *S. japonicum* and *S. mekongi* (Van 't Wout et al., 1995; van Dam et al., 2015a; van Dam et al., 2015b).

The advantage of the POC-CCA test is that it is a standardized urine test applicable in the field without the need for any extra equipment (fulfilling all 'ASSURED' characteristics). It has been mainly and widely validated for *S. mansoni* detection, but shows limited use for the other schistosome species (Kittur et al., 2016). However specificity is limited to some extent, because CCA has epitopes common with certain human components (Lewis-X structures) that sometimes end up in the urine causing false positive reactions (Polman et al., 2000). The UCP-LF CAA test, on the other hand, is applicable for all schistosome species and for various human liquid samples, such as urine and serum, as well as potentially saliva (Corstjens et al., 2014). In contrast to the POC-CCA assay, the UCP-LF CAA test format is not yet commercially available nor is its current format applicable for POC application because of a sample preparation procedure and the use of an UCP strip-reader. While the cost of the former is USD 1-1.5 per test, that of the latter, being a manual laboratory test, is at least 10-fold higher. However, as shown here, the UCP-LF CAA test does display a superior sensitivity by concentration of the clinical sample and may therefore detect single-worm infections (Corstjens et al., 2014). Still, as our results show that the POC-CCA assay is applicable for field diagnosis of *S. mekongi*, this assay should be the approach of choice for schistosomiasis diagnosis in Lao PDR and Cambodia with the current infrastructure.

We found a strong correlation of the test results of the urine and serum CAA tests and ELISA, while the correlations between the two CAA tests and the Kato-Katz and POC-CCA were weaker. These observations are consistent with previous studies in the People's Republic of China (van Dam et al., 2015b) and elsewhere (Knopp et al., 2015; Lamberton et al., 2014) and are largely a reflection of the different sensitivities of these diagnostic tests.

It should be mentioned that the results presented here are interpreted rather conservatively with respect to the cut-off threshold, leaving the POC-CCA trace scores and the UCP-LF CAA indecisive values as negatives. A more detailed comparison of the different assays using e.g., latent class analysis may shed a better insight in the actual status of trace and indecisive samples. Such additional analyses, incorporating also a quantitative analysis of the POC-CCA results using a gold strip reader, are being planned.

In agreement with previous evaluations of the various assays for circulating schistosome antigens in areas endemic for other schistosome species, we found that the POC-CCA is both more rapid and more sensitive than multiple Kato-Katz thick smears. In the present study

the number of positives identified by POC-CCA was significantly higher than those found by Kato-Katz in both counties. These results are in accordance with published results which showed that POC-CCA prevalence was between 1.5- and up to 6-fold higher than Kato-Katz prevalence estimates in areas with low infection intensity (Kittur et al., 2016). The comparable cost levels per determination for POC-CCA and Kato-Katz (Sousa-Figueiredo et al., 2009; Worrell et al., 2015) should not prevent the application of the rapid test in national schistosomiasis control programmes. Furthermore, people are more likely to provide urine samples than any other type of sample, leading to higher compliance.

While eggs continue to be excreted by the host for a few weeks after cure, both CCA- and CAA-levels drop quickly, sometimes turning negative within 1 week after treatment (de Jonge et al., 1989; Lamberton et al., 2014), making this approach a promising tool to monitor drug efficacy. The sensitivity of CCA-based tests is not as high as what the UCP-LF CAA assay or what DNA-based detection methods can offer (Lodh et al., 2013; Obeng et al., 2008), while the ultrasensitive SCAA500 format of the UCP-LF CAA test surpasses PCR in sensitivity (Stothard et al., 2014; Wilson et al., 2006). As many different diagnostic assay systems are now available, planning to assess geographic areas potentially endemic for schistosomiasis, multiple diagnostic approaches should be compared taking into account modelling and statistical methods in combination with knowledge how biological systems operate (Knopp et al., 2015; Koukounari et al., 2013).

8.2.4. Improved latrines have a small short term impact on the transmission of *Schistosoma mekongi, Opisthorchis viverrini* and other helminth infections on Mekong islands, Southern Lao PDR

Sanitation improvement such as constructing of ventilated improved pit latrines, provision of clean drinking water, and hygiene education affected to helminth and protozoa infection decreasing (Gelaye et al., 2014; Graham and Polizzotto, 2013; Pruss et al., 2002; Strunz et al., 2014; WHO, 2014; Wolf et al., 2014). Strunz and colleages conducted a systematic review of WASH on infection with STH and showed that WASH access and practices are generally related to reduced odds of STH infection. Use of treated water was associated with lower odds of STH infection (odds ratio [OR] 0.46, 95% CI 0.36–0.60). Access to sanitation was associated with decreased likelihood of infection with any STH (OR 0.66, 95% CI 0.57–0.76), *T. trichiura* (OR 0.61, 95% CI 0.50–0.74), and *A. lumbricoides* (OR 0.62, 95% CI 0.44–0.88), but not with hookworm infection (OR 0.80, 95% CI 0.61–1.06) (Strunz et al., 2014). In our study, we compared intestinal helminth infection rates before and after intervention consisting of a latrine construction and utilisation campaign in selected intervention villages. The intervention villages were compared with control villages where no latrine construction and utilisation campaign was conducted. In all villages a MDA against all endemic parasitic infections were performed twice, in particularly against *S. mekongi, O. viverrini* (using praziquantel), STH using albendazole), including *S. stercoralis* (by using Ivermectin). After the intervention an excellent latrine coverage of 100% was reached in the intervention villages while in the control villages the latrine coverage remained unchanged. The follow-up surveys were conducted 12 months after the latrine intervention was completed and the second MDA was performed. We found that in all study villages the infection rates of *S. mekongi*, *O. viverrini*, hookworm and *S. stercoralis* were significantly lower compared to the initial infection prevalence, regardless whether the villages belonged to intervention or control group. We attribute the reduction the MDA in all study villages. Given the considerable infection rates assessed at follow-up indicate that the transmission of these helminth species is on-going in all study villages and has led to the observed infection rates.

There is a difference in re-infection rates between intervention and control villages. However the differences are marginal. Obviously the latrines did not avoid a complete re-infection of the studied helminthiasis particularly *S. mekongi*, *O. viverrini*, hookworm and *S.*

stercoralis. The reason might be the continuing of open defecation of villagers even having latrine at home which was still high 35.7% in the intervention villages (Table 3). Regarding to human side of intervention villages, one of the reason why latrines were not totally in use was that the daily life of farmer or fisherman which have to go for work in early morning then they did defecation in the rice field or some islands nearby their work places. The explanation above was from our observation and interviewing some villagers but was not included in the questionnaires. Despite, other factors should be also considered to be the reasons of re-infection such as the infection of intermediate and animal reservoir hosts, personal hygiene, raw food consumption behaviour, etc. according to the life cycle of each parasite which can be the potential sources for the transmission and distribution.

Other reasons for the high infection rates might be that main risk factors for infection persist. For *S. mekongi*, given the villagers daily life relate to water contact in Mekong river which almost more than 90% such as bathing, clothes washing, fishing, etc. and *O. viverrini* infection relates to raw fish consumption behaviour which was deep cultural practice in this area. Those main factors support these trematodes parasite transmission and their re-infection in the studies areas.

Hookworm and *S. stercoralis* are transmitted by their larval form burrowing through the skin of the foot as someone squats to defecate on an area of soil previously used for defecation by others or whenever bare foot working in the rice field (Ericsson et al., 2001; Hotez et al., 2004). The KAPP showed that people in both control and intervention villages were practice bare foot more than 80% which allowed for those parasites re-infection.

Regarding to *S. mekongi*, it is important to note that the nearest and similar environment neighbouring islands for the intervention island were already done MDA with praziquantel by local health authority in a few months before our study implementation therefor we had chosen other islands to be the control villages. That was why the quite big different was observed in terms of the infection rate between intervention and control villages from both baseline and follow-up study (28.6% vs 1.8% and 22.6% vs 2.6%, respectively). It was might not good control group selection for this intervention. However, it was not affect to other helminthic infection.

Although this study did not collect data on the community mobilization specifically, the small providing of the project such as the pit and the slab then let the household members conducted the latrines by themselves. This such activity in our study demonstrated that community mobilization can be an effective, low-cost way to increase latrine ownership which many solid and nice latrines were built in the intervention villages.

9. Conclusions

We conclude that human intestinal helminth infections, namely *O. viverrini, S. mekongi, S. stercoralis* and hookworms are still highly endemic on the Mekong islands in Khong district. Particularly, we noted *S. stercoralis* infection was very high by using more sensitive tool as Baermann test and it should be noted by the national helminth control programme. Moreover, their heavy infections and multi-parasitism were still observed there in all studies of this thesis research.

The low prevalence of *O. viverrini* and *S. mekongi* infection in intermediate snail hosts point at on-going transmission. In addition, infection rates of locally caught *Cyprinoid* fish with *O. viverrini* and minute intestinal fluke (MIF) metacercariae were very high, pointing to a high risk of infection when they are consumed raw or undercooked. Animal reservoir hosts, particularly cats and dogs, have high *O. viverrini* infection rates, while only dogs are infected with *S. mekongi*. Therefore, an appropriate integrated control approach involving interventions targeting humans, animal reservoirs, and environmental modification might improve the effectiveness of interventions and lead to the elimination of infections.

Active schistosomiasis mekongi in Lao PDR and Cambodia might thus have been considerably underestimated previously. CCA-based assays are already available for use in the field, but tests targeting CAA still need the laboratory due to some of the sample preparation steps. Although the latter approach is the most sensitive antigen test, it would still be useful to apply POC-CCA testing for screening.

According to the result of a marginal short term impact the transmission of *S. mekongi, O. viverrini* and other helminth infections on Mekong islands, Southern Lao PDR. Hookworm and *S. stercoralis* were showed clearly reducing of the re-infection. The period of latrine intervention in this study could not stop the parasite re-infection. Therefore, long-term latrine intervention with eco-health combination approaches might be more effect to reduce all gastro-intestinal helminthiasis prevalence.

10. Further research needs

The research activities conducted within this PhD thesis have advanced our understanding of various aspects of helminth control in Lao PDR. However, they have also resulted in a serious of further most important research questions and needs. Some of them are:

- The overall epidemiological picture of *S. stercoralis* infection is still missing. Large scale investigation for *S. stercoralis* infection using the sensitive tool as Baermann test is needed to determine its true prevalence and exact distribution in order to target the control activities.
- Furthermore, still today the extend of morbidity associated with *S. stercoralis* infection is unknown and particularly needed in order to guide policy makers on the priority setting in the control of this helminth infection.
- Of particular importance are in this context patients with specific immunocompromised condition. More research is required to understand the effects of *S. stercoralis* infection in this patient group.
- Today helminth control follows the "preventive chemotherapy" strategy, resulting largely in mass-drug distribution of nematocidal and trematocidal drugs. However, the prevention of infection and re-infection must be achieved in order to free the preventive health services from the cumbersome mass-drug administration and keep the population free of infection. EcoHealth approaches have the potential to result in sustainable control approaches. However, more EcoHealth intervention for *O. viverrini* and other helminth is needed to be evaluated to find the appropriate approaches. Question such as cost-effectiveness of animal reservoir hosts interventions or impact on eating raw food behaviour changes intervention need to be evaluated.
- School health program together with ecohealth intervention which targets specifically to the endemic areas of *O. viverrini* and *S. mekongi* maybe good control approaches.
- Research on the epidemiology of cholangiocarcinoma among Lao population should be conducted to know the morbidity and how much its association to *O. viverrini* infection. This investigation would like to show the association of medical important between *O. viverrini* infection and cholangiocarcinoma among Lao people to encourage the high range of health administrators, local government units, researchers, non-government organizations, other government organizations to be aware.

- Given the advancement of the *S. mekongi* control in the last decade the eradication of this helminth from the Mekong is in reach. The evaluation of the POC-CCA urine test for the diagnosis of *S. mekongi* was very positive. However, POC-CCA showed an very high rate of *S. mekongi* positive person. These results need further confirmation before the test can be introduced as a standard in *S. mekongi* endemic area. In particular, the importance of cross-reactivity with other helminth infections and the change of POC-CCA positivity after treatment need to be addressed.
- Our study has shown after one year a latrine construction and utilisation programme is not measurable in terms of reduced incidence of *S. mekongi, O. viverrini* and *S. stercoralis.* Long-term latrine interventions best combined with eco-health approaches are required to assess the effects on transmission of gastro-intestinal helminthiasis and the cost-effectiveness.

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English, fluent in speaking, reading and writing

Thai, fluent in speaking, reading and writing

French, basic conversation

Profession: General Physician (MD), Lecturer of Medical parasitology

Domains of interest:

Research and control of parasitic infections and associated health conditions; management of interventions and operational research; teaching and training

Degrees

1995-2001: Bachelor of Medicine **(MD)**, National University of Laos, Faculty of Medicine.

2007-2010: Master of Science in Tropical Medicine **(MSc. TropMed)**,
Mahidol University, Faculty of Tropical Medicine, Bangkok, **THAILAND**

- Parasitology, Serodiagnosis
- MSc Thesis, *Gnathostoma* and gnathostomiasis in three provinces of Lao PDR

• Thesis Supervisor: Assoc. Prof. Jitra Waikagul *and*
Assoc. Prof. Paron Dekumyoy

2010-Now: **PhD student**, Department of Epidemiology and Public Health, Swiss Tropical and Public Health Institute **(Swiss TPH)**, University of Basel, **Switzerland**

• Control approach for *Opisthorchis viverrini* and co-infections in Lao PDR

• Thesis Supervisor: Assoc. Prof. Peter Odermatt

Training experiences

2003: Certificate of **"International Training Course on School-based Malaria and Soil-transmitted Helminthiasis Control for Programme Managers 2003".** Organized by JICA, ACIPAC (Asian Centre of International Parasite Control) at Mahidol University, Faculty of Tropical Medicine, Bangkok, **THAILAND**

2005: Certificate of **"Training Workshop on Managing the Integration of Culture into Development Programmes".** Organized by the Asean Foundation and SPAFA at Vientiane Capital, **Lao PDR**

2005: Certificate of **"Training Workshop on Medical Educational Assessment"** at Faculty of Medicine, University of Calgary, Alberta **(CANADA)**

2006: Training on Serodiagnosis, **using Immunoblot technique** for paragonimus infection, Faculty of Tropical Medicine, Mahidol University, Bangkok, **THAILAND**

2010: Certificate of **"Good Clinical Practice (GCP) for Investigators and Sub-Investigators"** at Swiss Tropical and Public Health Institute (Swiss TPH), University of Basel, Basel **(SWITZERLAND)**

2013: Certificate of **"Training Course on Scientific Manuscript Writing".** Organized by Research Institute for Tropical Medicine (RITM), Regional Training Center for Good Health Practices for the Western Pacific Region, held in RITM Training Center, Muntinlupa City, **PHILIPPINES**

2016: Training on Serodiagnosis, **ELISA test** for Cysticercosis, Sparganosis, Paragonimiasis and Clonorchiasis, Department of Parasitology and Tropical Medicine, Seoul National University College of Medicine, **KOREA**

Scientific meeting experiences

2006: Attended in **"Joint International Tropical Medicine Meeting 2006 and 6th Asia-Pacific Travel Health Conference (JITMM 2006-6th APTHC)"** and Oral presentation with a title of Epidemiological study on paragonimiasis in Kasy District, Vientiane Province, Lao PDR in **"5th Seminar on Food- and Water – borne Parasitic Zoonoses (5th FBPZ)"** in Bangkok **(THAILAND)**

2007: Oral presentation with a title of Paragonimiasis in Kasy District, Vientiane Province, Lao PDR in **"Joint International Tropical Medicine Meeting 2007"** in Bangkok **(THAILAND)**

2008: Oral presentation with a title of *Gnathostoma* and gnathostomiasis in Champasack Province, Southern of Laos in **"Joint International Tropical Medicine Meeting 2008"** in Bangkok **(THAILAND)**

2009: Oral presentation with a title of Gnathostomiasis in three provinces of Laos in **"6th Seminar on Food- and Water-borne Parasitic Zoonoses (6th FBPZ)"** in Bangkok **(THAILAND)**

2011: Attended in **"11th Regional Network for Asian Schistosomiasis and Other Helminth Zoonoses".** 17-19th October 2011, Siem Reap City, **Cambodia**

Teaching

2002-Now: **Lecturer of Parasitology** at University of Health Sciences, Vientiane Capital, Lao PDR.

2005-2006: **Course coordinator** for new curriculum development (Integrated curriculum) of Faculty of Medicine, National University of Laos.

Publication List

1. **Youthanavanh Vonghachack**, Dalouny Bouakhasith, Jun Kobayashi, Paron Dekumyoy, Jitra Waikagul. **Paragonimiasis in Kasy District, Vientiane Province, Lao PDR, using immunoblot test.** 2006 (principal investigator) *(unpublished)*
2. Hyun-Ouk Song , Duk-Young Min , Han-Jong Rim , **Vonghachack Youthanavanh** , Bouakhasith Daluny , Vongsouvan Sengdara, Banouvong Virasack and Phommasak Bounlay. **Skin Test for Paragonimiasis among Schoolchildren and Villagers in Namback District, Luangprabang Province, Lao PDR**. *Korean J Parasitol. Vol. 46, No. 3: 179-182, September 2008.*

3. Somphou Sayasone, **Youthanavanh Vonghachack**, Monely Vanmany, Oroth Rasphone, Smarn Tesana, Jürg Utzinger, Kongsap Akkhavong, Peter dermatt. **Diversity of human intestinal helminthiasis in Lao PDR**. *Transactions of the Royal Society of Tropical Medicine and Hygiene (2009) 103, 247-254.*
4. Woon-Mok Sohn, Jae-Sook Ryu, Duk-Young Min, Hyun-Ouk Song, Han-Jong Rim, **Youthanavanh Vonghachack**, Daluny Bouakhasith,Virasack Banouvong. **Indochinamon ou (Crustacea: Potamidae) as a New Second Intermediate Host for *Paragonimus harinasutai* in Luang Prabang Province, Lao PDR.** *Korean J Parasitol. Vol. 47, No. 1: 25-29, March 2009.*
5. Urusa Thaenkham, Supaporn Nuamtanong, Surapol Sa-nguankiat, Tippayarat Yoonuan, Sarun Touch, Khemphavanh Manivong, **Youthanavanh Vonghachack**, Megumi Sato, Jitra Waikagul. **Monophyly of *Opisthorchis viverrini* populations in the lower Mekong Basin, using mitochondrial DNA nad1gene as the marker.** *Parasitology International 59 (2010) 242 –247.*
6. **Youthanavanh Vonghachack**, Paron Dekumyoy, Tippayarat Yoonuan, Surapol Sa-nguankiat, Supaporn Nuamtanong, Urusa Thaenkham, Bounlay Phommasack, Jun Kobayashi, Jitra Waikagul. **Sero-epidemiological survey of gnathostomiasis in Lao PDR.** *Parasitology International (2010).*
7. Soukhathammavong P, Odermatt P, Sayasone S, **Vonghachack Y**, Vounatsou P, Hatz C, et al. **Efficacy and safety of mefloquine, artesunate, mefloquine-artesunate, tribendimidine, and praziquantel in patients with Opisthorchis viverrini: a randomised, exploratory, open-label, phase 2 trial**. *Lancet Infect Dis 2011*
8. Lovis L, Mak TK, Phongluxa K, Aye Soukhathammavong P, **Vonghachack Y**, Keiser J, et al. **Efficacy of praziquantel against *Schistosoma mekongi* and *Opisthorchis viverrini*: a randomized, single-blinded dose-comparison trial**. *PLoS Negl Trop Dis 2012*
9. Forrer A, Sayasone S, Vounatsou P, **Vonghachack Y**, Bouakhasith D, Vogt S, et al. **Spatial distribution of, and risk factors for, *Opisthorchis viverrini* infection in southern Lao PDR**. *PLoS Negl Trop Dis 2012*
10. Phongluxa K, Xayaseng V, **Vonghachack Y**, Akkhavong K, van Eeuwijk P, Odermatt P. **Helminth infection in southern Laos: high prevalence and low awareness.** Parasit Vectors. 2013;6(1):328. doi: 10.1186/756-3305-6-328.

11. Aye Soukhathammavong P, Rajpho V, Phongluxa K, **Vonghachack Y,** Hattendorf J, Hongvanthong B, et al. **Subtle to severe hepatobiliary morbidity in *Opisthorchis viverrini* endemic settings in southern Laos.** Acta Trop. 2015;141(Pt B):303-9. doi: 10.1016/j.actatropica.2014.09.014. Epub Sep 29.
12. **Vonghachack Y**, Sayasone S, Bouakhasith D, Taisayavong K, Akkavong K, Odermatt P. **Epidemiology of *Strongyloides stercoralis* on Mekong islands in southern Laos**. *Acta Trop 2015 Jan;141(Pt B):289-94*
13. Sayasone S, Odermatt P, **Vonghachack Y**, Xayavong S, Senggnam K, Duthaler U, et al. **Efficacy and safety of tribendimidine against *Opisthorchis viverrini*: two randomised, parallel-group, single-blind, dose-ranging, phase 2 trials.** Lancet Infect Dis. 2016;26(16):30198-0.