“They get under your skin: *Strongyloides stercoralis* and hookworm distribution, risk profiling, and control in Cambodia”

INAUGURALDISSERTATION

zur

Erlangung der Würde eines Doktors der Philosophie

vorgelegt der

Philosophisch-Naturwissenchaftlichen Fakultät

der Universität Basel

von

Armelle Forrer

aus Winterthur, Zürich, Schweiz

Basel, 2018

Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakultät auf Antrag von

Prof. Dr. Marcel Tanner, PD Dr. Peter Odermatt, Prof. Dr. Russel Stothard

Basel, den 20. Juni 2017

Prof. Dr. Martin Spiess

Dekan

Summary

Background

Strongyloides stercoralis is a highly neglected intestinal nematode that has been overlooked and under-reported for decades, due to the use of inadequate diagnostic techniques in health services of endemic countries and in field studies.

S. stercoralis occurs worldwide but thrives in regions with warm climate and poor sanitation. Recent estimates suggest prevalence rates between 10% and 40% in the tropics and subtropics. Globally, at least 200-370 million people would be infected worldwide. However those are rough figures based on few data and assumptions and need to be better estimated. This parasite is a major public health issue in Cambodia, where prevalence rates between 25% and 40% have recently been found in the South and the North, respectively. Still, national estimates are not yet available and the zones of high, moderate or low infection risk remain to be identified.

Because it can be life-threatening, *S. stercoralis* is well known in hospitals of developed countries. *S. stercoralis* has the unique ability among parasitic nematodes to replicate within its host. Known as "autoinfection", it may lead to infections persisting over decades and to hyperinfection, a condition that is 100% fatal if untreated. However, the morbidity associated with chronic infections in endemic settings has rarely been studied and assessing its extent is of major importance to better estimate the burden and full public health impact of this parasite.

The mainstay of the WHO's "preventive chemotherapy" control strategy against STH is regular chemotherapy with single-dose of albendazole or mebendazole, either through targeted treatment of specific at-risk groups or by mass-drug administration to entire populations. However *S. stercoralis* is not addressed by those control programmes because delivered drugs have are not efficacious against it. The drug of choice against *S. stercoralis* is ivermectin, of which a single oral dose has been shown to be highly efficacious.

To date, there is no control strategy against *S. stercoralis*. In face of the very high *S. stercoralis* infection rates and the evidence of associated morbidity in Cambodia, control programmes are needed. Pre-requisite to programme designing are several key features, including the impact of treatment in endemic communities and age groups that are the most affected and should be targeted by control measures.

Aims and objectives

This main objective of this work was to contribute to the knowledge-base to help guiding *Strongyloides stercoralis* control efforts in Cambodia, while secondary objectives included profiling hookworm infection risk in the region, either alone or in co-occurrence with *S. stercoralis*.

Five main objectives were pursued: (i) to assess the community-effectiveness of annual targeted ivermectin treatment (200µ/kg BW, single dose) against *S. stercoralis*, with regard to rates of, and risk factors for, re-infection, treatment efficacy and morbidity resolution in an endemic population, (ii) to assess the cost-effectiveness of targeted ivermectin treatment (200µ/kg BW, single dose) *vs.* mass drug administration to school-aged children or all age groups for the control of *S. stercoralis* in Cambodia, (iii) to estimate national prevalence and assess the national distribution of *S. stercoralis* in Cambodia, (iv) to assess the distribution of hookworm-*S. stercoralis* co-infection in North Cambodia, (v) to assess the distribution of hookworm infection risk and intensity and investigate associated risk factors at provincial level in Southern Lao PDR .

Methods

A two-year community-based cohort study consisting in a baseline survey and two-follow up surveys, 12 and 24 months after baseline, was conducted between 2012 and 2014 in 8 villages of Preah Vihear province, North Cambodia. The cohort consisted in all *S. stercoralis* cases detected at baseline and a random sample of 300 *S. stercoralis* negative participants living in two of the study villages. At each survey, data on demography, socioeconomic status, water, sanitation, behaviour and knowledge on worms were collected. *S. stercoralis* was diagnosed using combined Baermann method and Koga Agar Plate technique (KAP) on two stool samples. The Kato Katz method used on two samples and the formalin-ether concentration technique (FECT) on one sample were used to diagnose other helminth and protozoan infections, respectively. All *S. stercoralis* cases were treated with a single oral dose of ivermectin (200µg/kg BW). A before-after treatment survey was conducted among a subsample of about 300 individuals to investigate clinical signs resolved by treatment. Mixed logistic regression models were used to investigate risk factors for *S. stercoralis* prevalent and incident risk.

Using results as well as cost data collected during the above-mentioned study or in the literature, an economic analysis assessing the cost-effectiveness of three potential alternative control approaches for *S. stercoralis* was conducted. The three options were (i) targeted treatment (i.e. following diagnosis with combined Baermann and KAP methods on two samples), (ii) mass drug administration to risk groups already targeted by STH control programmes in Cambodia, i.e. children and women of child bearing age, and (iii) mass drug administration to entire communities.

A nation-wide community-based cross-sectional survey was conducted in 2016 among the general population aged 6 years and above in all 25 provinces of Cambodia, to assess the national prevalence and distribution of *S. stercoralis*. *S. stercoralis* diagnosis was performed using a serological test detecting antibodies in urine and an individual questionnaire was administrated to collect demographic, behavioural and knowledge data, which were combined to remote sensing environmental data. Geostatistical logistic regression models were used to assess the factors associated with, and the distribution of, *S. stercoralis* infection risk, and to predict *S. stercoralis* infection risk throughout the country.

A community-based large scale survey was conducted in 2010 in 60 villages of Preah Vihear province, North Cambodia. *S. stercoralis* was diagnosed using combined Baermann method and the Koga Agar Plate technique (KAP) on two stool samples. Hookworm was diagnosed using Kato Katz on two stool samples. Demographic, behavioural, knowledge, water, sanitation and socioeconomic data were combined with remotely sensed environmental data. Geostatistical multinomial regression models were used to investigate the risk factors for, and the distribution of, *S. stercoralis* and hookworm mono- and co-infection in Preah Vihear province, and to predict *S. stercoralis* and hookworm mono- and co- infection risk throughout the country.

A community-based large scale survey was conducted in 2007 in 51 villages of Champasack province, Lao PDR. Helminths were diagnosed with duplicate Kato-Katz thick smears. Data on demography, socioeconomic status, water, sanitation, and behaviour were combined with remotely sensed environmental data. Bayesian mixed effects logistic and negative binomial models were utilized to investigate risk factors and spatial distribution of hookworm infection and intensity, and to make predictions for non-surveyed locations.

Findings

In the cohort study, 33% of the 3,096 participants present at baseline were infected with *S. stercoralis*. The cohort followed-up for two years included 1,269 participants. Ivermectin treatment was highly beneficial to endemic communities, with a cure rate estimate to be of 96.6% and treatment resulting in re-infection rate below 15% one year after treatment. While *S. stercoralis* prevalence tends to increase with age, probably to the maintenance of infection through time, the risk acquiring a new infection after treatment did not vary across age groups. Community-level sanitation coverage impacted on re-infection rates: the higher the coverage, the lower the re-infection risk.

Symptoms specifically associated with *S. stercoralis*, i.e. excluding other helminth infections and pathological protozoa, were identified in a multi-parasitic setting. Gastrointestinal, dermatological, and respiratory symptoms were less prevalent in 103 mono-infected participants after treatment with a single oral dose of ivermectin (200µg/kg BW). Treatment resolved urticaria (66% *vs.* 11%, OR: 0.03, 95% CI: 0.01 - 0.1) and abdominal pain (*81 vs.* 27%, OR: 0.07, 95% CI: 0.02 - 0.2) in most patients. We also found that children heavily infected with *S. stercoralis* were more likely to suffer from malnutrition and physical development retardation, particularly in case of high parasite load.

All interventions assessed by the economic study were cost effective compared to Cambodia Gross Domestic Product. MDA interventions were the most cost-effective a few exceptions aside, at $108 and $107 per case cured, for the interventions targeting children and women or entire communities, respectively. The targeted treatment alternative was more cost-effective when prevalence was below 20%. The cost to treat one person was $29 with any MDA option. However those costs are too high to be entirely supported by the Ministry of Health and currently preclude any deployment of *S. stercoralis* control in Cambodia.

In Cambodia, almost a third (30.5%) of the 7,246 participants in the national survey was infected with *S. stercoralis.* The parasite was ubiquitous in the country, with prevalence rates below 20% only in five south-eastern provinces. Province-level prevalence ranged between 10.9% and 48.2%. Infection risk increased with age both in men and women although girls aged less than 13 years and women aged 50 years and above had lower odds of infection than their male counterparts. Open defecation was associated with higher odds of infection while declaring having some knowledge about health problems caused by worms was protective. Infection risk was positively associated with night maximum temperature, minimum rainfall, and distance to water, and negatively associated with land occupied by rice fields.

In Preah Vihear province, North Cambodia, *S. stercoralis* and hookworms infected 48.6% and 49.2% of the 2,576 study participants, respectively, with 44% of all cases being co-infections. Females, preschool-aged children, adults aged between 19 and 49 years, and participants who reported regularly defecating in toilets, systematically boiling drinking water and having ever been treated with anthelmintic drugs had lower odds of co-infection. The geographical distribution of hookworm and *S. stercoralis* mono-infections were mostly explained by climatic and environmental factors, but the distribution of the co-infection was not, suggesting the influence of other processes, such as socioeconomic status or health-related behaviour.

In Champasack Province, southern Lao PDR, hookworm prevalence was of 48.8% among the 3,371 participants in the study, with most infections (91.7%) being of light intensity. Lower hookworm infection levels were associated with higher socioeconomic status. The lowest infection levels were found in preschool-aged children. Overall, females had lower odds of infection, but women aged 50 years and above harbored the heaviest hookworm infection intensities. Hookworm was widespread in Champasack province with little evidence for spatial clustering. Infection risk was somewhat lower in the lowlands, mostly along the western bank of the Mekong River, while infection intensity was homogeneous across the Champasack province. Hookworm transmission seemed to occur within, rather than between villages in Champasack province. Risk maps of hookworm infection and intensity suggest that control efforts should be intensified in the Champasack province, particularly in mountainous areas.

Conclusions

S. stercoralis is ubiquitous in Cambodia where it infects almost a third of the population. Let alone the risk of hyperinfection that remains unknown in endemic settings, *S. stercoralis* chronic infections are responsible for significant gastrointestinal and dermatological morbidity. This combination of high prevalence and morbidity confirms the urgent need for control programmes delivering ivermectin in Cambodia.

However, the current high price of ivermectin, precludes the deployment of *S. stercoralis* control in the country. Affordable generics, subsidies or donations of ivermectin are needed to start tackling *S. stercoralis* in Cambodia.

The example of Cambodia shows that estimating *S. stercoralis* prevalence, despite the need of time and resource consuming diagnostic approaches, is feasible even in countries where resources are scarce. Hopefully, the case of Cambodia will help triggering interest from the scientific and public health community for this long neglected parasite that is likely to be extremely common, as least in South East Asia.

It is to be hoped that Cambodia will be the first of a long list of countries providing prevalence and burden estimates for *S. stercoralis*, which in turn might help raising awareness and improve access to ivermectin for *S. stercoralis* control so the infection can be adequately managed and the health of affected populations can be improved.

Acknowledgements

In 2005 I enrolled in an e-learning university certificate with the aim of getting acquainted with public health, the wide field I chose for my professional reconversion. This is how I discovered epidemiology and I instantly knew that this was what I wanted to do. It had it all, science, health, people, and trying to help improving some lives. This is how, no less than ten years ago, I first entered the Swiss TPH, at that time, the Swiss Tropical Institute, to attend the Master in Epidemiology. Time flies. It will have taken a couple of interruptions, a few jobs… and ten years overall to be finalizing this thesis. Those years did not all go completely smoothly and required stubbornness as well as extended help and support from my family and friends. Yet, I would go all over it again whatever the obstacles. I am not sure how all this would have gone, and if this thesis would be there without the presence and support of David, no matter what, all along this adventure. Engaging in those studies and embracing epidemiology was one of the best decisions I ever made. I feel incredibly lucky to have had the opportunity to go through all this and be there today.

I am greatly and sincerely grateful to my supervisor PD Dr Peter Odermatt to have taken me on board and given me the opportunity to discover how fascinating this wormy world is. I deeply thank him for offering the privilege to work with him and his teams for all those years, for his teaching, his trust, for always having been there for me, for his communicative enthusiasm, and for always being open to discussion and supportive.

My deepest thanks go to PD Dr Penelope Vounatsou for getting me acquainted with statistics and then, for taking me through a challenging but fascinating journey into her Bayesian world and spatial statistics.

I am sincerely grateful to PD Dr Christian Schindler, who accompanied me through a part of my studies and who is an amazing teacher and statistical supervisor. Christian helped me realize that modelling is not only a science but also an art. Analysing data is a world of infinite shades of grey rather than of black and white, and that contributes to making it so stimulating and fascinating.

My stays in Cambodia would not have been the same without Molyden Vann. I will never thank her enough for having been there for me, for making everything easy, for her great efficiency and for her warm and kind presence which were invaluable.

My warmest thanks go to Dr Virak Khieu and Dr Fabian Schär for collecting the excellent data that I was lucky to analyse. I am very grateful to Virak Khieu for his patience in answering all kinds of questions, and for the amazing moments in the field.

I am also extremely grateful to Dr Sinuon Muth, to have, despite her overly busy schedule, taken the time to answer so many questions, and have told me a little bit of the amazing work she accomplished in Cambodia. Sinuon Muth has been awarded a "NTDs hero award", and that for sure does not even do justice to what she accomplished in the field of helminth control in her country.

Everything presented in this work is the fruit of teamwork and I also would like to sincerely thank Ms. Sophal Tess, Ms. Sam Ath Kong, Ms Sileang Sin, Ms. Pouthleakhana Heng, Mr Chetana Ten, Mr Chanmakara Mean, Mr Nhel Vann, and Dr Saomony Chhay, for their dedication and excellent work in the field that produced high quality data. Many thanks go of course to Dr Somphou Sayasone, Youthanavanh Vonghachak, and the field team who worked in Champasack. I also sincerely thank Dr Hanspeter Marti, Dr Jan Hattendorf, Prof. Christoph Hatz, Dr Andreas Neumayr and Amena Briet for their advice. Finally, I would like to warmly thank Dr Frédérique Chammartin for her help and advice, as well as for our discussions.

I would like to thank Prof. Marcel Tanner and Prof. Jürg Utzinger for leading the SwissTPH, where the learning and human atmosphere is unique, and Prof. Nicole Probst-Hentsch, head of Epidemiology and Public Health Department. My sincere thanks also go to the administrative team of the Swiss TPH, particularly to Christine Mensch and Margrith Slaoui who answered any help request with a smile and a kind word, and addressed them with an amazing efficiency. I would also like to thank the library staff and the IT staff who were always there to help when needed.

Finally, I would like to deeply thank my family, in particular my dad and my grand-parents who always believed in me and supported me whatever I chose to do, including engaging in a PhD at the age of forty-one. I am also very grateful to Ysabel Fougery, Adriana Zanetti, and Astrid Erbsen for their support and friendship that accompanied me all along.

I would also like to acknowledge the UBS Optimus Foundation, Zürich, Switzerland, for funding all the studies in Cambodia, the Swiss National Foundation for funding the study in Lao PDR, and the Freiwillige Akademische Gesellschaft (FAG) for providing extra funds that helped finalizing this work.

Contents

List of figures

List of Tables

Chapter 1

Introduction

1.1 The threadworm *Strongyloides stercoralis*, an overlooked soil-transmitted helminth

Strongyloides stercoralis is an intestinal nematode which larvae living in faecally-polluted soil infect humans transcutaneously. This infection mode is the same as the well-known hookworm’s, which belongs to the group of soil-transmitted helminths (STH) together with *Ascaris lumbricoides* and *Trichuris trichiura*.

Infection with those three STH is the most common Neglected Tropical Disease (NTD) with over 1.5 billion cases worldwide, mostly in low- and middle-income countries (LMIC) and a global burden estimated at 5.18 million Disability-Adjusted Life Years (DALYs) lost in 2010 (Pullan et al., 2014, Murray et al., 2012). Yet, those figures do not reflect the burden of STH infection in full, as they do not include any estimates for *S. stercoralis*, which has been overlooked for decades (Krolewiecki et al., 2013).

1.1.1 *Strongyloides stercoralis* biology & life cycle

Among the 52 species belonging to the *Strongyloides* genus, which hosts include reptiles, birds, and a wide range of mammals, only three are known to infect humans. *S. stercoralis* is by far the most common. *S. fuelleborni* and *S. fuelleborni kellyi* infect humans exclusively in tropical Africa and in New Guinea, respectively, and have little clinical importance (Grove, 1996, Schär et al., 2013). *S. stercoralis* has first been found in 1876 in Vietnam veterans by a physician of the French navy. Research conducted in the early 20th century elucidated its transmission mode and life cycle, and described pathological manifestations in humans (Grove, 1996). The main host of *S. stercoralis* are humans but the parasite can also infect dogs and cats which can act as reservoir (Toledo et al., 2015).

The life cycle of *S. stercoralis,* presented in Figure 1.1, is complex and has two stages, a parasitic stage that occurs within the host and a free-living stage taking place in the external environment. The most singular and striking aspect of *S. stercoralis* life cycle is its ability to complete its life cycle without leaving its host, which is unique among STH. While hookworm eggs exclusively hatch in the environment, *S. stercoralis* first-stage can reach the infective state within the intestine and re-infect the host by penetration of the colon mucosa or perianal skin. This is the so-called “auto-infection”, which can maintain an infection for decades; the longest ever reported infection having lasted about 65 years (Grove, 1996, Keiser and Nutman, 2004, Leighton and MacSween, 1990). This ability is of major importance since it leads both to perpetuating infections as well as potentially fatal massive infestations.

After skin penetration, the most commonly accepted infection route is via the lymphatic system and the bloodstream, with parasites entering the blood vessels and migrating to the lungs where they are passed up the respiratory tree, arrive in the pharynx, are swallowed and eventually reach the small intestine where they develop into adulthood. However the filariform larvae are also capable of migrating through connective tissues, and the oral route has not been excluded (Grove, 1996). After having reached the anterior small intestine, the larvae mould twice and exclusively become adult parthenogenetic parasitic females. Measuring about 2 mm, they live in the submucosa of the anterior small intestine and produce eggs that hatch directly in the lumen. A female produces about 10 larvae per day, with most chronically infected patients excreting between 100 and 2000 larvae per day, corresponding to 0-20 larvae per grams of faeces (Grove, 1996). From infection to the first eggs shed, the cycle takes about one month (Grove, 1996, Toledo et al., 2015).

Excreted larvae survive 1 to 2 weeks in the external environment, where they can take two routes of development. Either they directly develop into filariform infective larvae (homogonic development) and infect a new host, or they can mature into adult males and females and sexually produce eggs (heterogonic development) which never develop into a second free living cycle but will eventually mature into infective larvae. Factors governing the mode of development, which include genetic control and the influence of external temperature, are unclear and mostly rely on knowledge on other *Strongyloides* species. Some authors observed that tropical strains are more likely to develop through sexual reproduction whatever the temperature, while temperate strains tend to undergo a direct development, especially at lower temperatures.

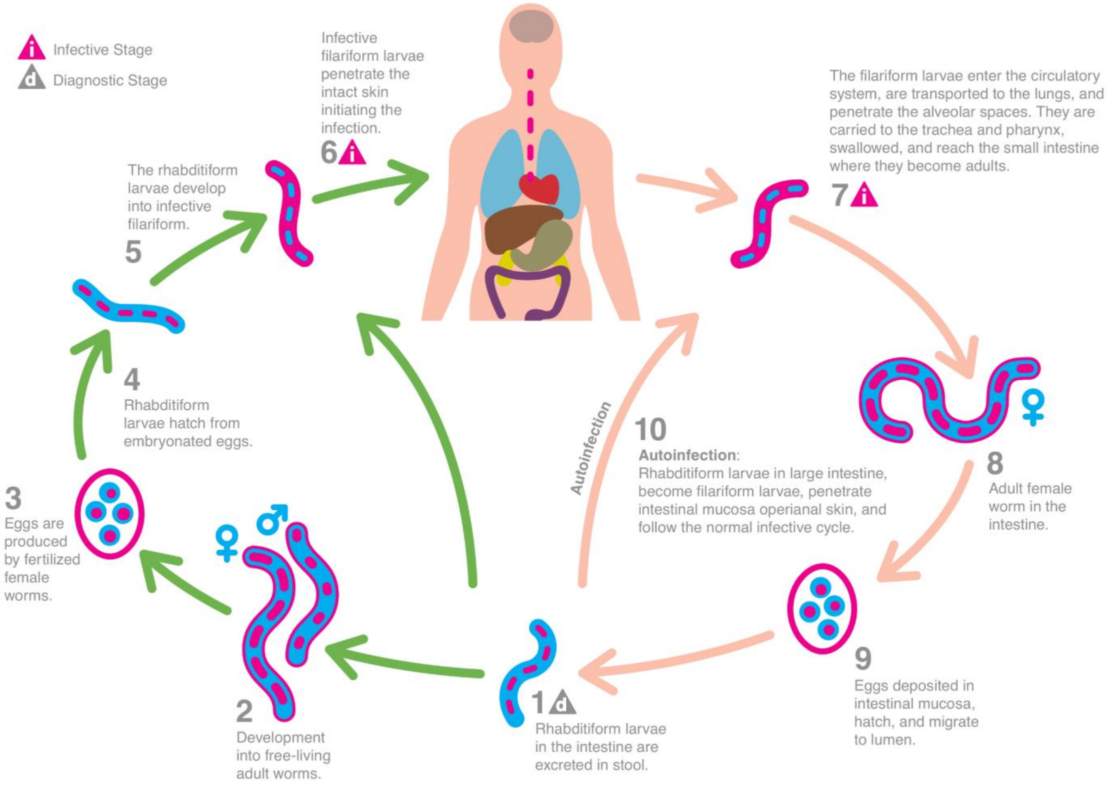


Figure 1.1: *Strongyloides stercoralis* life cycle

Reproduced from Schär et al, 2013 (Schär et al., 2013).

1.1.2 Strongyloidiasis

The most common symptoms associated with chronic *S. stercoralis* infections affect the intestinal tract and the skin. Although it is widely reported that most infections remain asymptomatic, chronic strongyloidiasis commonly involves diarrhea, abdominal pain, heartburn, borborygmus, anorexia, nausea and vomiting as well as dermatological symptoms, mostly urticarial rashes, pruritus ani and larva currens (Toledo et al., 2015, Nutman, 2016). The latter is an intermittent urticarial linear, serpiginous, migratory eruption, due to the migration of larvae under the skin, mainly located on the lower trunk, bottom and thighs. The location and high speed of the migration, between 5 and 10 centimetres per hour, make larva currens a highly specific symptom of strongyloidiasis (Becker et al., 2011, Khieu et al., 2013b, Toledo et al., 2015, Nutman, 2016, Grove, 1996). Indeed, the speed is key in differentiating larva currens from larva migrans caused by *Ancylostoma braziliense*, another migratory eruption but of slow motion (Hotez et al., 2004). Respiratory symptoms, i.e. cough, wheezing, dyspnea, are rare during chronic infections but can occur shortly after infection due to the migration of larvae through the respiratory system (Toledo et al., 2015). Finally, peripheral eosinophilia and elevated IgE are common in strongyloidiasis patients (Nutman, 2016, Concha et al., 2005).

Although no standards have yet been defined to reflect the association between morbidity and *S. stercoralis* infection intensity, a study conducted in north Cambodia among 21 patients with high worm loads (≥ 10 larvae per gram of faeces) found that abdominal pain, diarrhea, and urticaria were resolved by ivermectin treatment in most of those patients (Khieu et al., 2013b). Interestingly, a recent study conducted in a setting co-endemic for hookworm and *S. stercoralis* in Côte d’Ivoire found that self-reported morbidity was higher among patients infected with *S. stercoralis* than with hookworm (Becker et al., 2011).

The extent of the morbidity associated with *S. stercoralis* in endemic settings has rarely been assessed, and remains unclear. One difficulty is that symptoms are subtle and non-specific, i.e. common to infections caused by other helminths of protozoa. Additionally, because infections are chronic and long-lasting, mild symptoms are likely to be perceived as normal and under-reported. Estimating the morbidity associated with chronic strongyloidiasis is of major importance to better estimate the full public health impact of this parasite, including in its relation with malnutrition and poverty. Documenting morbidity is instrumental to providing global burden estimates burden (number of cases and health impacts) that have long been lacking.

1.1.3 Hyperinfection

The auto-infection ability of *S. stercoralis* is responsible for severe strongyloidiasis, which mostly occurs in patients with compromised immune functions due to concomitant diseases or corticoid therapy, and is 100% fatal if untreated (Keiser and Nutman, 2004, Fardet et al., 2007, Marcos et al., 2008). The acceleration of the autoinfection process results in hyperinfection, i.e. a massive invasion of the intestine and/or lungs. There is no strict definition of a quantitative threshold between autoinfection and hyperinfection but a clear hallmark is an increase of larvae in the stool (Keiser and Nutman, 2004, Nutman, 2016). When larvae invade other internal organs, the condition is called disseminated strongyloidiasis, a condition with a mortality rate as high as 85% (Keiser and Nutman, 2004). Clinical manifestations can be insidious or acute, vary in type and intensity and depend on the intensity of hyperinfection and the affected organs (Keiser and Nutman, 2004, Toledo et al., 2015, Concha et al., 2005). Constitutional symptoms include fever, chills, fatigue, weakness, and body pain (Keiser and Nutman, 2004). The most commonly targeted organs are the bowel, the lungs, and the central nervous system, and the most common manifestations are larva currens and non-specific gastrointestinal symptoms (Grove, 1996, Nutman, 2016, Keiser and Nutman, 2004, Toledo et al., 2015). Bacterial blood infection (bacteraemia), meningitis or infection of any organ can occur due to gut flora infiltrating through damaged tissues, or with gram-negative bacteria being transported on the surface, or digestive system of *S. stercoralis* larvae (Keiser and Nutman, 2004, Nutman, 2016, Concha et al., 2005, Mahmoud, 1996).

The major cause of hyperinfection is the use of immunosuppressive drugs to prevent transplantation graft rejection, allergies, autoimmune conditions or inflammatory disorders (Keiser and Nutman, 2004). Glucocorticoids are the most widely used and commonly associated with the evolution of infection into hyperinfection, even at low doses or with short-course treatments (Keiser and Nutman, 2004, Nutman, 2016). In a review including 151 patients with severe strongyloidiasis due to corticoid treatment, the median prednisone dosage was 52 mg ± 40mg, with 84% of patients having received a cumulative prednisone equivalent of 1000 mg and above, while treatment duration could be as short as 4 days, although 74% of the cases had a treatment course of 1 month or longer (Fardet et al., 2007). The mechanisms through which corticoids trigger hyperinfection are unknown but a commonly accepted explanation would be the suppression of eosinophilia by corticosteroids and the decreased response of cell-mediated immunity through the inactivation of lymphocytes (Keiser and Nutman, 2004, Toledo et al., 2015, Marcos et al., 2008). Interestingly, it has also been hypothesized that corticoids would trigger *S. stercoralis* replication because of their chemical resemblance to moulting hormones (Grove, 1996).

Another widely documented risk factor for hyperinfection is concomitant infection with Human T-cell Lymphotropic Virus Infection (HTLV-1). The HTLV-1 virus would decrease the T-helper (Th) 2 immunological response which is activated in response to helminthic infections and is essential to protect against hyperinfection. An increased Th1 response would result in a higher production of gamma interferon (INF-γ), one consequence of which is a reduction in specific IgE and the failure of eosinophil activation, which both have been found to be important in the response to helminthic infections (Keiser and Nutman, 2004, Herbert et al., 2000, Nutman, 2016, Ramanathan and Nutman, 2008).

The association between hyperinfection and the human immunodeficiency virus (HIV) infection, arguably the most well known cause of immunosuppression, has been reported only rarely, despite the large overlap of the two conditions (Grove, 1996, Toledo et al., 2015). Although those conditions co-occur mostly in LMIC where disseminated strongyloidiasis was the less likely to be detected and reported, the rarity of cases suggested that HIV infection does not lead to *S. stercoralis* infection, and there is no evidence so far that AIDS (acquired immune deficiency syndrome) and/or low CD4 cell count will cause hyperinfection (Grove, 1996, Concha et al., 2005, Keiser and Nutman, 2004, Nutman, 2016).

Importantly, severe strongyloidiasis has also been observed in patients with no evidence of clinical immunosuppression, particularly in developing countries (Olsen et al., 2009). A possible underlying cause might be protein-calorie malnutrition, which is known to impair immunity and is a major cause of immunodeficiency in developing countries (Olsen et al., 2009, Marcos et al., 2008). Protein-calorie malnutrition has been found associated with hyperinfection, although rarely given the extensive overlap of malnutrition and helminth infections (Nutman, 2016, Foreman et al., 2006, Stephenson et al., 2000a). Malnutrition could be responsible for severe strongyloidiasis cases that were identified in developing countries but could not be related to known causes of immunosuppression (Cimino and Krolewiecki, 2014, Olsen et al., 2009, Concha et al., 2005). It is unknown whether the association between malnutrition and *S. stercoralis* is due to impaired immunity per se or to modified metabolism of endogenous cortisol (Keiser and Nutman, 2004). However hyperinfection cases in endemic countries appear, altogether, to be missed, and reports from regions where both conditions may overlap are extremely rare, which might just reflect the lack of case detection and/or specialized studies in potentially affected areas (Olsen et al., 2009, Keiser and Nutman, 2004).

The burden caused by severe strongyloidiasis in endemic countries remains unknown and might be substantial. Given that low doses and short term treatment courses with corticosteroids can trigger the parasite multiplication, the increasing use of immunosuppressive therapies in countries going through economic transition and the wide availability of over-the-counter drug cocktails containing corticoids, is of concern. Indeed, *S. stercoralis* could lead to serious public health issues if not detected and controlled (Olsen et al., 2009). Additionally, the epidemiology of hyperinfection in endemic countries needs to be documented to correctly assess *S. stercoralis* burden.

1.1.4 Diagnosis

The most widely diagnostic technique used in the field to detect STH, the Kato-Katz technique, fails to detect *S. stercoralis* larvae and other standard methods like stool microscopy or the formalin-ether concentration technique have poor performances for the detection of *S. stercoralis* (Katz et al., 1972, Schär et al., 2013, Buonfrate et al., 2015a, Requena-Méndez et al., 2013). The Baermann technique and the Koga agar plate method are the two coprological methods that better perform for the detection of *S. stercoralis*, but multiple stool samples are required to ensure a satisfactory sensitivity, due to the low and irregular larval output (Schär et al., 2014, Albonico et al., 2016, Uparanukraw et al., 1999, Sato et al., 1995). In absence of a gold standard, the best current approach for field activities is to combine the results obtained on two stool samples examined both with the Baermann method and the Koga Agar Plate technique (KAP), which achieves 92.8% sensitivity, while increasing the number of stool samples to three yields a sensitivity of 98.6% (Baermann, 1917, Khieu et al., 2013a, Cimino and Krolewiecki, 2014, Koga et al., 1991). To ensure satisfactory performances, those coprological approaches are therefore cumbersome, time- and labour-consuming and are difficult to implement for large scale surveys (Schär et al., 2013).

Other diagnostic approaches include molecular diagnosis techniques, i.e. polymerase chain reaction (PCR) and real-time PCR that detect the parasite’s DNA in faeces, and immunologic tests based on the detection of parasite-specific antibodies or antigens in blood serum (Requena-Méndez et al., 2013, Levenhagen and Costa-Cruz, 2014, Bisoffi et al., 2014). Real-time PCR has the highest sensitivity on a single stool sample, but a high sensitivity can be ensured only by combination with Baermann or KAP on multiple stool samples (Albonico et al., 2016). Additionally, the cost of molecular techniques limits their use for prevalence studies in endemic countries (Buonfrate et al., 2015a).

Serological tests such as indirect immunofluorescence microscopy (IFAT) or Several Enzyme linked Immunosorbent Assays (ELISAs) are highly sensitive but their specificity might be of concern in areas endemic for other nematodes due to cross-reaction with other helminths species (Requena-Méndez et al., 2013, Siddiqui and Berk, 2001). Another issue is that they may overestimate prevalence in endemic areas since those methods -which rely on the detection of parasite-specific antibodies or antigens that can still be present long after contact with the parasite or cure- cannot distinguish current from past infections (Requena-Méndez et al., 2013). This aspect would not impair the parasite detection in areas naïve to treatment but would result in limited use for monitoring and post-treatment follow-up in endemic areas (Levenhagen and Costa-Cruz, 2014). A solution consisting in monitoring the antibody titres after treatment has been proposed but it unfortunately has two important limitations: first, it involves a 6-12 months follow-up of patients and second, the definition of a cut-off for cure identification is difficult, due to the variability of antibody titres across individuals, immunological conditions and regions (Levenhagen and Costa-Cruz, 2014). However, serological tests using a recombinant antigen called NIE -which has the advantage to be easily produced in large quantities- (NIE-ELISA), used on dried blood spots collected with finger prick or, a new approach developed in Thailand using an antigen from *S. ratti* to detect antibodies in urine might eventually consist good alternatives for surveys in endemic settings (Buonfrate et al., 2015a, Eamudomkarn et al., 2015).

1.1.5 Treatment

Ivermectin and benzimidazoles can be used to treat *S. stercoralis*. Ivermectin, which targets both adults and larvae, is the drug of choice (WHO, 2009, Nutman, 2016, Henriquez-Camacho et al., 2016). A single oral dose of 200µg/kg Body Weight (BW) or twice this dosage on consecutive days have been shown to be well tolerated and highly efficacious against chronic strongyloidiasis (Suputtamongkol et al., 2011, Igual-Adell et al., 2004, Gann et al., 1994, Keiser and Utzinger, 2010, WHO, 2009).

There are little guidelines for the treatment of *S. stercoralis* in immunocompromised patients and no control trials have been conducted on hyperinfection (Nutman, 2016, Marcos et al., 2008). However, ivermectin is also considered as the treatment of choice for hyperinfection cases, with oral doses of 200µg/kg BW being administered until stool exams remain negative for a minimum 2 weeks (Mejia and Nutman, 2012, Marcos et al., 2008). For disseminated strongyloidiasis, and in case of malabsorption, ivermectin can also be administered subcutaneously or via the rectum (Mejia and Nutman, 2012, Marcos and Gotuzzo, 2013).

Albendazole, which efficacy is lower than that of ivermectin, probably because it only targets adult parasites, is well tolerated in adults and children and a 400mg dose once or twice daily for 3-7 days achieves reasonable cure rates (Nutman, 2016, Keiser and Utzinger, 2010). Albendazole is a reasonable option as second-line therapy to treat both uncomplicated chronic strongyloidiasis and hyperinfection cases (Keiser and Nutman, 2004, Mejia and Nutman, 2012). Thiabendazole (25 mg/kg/day) is highly efficacious and was the treatment of choice before being supplanted, due to side effects, by ivermectin (Mejia and Nutman, 2012, Siddiqui and Berk, 2001). Mebendazole needs long treatment periods to show some efficacy, however suboptimal, due to poor absorption and a limited effect on larvae cannot prevent autoinfection (Keiser and Nutman, 2004, WHO, 2009).

For control, for which efficacious single oral doses are needed for obvious practicability issues, the only current option is a single ivermectin oral dose of 200µg/kg BW.

1.1.6 Epidemiology

Specific, time- and labour-consuming diagnostic approaches are necessary to detect *S. stercoralis* so the use of more practical but inadequate diagnostic techniques in health services of endemic countries and in field studies has resulted in the large under-reporting and overlooking of this parasite (Olsen et al., 2009, Schär et al., 2013, Bisoffi et al., 2013, Cimino and Krolewiecki, 2014).

S. stercoralis is ubiquitous and thrives in the tropics and subtropics where sanitation conditions are poor (Schär et al., 2013, Olsen et al., 2009). Infection can persist for decades. The parasite is regularly found also in high-income countries among travellers, migrants or refugees who contracted it in endemic countries where from they came or travelled through, and is common among aboriginal Australians (Schär et al., 2013, Johnston et al., 2005). Of note, *S. stercoralis* transmission also occurs in cooler climates and although rare, autochthonous cases have been reported in temperate regions including Europe (Duvignaud et al., 2016, Rodriguez Calabuig et al., 2001, Sanchez et al., 2001, Hirata et al., 2007, Glize and Malvy, 2014).

Recent estimates show that *S. stercoralis* is very common and occurs in many countries at high prevalence rates (Schär et al., 2013). Figure 1.2 presents a world map with model-based estimates of *S. stercoralis* prevalence accounting for diagnosis sensitivity and based on community-based studies conducted since 1989 (Schär et al., 2013). It was estimated that *S. stercoralis* prevalence would range between 10% and 40% of the general population in many tropical and subtropical countries and could possibly reach up to 60% in regions with socio-ecological conditions particularly favourable to the parasite (Schär et al., 2013).

However a major challenge in the estimation of the parasite global prevalence is the large variety of diagnostic approaches used and the lack of comparability (Schär et al., 2013). Along the same line, the rare available estimates of *S. stercoralis* prevalence cannot be readily compared as they were acquired using highly variable diagnostic approaches including serology and various combinations of coprological techniques. Prevalence rates of 21% and 20% have been reported in Ethiopia and Bolivia, while in Yunnan, China, 12% of villagers were found infected with *S. stercoralis* (Albonico et al., 2016, Steinmann et al., 2007a).

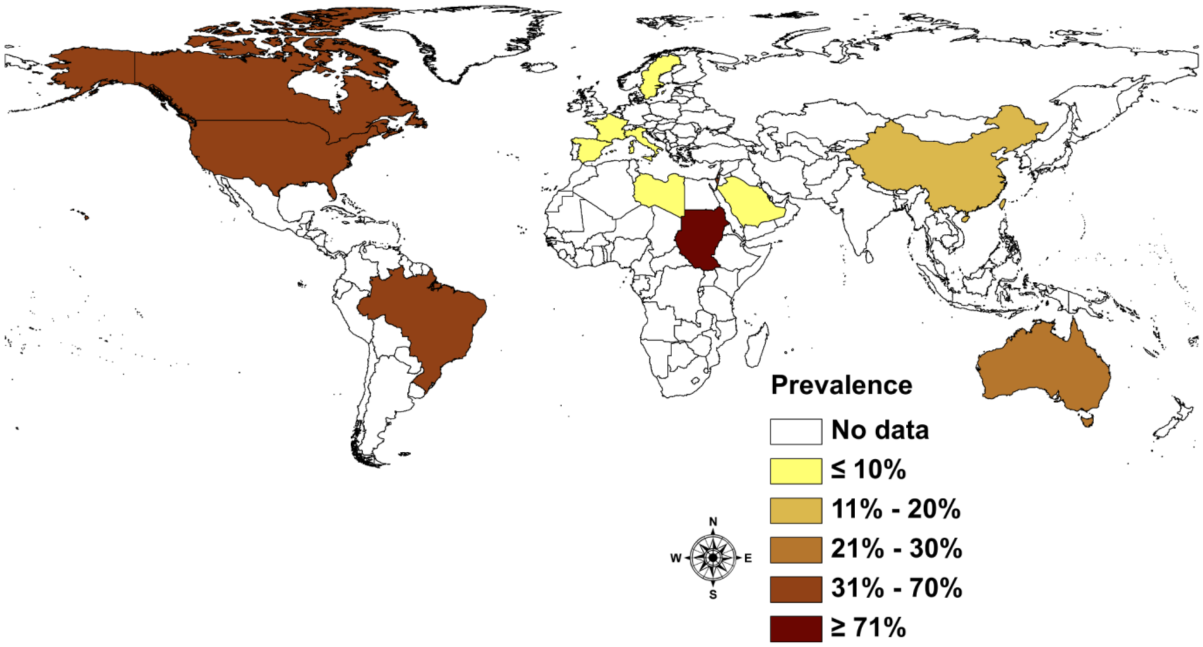


Figure 1.2: Map of *S. stercoralis* prevalence estimated in a meta-analysis using community-based studies(Schär et al., 2013)

The most commonly reported global figure is 30-100 million *S. stercoralis* cases but looking at surveys using the Baermann technique and/or coprological diagnostic approaches, *S. stercoralis* appears much more common. Indeed, detected at a ratio to hookworm of 1/4 to 1/1, a more reasonable but still conservative estimate would be half of hookworm prevalence, that is, 200-370 million cases worlwide (Bisoffi et al., 2013). However those are rough figures based on few data and need to be better estimated. Overall, lower prevalence rates would be expected in countries undergoing onchocerciasis control due to the regular community-based distribution of ivermectin (Anselmi et al., 2015, Mohammed et al., 2012, Krotneva et al., 2015, Henriquez-Camacho et al., 2016, Nutman, 2016).

In Southeast Asia, where ivermectin is not available, it has recently been estimated, based on existing studies and accounting for diagnostic sensitivity that *S. stercoralis* prevalence would range between 0.1% in Vietnam to 36% in Malaysia, with rates over 20% also in Cambodia, Thailand, and Lao PDR (Schär et al., 2015).

Finally, *S. stercoralis* is a major public health issue in Cambodia, where, up to recently, the few studies documenting *S. stercoralis* indicated prevalence rates between 2.6 and 20.2% (Jex et al., 2011, Schär et al., 2013). However, recent large-scale community-based studies conducted in two rural provinces of Cambodia between 2009 and 2011 found prevalence rates of 25% in the South and up to 45% in the North (Khieu et al., 2014c, Khieu et al., 2014b).

1.1.7 Risk factors for *S. stercoralis and* hookworm infection

Risk factors for infection with STH pertain to the parasites transmission route and exposure to the infective larvae. They encompass demographic, socio-economic, personal hygiene, health knowledge, sanitation conditions as well as environmental factors.

Transmission is sustained by open defecation and infection occurs due to exposure to contaminated soil, either by bare feet or by prolonged contact with soil, as in farming or mining activities (Schär et al., 2013, Brooker et al., 2004a, Toledo et al., 2015). Access to, and use of, improved sanitation facilities, i.e. preventing contact with human excreta as well as access to safe water and hygienic behaviour are key determinants of STH infections, including *S. stercoralis* (Strunz et al., 2014, Freeman et al., 2013b, Freeman et al., 2014, Ziegelbauer et al., 2012, Echazú et al., 2015). Two meta-analyses found that improved sanitation reduced odds of STH infection and another one found reduced odds of diarrhea (Strunz et al., 2014, Ziegelbauer et al., 2012, Fewtrell and Colford, 2005). Footwear was also found to reduce the odds of hookworm and *S. stercoralis* infection in two and one meta-analysis, respectively (Tomczyk et al., 2014, Strunz et al., 2014).

Hookworm infection risk increases with age, with adults being more likely to be infected than children, a characteristic that contrasts from the two other STH that are more prevalent in children (Brooker et al., 2004a, Hotez et al., 2004, Hotez et al., 2008). *S. stercoralis* prevalence has also been found to plateau in adulthood in north Cambodia but no association with age was found in Jamaica, Zanzibar and South Cambodia (Khieu et al., 2014b, Knopp et al., 2010b, Khieu et al., 2014c). The pattern of *S. stercoralis* prevalence in association with age remains unclear and needs to be further documented.

Males are commonly more infected by hookworm than females, although, in some regions, females exhibit higher infection intensities (Brooker et al., 2004a, Bethony et al., 2002, Pullan et al., 2010b). Sex difference in hookworm infection is commonly attributed to increased exposure rather than genetic susceptibility, although the role of the latter remains unclear, particularly with regard to infection intensity (Brooker et al., 2004a, Inpankaew et al., 2014). The few studies that investigated the association between sex and *S. stercoralis* infection also found that men were at higher risk for this infection (Steinmann et al., 2007b, Knopp et al., 2010b, Khieu et al., 2014b, Khieu et al., 2014c).

STH infections are widely recognized as infections of poverty, both at large scale as they are endemic in LMIC countries, but also within communities, the most poor having a higher risk of infection. However, in the case of *S. stercoralis*, the relationship with socio-economic status at small scale remains unclear, with no association found between the socioeconomic level as assessed by a household asset-based wealth index either in north Cambodia or Zanzibar (Knopp et al., 2008b).

HTLV-1 infection was associated with increased risk of *S. stercoralis* chronic infection in studies conducted in regions endemic for HTLV-1, i.e. in Japan, the Caribbean and among indigenous populations of Australia. However, the association was not identified in all studies, but was mostly found in studies using coprological diagnostic techniques, rather than serological diagnosis (Carvalho and Da Fonseca Porto, 2004, Tanaka et al., 2016). Two explanations have been put forward. First, the lack of association in studies using serological techniques might be due to cross-reactivity with other helminths (Carvalho and Da Fonseca Porto, 2004). Second, the impaired local immunity due to decreased total and *S. stercoralis*-specific IgE levels observed in co-infected patients eases the parasite proliferation and therefore its detection in the stool (Carvalho and Parise, 2006, Newton et al., 1992, Robinson et al., 1994).

Alcoholism has been found associated with *S. stercoralis* infection, with increasing prevalence across groups with increasing daily ethanol intake (de Oliveira et al., 2002, Marques et al., 2010, Zago-Gomes et al., 2002). It has been hypothesized that the immune system should be able to eradicate *S. stercoralis* infection, but there is no formal evidence on this phenomenon (Grove, 1996). Would that be confirmed, the immune response impairment due to alcoholism could be an explanation for increased susceptibility to infection. Other possible reasons for a higher risk of *S. stercoralis* infection among alcoholics include higher exposure due to risky or unhygienic behaviour, or a higher infection detection rate due to greater amounts of larvae in stool of alcoholics (de Oliveira et al., 2002, Marques et al., 2010). The latter could be due either to low bowel motility favouring autoinfection, or to higher parasite survival and autoinfection rate due to decreased immune functions (Marques et al., 2010). Another suggested mechanism relates to the increased corticoid production due to ethanol and the possible action of steroid metabolites similar to helminth moulting hormones (Marques et al., 2010, Zago-Gomes et al., 2002).

1.2 The three "major soil-transmitted helminths"

STH belong to the group of Neglected Tropical Diseases (NTDs) which includes 18 heterogeneous communicable diseases sharing two characteristics: they disproportionally affect the poorest and have suffered from a lack of interest from the research and public health community as well as scarce funding for their management (Utzinger et al., 2012, Boatin et al., 2012, Hotez et al., 2009).

Infection with STH occurs through contact with soil contaminated with human faeces infected with eggs or larvae, due to poor sanitation conditions (Pullan et al., 2014). *A. lumbricoides* and *T. trichiura* infection route is faecal-oral, with eggs ingested through contact with soiled hands, whereas hookworm larvae penetrate intact skin. STH infections may cause excess mortality, but they are mainly responsible for chronic and debilitating conditions (Vos et al., 2012). Their morbidity, estimated at 4.98 million years lived with disabilities (YLD) by the global disease burden study (GBD) study in 2010 is actually comparable to the disabilities caused by malaria or HIV/AIDS which were estimated at 4.07 and 4.34 million YLDs in 2010, respectively (Vos et al., 2012). STH mostly affect rural populations of warm LMIC countries where lack of sanitation prevails and are tightly linked to poverty and economic underdevelopment. They are widely recognized as poverty-promoting diseases through a vicious cycle of gastro-intestinal symptoms, malnutrition, physical development impairment and loss of productivity. They are one of the most important causes of physical and intellectual growth impairment (Hotez et al., 2008, Bethony et al., 2006, Hotez, 2003, de Silva, 2003, Yap et al., 2014).

Among STH, hookworm, which refers to two species, *Ancylostoma duodenale* and *Necator americanus,* causes the highest burden, with 439 million cases and 3.2 million DALYs in 2010 (Murray et al., 2012, Pullan et al., 2014). *N. americanus* is the most common species worldwide and is endemic in Southeast Asia (Brooker et al., 2004a).

Hookworms are well known parasites that have been extensively studied for decades. In addition to the human hookworms cited above, there are three zoonotic hookworm species, the hookworm of dogs *Ancylostoma caninum*, and the canine and feline hookworms *Ancylostoma braziliense* and *Ancylostoma ceylanicum*. Both *A. caninum* and *A. ceylanicum* are present in Southeast Asia but the latter is particularly common in dogs and in humans among whom it can cause patent infections with symptoms including stomach pain, diarrhea and anaemia, even in well-nourished individuals (Conlan et al., 2012, Ngui et al., 2012, Inpankaew et al., 2014, Traub et al., 2008). The molecular characterization of hookworm infections among inhabitants of a rural village of North Cambodia showed that half of humans infected with hookworms harboured *A. ceylanicum*, mostly as mono-infection, while 94.4% of dogs were also mono-infected with *A. ceylanicum* (Inpankaew et al., 2014). The rest of human hookworm cases involved *N. americanus* and only three cases (2.4% of all human cases) were due to *A. duodenale* (Inpankaew et al., 2014). Hookworms have a direct life cycle. In brief, infective (third-stage) larvae penetrate skin, and reach the heart and the lungs through the bloodstream. Coughed, then swallowed, larvae reach the small intestine where they mature into adults and attach to the mucosa. Females shed eggs that are excreted with faeces and hatch at the surface of the soil. Each egg will produce one first-stage larvae within 1-2 days. After moulting twice, they will become non-feeding infective larvae that can survive for several weeks under adequate warmth and moisture conditions until they have consumed their metabolic reserves (Brooker et al., 2004a, Hotez et al., 2004).

An important feature of helminth and STH infections is that morbidity arises with the intensity of infection, i.e. high worm loads, whereas light infections are often asymptomatic. Symptoms of chronic light hookworm infection include mild gastrointestinal symptoms, weakness, weight loss and high eosinophilia (Brooker et al., 2004a).

The major clinical feature of hookworm infection is intestinal blood loss caused by the parasite attachment to the intestinal mucosa and sub-mucosa. Hookworm disease, characterized by iron deficiency anaemia, is responsible for over half of hookworm morbidity and arises in case of moderate or heavy infection, respectively reflected by egg outputs of 2,000-3,999 eggs per gram (EPG), and ≥4,000 EPG (WHO, 2002, Brooker et al., 2004a, Smith and Brooker, 2010). Children and women of childbearing age (WBCA), who are more likely to have low or depleted iron stores, or individuals with high parasite loads, are the most affected (Brooker et al., 2004a). Hookworm disease is of major public health importance as it negatively impacts children growth and cognitive development, with consequences on worker productivity at adult age, but also increases maternal and infant mortality due to low birth weight (Bethony et al., 2006, Brooker et al., 2008, Smith and Brooker, 2010).

1.3 Preventive chemotherapy to control soil-transmitted helminths

In the past decades, the recognition of the high burden due to STH has led to the design of intervention strategies to mitigate their morbidity and tackle the huge public health problem they represent in Asia, sub-Saharan Africa and the Americas (Prichard et al., 2012, WHO, 2005, Boatin et al., 2012). In public health, the “control” of an infectious disease is defined as the “reduction in the incidence, prevalence, morbidity or mortality to a locally acceptable level” (Dowdle, 1998) (WHO, 2010, Prichard et al., 2012, Dowdle, 1998).

For STH, the goal of control is to reduce morbidity due to infection with *A. lumbricoides*, *T. trichiura* and hookworms, including the incidence of severe manifestations that can occur due each species, such as hookworm disease and anaemi,a but also intestinal obstruction due to heavy *Ascaris* infection or *Trichuris* dysentery syndrome (Hotez, 2003). To achieve morbidity control, the mainstay of the WHO's "preventive chemotherapy" (PC) control strategy against STH is regular chemotherapy with single-dose of albendazole or mebendazole, either through targeted treatment of specific at-risk groups or by mass-drug administration (MDA) to entire populations in areas where STH prevalence exceeds 20% (WHO and Crompton, 2006, WHO, 2010). The current PC control strategy advises for deworming of all school-aged children once a year in low-risk areas, i.e. with prevalence ranging from 20 to 50%, and twice a year high-risk communities, i.e. with a prevalence rate of 50% and above (WHO and Crompton, 2006). Preschool-aged children and women of child-bearing age, including pregnant women in the 2nd and 3rd semester, and breastfeeding women, should also be treated, whatever the prevalence level (WHO and Crompton, 2006).

A main advantage of PC is its high cost-effectiveness but an important drawback is its lack of sustainability. As long as environment contamination occurs, individuals get re-infected, so treatment must be administrated regularly (Yap et al., 2013, Jia et al., 2012, Prichard et al., 2012). However, probably over time, parasite load reduction due to treatment results in the reduction of the eggs and larvae reservoir in the environment and might, depending on infection levels among untreated groups, impact community-level transmission.

Depending on the species, re-infection rates a year after treatment range from 57% for hookworm to 82% for *T. trichiura* and up to 94% for *A. lumbricoides* (Jia et al., 2012). Additionally, although drug resistance has not yet occurred in humans, is it a major issue in veterinarian medicine and might emerge, particularly if control measures were to be intensified and drug pressure increased (Prichard et al., 2012). This might eventually happen and maybe sooner than later in areas where the goal of deworming would be switched from morbidity control to elimination, i.e. the local interruption of transmission, a measure that has been increasingly considered among the STH control research community (Prichard et al., 2012, Anderson et al., 2015, Truscott et al., 2014, Brooker et al., 2015).

Health education and sanitation improvement are also recommended by the WHO because they contribute to reducing transmission and are necessary for sustainable control (World-Health-Assembly, 2001). Yet, sanitation measures have not concretely been included in STH intervention packages because implementing them is particularly challenging mostly due, among other factors, to their high cost, complexity, need for cross-sectorial collaboration, and lack of perceived need by communities (Campbell et al., 2014, Freeman et al., 2013b).

In Cambodia, the tropical climate and poor sanitation conditions are most favourable to worm infections, where STH are a major public health concern. Although prevalence data are not available for most regions, 47% of schoolchildren were estimated to be infected with one of the major STH species in the early 00s (*A. lumbricoides, T. trichiura*, hookworms), with prevalence rates found to be over 50% in 15 provinces and reaching up to 70% (Sinuon et al., 2005, Jex et al., 2011).

Nation-wide school-based deworming was launched in 2002 as a response to those alarming estimates and reached the WHO target of 75% coverage in 2004 (Sinuon et al., 2005). Between 2006 and 2011, a survey conducted at national scale among schoolchildren and adults found that the most prevalent helminth was hookworm, with 9.6% of participants infected, and particularly high prevalence rates in the North and North West, up to 22.3% and 22.1% in Oddar Manchey and Siem Reap province, respectively (Yong et al., 2014). However, this study used a single Kato-Katz thick smear for the diagnosis, an approach that has a low sensitivity, particularly in low-intensity settings (Booth et al., 2003, Knopp et al., 2008a, Knopp et al., 2009b, Tarafder et al., 2010, Nikolay et al., 2014). Interestingly, a large scale survey conducted in the Northern Province of Preah Vihear found a hookworm prevalence of 46.7% using the Kato-Katz technique on two samples (Khieu et al., 2014b).

Cambodia benefits from a well-established STH control delivery network working through health centres with community health workers. Schoolchildren are dewormed twice a year at school while preschool-aged children are reached in communities through Vitamin A distribution and Immunization campaigns. Lately, because they are an important risk group for hookworm infection, women have also been targeted in factories (Montresor et al., 2008, Sinuon et al., 2005, National Center for Parasitology, 2014).

1.4 Identification of parasite geographical distribution and high risk areas

Because resources are limited, everywhere and particularly in LMIC, spatial modelling is a unique tool to identify priority zones for control so the allocation of scarce resources can be optimized. Bayesian geostatistical models (spatial models that integrate the geographical distribution of a disease and the association between its occurrence and environmental factors) are commonly used to help identifying priority zones for helminth control at provincial, national, or regional scale (Chammartin et al., 2013a, Brooker et al., 2003, Clements et al., 2010, Chammartin et al., 2013b, Chammartin et al., 2014b, Raso et al., 2005, Raso et al., 2006b, Karagiannis-Voules et al., 2015a, Forrer et al., 2012, Khieu et al., 2014b). Those models also allow understanding the relationship between the environment and the geographical distribution of parasite infection levels. Two climatic factors known to influence STH distribution, impacting larvae development and survival in the external environment, are temperature and rainfall which condition atmospheric and soil humidity, while the latter also depends on vegetation coverage (Brooker et al., 2004b, Chammartin et al., 2013a, Khieu et al., 2014b, Chammartin et al., 2014b, Soares Magalhães et al., 2011a). Hookworm infection geographical clusters tend to be more limited both at small and large scale than that of *A. lumbricoides* or *T. trichiura*. A possible reason is that as opposed to eggs, hookworm larvae can migrate vertically in the soil and avoid desiccation (Brooker et al., 2004a). *S. stercoralis* distribution has not yet been investigated at large scale but small scale mapping in North Cambodia indicated a very low propensity of *S. stercoralis* infection to cluster in space, although this does not preclude that infection will cluster at larger scale due to climatic influence. Finally, multiparasitism being common, geostatistics also help identifying areas of species overlapping thereby providing important information for integration of control measures.

1.5 Knowledge gaps for *S. stercoralis* control

"Strongyloidiasis-the most neglected of the neglected tropical diseases?", "*Strongyloides stercoralis*: there but not seen", "*Strongyloides stercoralis*: a plea for action", titles of many papers reflect the extent of the parasite neglect. While in 2004, the third meeting of the Partners for Parasite Control held at WHO recommended taking measures for the control of *S. stercoralis* in endemic areas, Olsen and colleagues reported in 2009 that no specific measures had been implemented so far (WHO, 2005). In the meantime, -mostly unpublished- prevalence studies conducted in endemic countries of Africa, Asia and South America, indicate high variability of prevalence across settings, although comparisons are hindered by the variability of diagnostic approaches (Albonico et al., 2016). The absence of an available rapid diagnostic test is a major issue in *S. stercoralis* assessment at global level, and as a result, of its public health management (Albonico et al., 2016).

Many epidemiological aspects of *S. stercoralis* infection are to date unknown or poorly documented, including accurate prevalence and intensity estimates, the location of endemic settings, the risk factors for infection and post-treatment re-infection rates, or the extent of morbidity associated with infection in high-risk areas (Olsen et al., 2009, Bisoffi et al., 2013, Krolewiecki et al., 2013). Symptoms of chronic infections are unknown in endemic communities, including the relationship between larval output and clinical features, and there is dearth of data on hyperinfection cases in LMIC.

Overall, new estimates based on surveys using adequate diagnosis approaches -allowing comparison across settings- as well as reliable data on morbidity are needed to appraise the parasite burden and successfully having *S. stercoralis* recognized as a public health problem. A major step would be the integration of *S. stercoralis* into the WHO strategy for helminth control. Nevertheless, although wanting, current evidence calls for action against this parasite (Olsen et al., 2009, Bisoffi et al., 2013, Krolewiecki et al., 2013, Khieu et al., 2014b).

To date, there is no control strategy against *S. stercoralis*. A major issue is the access to ivermectin which high cost prevents its use at large scale in countries where it is not subsidised for onchocerciasis control (Albonico et al., 2016). In Cambodia, a tablet produced by a certified good manufacturing practice company is available at 10 USD, while up to five tablets may be needed to treat one individual.

With specific regard to Cambodia, the endemicity of *S. stercoralis* in the country requires action, but key information is needed to guide control efforts. A first step is to identify target age groups, i.e. groups the most at risk for infection and/or morbidity, which would help determining whether control should be integrated in the existing school-based deworming programme, or if control should be community-based and target entire populations. Second, the impact of chemotherapy-based control should be assessed both in terms of cure rates and health effects. Thirdly, a major issue is the affordability and effectiveness of the various control options. Cost-effectiveness studies are needed to identify the most cost-effective control approaches (targeted treatment *vs.* MDA, target age groups) so the allocation of limited resources can be optimized. Fourthly and finally, an estimate of case number in Cambodia, together with a number of needed pills would help advocating for ivermectin subsidisation or donation so control can be rolled out.

Chapter 2

Aims of the thesis, study objectives and research questions

2.1 Aims of the thesis

The main aim of this work was to contribute to the knowledge-base to guide control efforts in Cambodia, by assessing the rates of, and risk factors for, *S. stercoralis* infection one year after ivermectin treatment, as well as clinical signs associated with chronic infection in endemic communities of North Cambodia. A second goal was to assess the cost-effectiveness of targeted *vs.* mass drug administration of ivermectin treatment against *S. stercoralis*. A third objective was to assess *S. stercoralis* prevalence and distribution across Cambodia to identify priority zones for control. Finally, an additional aim was to explore the geographical distribution and factors underlying the distribution of *S. stercoralis* and hookworm infection in the region at provincial level, including co-infection with both parasites in a Northern Province of Cambodia endemic for both parasites and assess risk factors for co-infection, and hookworm infection risk and intensity in an adjacent province of Lao PDR.

2.2 Main objectives

Five main objectives were pursued.

Objective 1: To assess the community-effectiveness of annual targeted ivermectin treatment (200μ/kg BW, single dose) against *S. stercoralis,* with regard to rates of, and risk factors for, re-infection, treatment efficacy and morbidity resolution in an endemic population.

Objective 2: To assess the cost-effectiveness of targeted ivermectin treatment (200μ/kg BW, single dose) treatment *vs.* mass drug administration to school-aged children or all age groups for the control of *S. stercoralis* in Cambodia

Objective 3: To assess *S. stercoralis* national prevalence and distribution in Cambodia

Objective 4: To assess the distribution of hookworm-*S. stercoralis* co-infection in North Cambodia

Objective 5: To assess the distribution of hookworm infection risk and intensity and investigate associated risk factors at provincial level in Southern Lao PDR

2.3 Research questions

Within each objective, the following specific research questions were specified.

Objective 1: community-effectiveness of ivermectin treatment against *S. stercoralis*

1. What is the cure rate achieved by a single oral dose (200 µg/kg BW) of ivermectin against *S. stercoralis* in endemic settings of Preah Vihear Province, North Cambodia?
2. What is the *S. stercoralis* re-infection rate one year after ivermectin treatment in endemic settings of Preah Vihear Province, North Cambodia?
3. Which age groups are most at risk for *S. stercoralis* incident infection and should be targeted by control?
4. What are the underlying risk factors associated with *S. stercoralis* incident infection risk of Preah Vihear Province, North Cambodia?
5. What are clinical signs associated with *S. stercoralis* chronic infection in endemic settings of Preah Vihear Province, North Cambodia?
6. What are the symptoms associated with *S. stercoralis* chronic infection resolved by a single oral dose (200 µg/kg BW) of ivermectin in endemic settings of Preah Vihear Province, North Cambodia?
7. Is there an association between *S. stercoralis* infection and malnutrition in children?

Objective 2: cost-effectiveness of targeted treatment *vs.* MDA as control options for *S. stercoralis*

1. What are the incremental cost-effectiveness ratios of targeted single oral dose (200 µg/kg BW) of ivermectin *vs.* mass drug administration to schoolchildren or entire communities?
2. What is the most cost-effective control approach?

Objective 3: distribution and number of *S. stercoralis* cases at national level, Cambodia

3. What is the prevalence of *S. stercoralis* in Cambodia?
4. What is the geographical distribution of *S. stercoralis* infection risk at national level in Cambodia?

Objective 4: distribution of, and risk factors for, hookworm-*S. stercoralis* infection risk in northern Cambodia

1. What is the geographical distribution of *S. stercoralis*-hookworm co-infection in Preah Vihear province, North Cambodia?
2. What are the underlying determinants associated with the geographical distribution of *S. stercoralis*-hookworm co-infection in Preah Vihear province, North Cambodia?

Objective 5: distribution of, and risk factors for, hookworm infection risk in southern Lao PDR

1. What are the distributions of hookworm infection risk and intensity in Champasack Province, Southern Province of Lao PDR?
2. What are the risk factors associated with hookworm infection risk and intensity in Champasack Province, Southern Lao PDR?

Chapter 3

Material and methods

3.1 Ethical considerations

All studies were approved by the National Ethics Committee for Health Research (NECHR) or Lao PDR or Cambodia, respectively, and by a Swiss ethics committee, either the Ethics Committee of Basel and Baselland (EKBB, before 2014), or the Ethics Committee North-West and Central Switzerland (EKNZ, after 2014).

Goals and procedures were explained in detail to all participants prior to enrolment in all studies. In Champasack Province, Lao PDR, where literacy is low, the study information sheet in the local language was read to all household members and their questions answered. Individual oral consent was obtained from all adult participants, while heads of household additionally signed written informed consent. In Preah Vihear, North Cambodia, the procedure was the same for the 2010 cross-sectional and the 2012-2014 cohort studies. Written informed consent was obtained from all adult participants before enrolment, while for participants aged less than 18 years, written consent was given by a parent or legal guardian. Finally, all participants in the national survey were explained the study goals and procedures before enrolment. All participants aged 16 years and above provided written informed consent and parents or legal guardians provided consent for participants aged 6–15 years.

3.2 Study area

3.2.1 Study countries

Cambodia and Lao PDR are two adjacent countries of Southeast Asia. They respectively counted 15.1 and 6.8 million inhabitants in 2013 and 2015. They have a tropical monsoon climate with the rainy season occurring between May and October. Both countries have experienced strong economic development in the past decades and moved up to the

World Bank group of “lower middle income countries”, although at the very bottom of this category. Their Human Development Index (HDI), a summary measure of a long and healthy life, access to knowledge, and a decent standard of living, were 138/188 and 143/188 for Lao PDR and Cambodia, respectively, in 2015 (UNDP, 2016b, UNDP, 2016a).

Cambodia’s landscape is dominated by a large central plain run through by two major rivers: the Mekong River which crosses the country from North to South and the Tonle Sap River which flows from a large inland lake situated in the Northwest, the Tonle Sap, down to Phnom Penh. This large plain is surrounded by low-range mountains, particularly in the South and South West, which coast line opens onto the Gulf of Thailand. About 20% of land area is used for agriculture and rice fields occupy 75% of the cultivated areas (FAO, 2014). Rice fields are found across the entire country in the lowlands, and occupy most of the land surface in the alluvial plains of the Tonle Sap along a Northwest-Southeast axis and the Mekong in the South. In 2000, 49% of the country surface was occupied by forests, but 1.44 million hectares of forests were lost by 2014, with Cambodia losing its forests at one of the fastest rates in the world (Global-Forest-Watch, 2016, NASA-Earth-Observatory, 2017, Open-Development-Cambodia, 2016).

Cambodia underwent several decades of political instability which started in the mid-seventies with the Khmer rouge genocide which exterminated at least 1.7 million people and specifically targeted educated citizens, i.e. doctors, teachers, engineers … namely, any literate people. In the early nineties Cambodia had to re-build a completely destroyed infrastructure and re-develop everything, including its educational and health systems. In the past decades, the country experienced a marked economic development mostly driven by the garment sector, construction and services. In 2015, Cambodia had a gross national income per capita (GNI) of 1,070 US$, with agriculture -mostly rice growing- mainly consisting in subsistence farming, being the fourth important economic sector in the country. Despite significant progress, including in reaching the 1st Millennium Development Goal (MDG) to halve poverty in 2009, 17.7% of the population was still under the poverty line, with 90% of the poor living in rural areas, and almost half of the population actually remained on the edge of the poverty line (WorldBank, 2017a, WorldBank, 2017b). Cambodia also accomplished important achievements in matters of health, with malaria and HIV/AIDS being under control and the MDG child and maternal mortality targets met. However, a major challenge is child malnutrition, which is rampant particularly in rural areas, despite excellent progress made since the nineties. In 2014, a third (32.4%) and 9.6% of under-5 children were stunted or underweight respectively (International-Food-Policy-Research-Institute, 2016). Access to sage water and sanitation remains a major challenge in rural areas, where as much as 79.3% of the population lived in 2015. In contrast to the urban population, of which 88% and 100% had access to improved sanitation and water, respectively, those proportions were only 31% (improved sanitation) and 69% (improved water) for the rural population in 2015. (WHO/UNICEF-Joint-Monitoring-Programme-(JMP)-for-Water-Supply-and-Sanitation, 2015).Those poor water and sanitation conditions result in open defecation, which, combined to the humid tropical climate, creates most favourable conditions for the development and subsistence of protozoa and helminths in the environment.

Regarding helminth control, Cambodia is an exemplary country, and was the first to reach the WHO target of 75% coverage of school-based deworming (primary schools) in 2004. Cambodia has also eliminated lymphatic filariasis as a public health problem in 2016, a target that has been so far met by only eight countries worldwide (WHO, 2016a, Montresor et al., 2008, Sinuon et al., 2005). However, STH, which remain highly prevalent, are still an important public health problem in the country (Khieu et al., 2014b, Khieu et al., 2014c, Moore et al., 2015, Yong et al., 2014, Kuong et al., 2016).

Lao PDR is a landlocked country mostly bordered by Thailand in the West and Vietnam in the East, and by China and Cambodia in the North, and the South, respectively. Up to 70% of its surface is occupied by mountains, highlands and plateaux. Its western border is run along or through by the Mekong river. The country economic growth is mostly due to its natural resources (water, mineral compounds, and forests) as well as services and construction. Regarding Millennium Development Goals, Lao PDR halved poverty by 2013, reduced maternal mortality by 75% by 2015, and achieved the MDG target for water and sanitation in 2015, with 76% and 71% of the population having access to improved water and sanitation, respectively (UNDP, 2015).

However the poverty rate was still high in 2012, with almost half of the population (46.9%) living under the 3.10 US$ poverty line and as many as 16.7% living below the extreme poverty line of 1.90 US$ (WorldBank). Helminths are endemic in the entire country, where the liver fluke *Opisthorchis viverrini* and STH are particularly highly prevalent (Sayasone et al., 2009, Sayasone et al., 2014, Sayasone et al., 2007, Chai et al., 2005, Phongluxa et al., 2013).

3.2.2 Study sites

The *S. stercoralis* national survey was conducted on all 25 provinces of Cambodia. Other studies conducted in Cambodia took place in the Preah Vihear province. The hookworm study from Lao PDR, was conducted in Champasack province.

a. Preah Vihear Province, Cambodia

Preah Vihear is a rural province of North Cambodia sharing its North border with the Champasack province, Lao PDR. There is no major river going through Preah Vihear province which is mostly flat and occupied by savannahs and shrub lands, but also rice fields, and forests. However the province has lost about 10% of its tree cover between 2001 and

2014 (Genta, 1989). In 2013, Preah Vihear province hosted 235,370 people, 87.6 % of whom lived in rural areas with most of the urban population living in the province capital, Tbaeng Meanchey (National Institute of Statistics, 2013b, United-Nations-Population-Fund, 2014). Preah Vihear is one of the poorest provinces of Cambodia (Asian-Development-Bank, 2014). With a poverty incidence of 62.2% in 2014, it even has been ranked at the most poor province by the Multidimensional Poverty Index (MPI), a multidimensional poverty measure integrating education, health and living standard dimensions. As a comparison, the poverty incidence of whole Cambodia as assessed by the MPI would be 33.0% (OPHDI, 2016).

b. Champasack province, Lao PDR

Champasack province has a surface of 15,415 km2 and hosted a population of 603,370 according to the 2005 national census (Census 2005, Lao Statistics Bureau, Vientiane; http://www.nsc.gov.la). It is run through from North to South by the Mekong River. The landscape is dominated by the river floodplains and mostly occupied by rice fields and flatlands. Farther from the floodplain and east of the Mekong River, the topography gets hillier and is covered in forests, while the North East third is mountainous and culminates at 1,617 m above sea level. Helminths including STH, *Opisthorchis viverrini* and *Schistosoma mekongi* are endemic in the province (Muth et al., 2010, Sayasone et al., 2011, Sayasone et al., 2014, Sayasone et al., 2009, Forrer et al., 2012).

3.3 Diagnosis and treatment

Direct parasitological diagnostic techniques on faecal samples were employed in the cross-sectional and cohort studies conducted in Preah Vihear, Northern Cambodia, and Champasack Province, southern Lao PDR.

In Champasack province, hookworm was diagnosed with duplicate 41.7 mg thick smears prepared from a single stool sample. Further details are provided in the corresponding chapter.

In Preah Vihear province, *S. stercoralis* infection was diagnosed used combined results of examination on two stool samples on consecutive days by the Baermann test and the Koga Agar culture technique (Baermann, 1917, Koga et al., 1991). This approach has 92.8% sensitivity and ensures a higher detection rate of *S. stercoralis*. Indeed, the examination of only one stool sample has been shown to be of relative low sensitivity (Marti and Koella, 1993, Dreyer et al., 1996, Knopp et al., 2008a, Khieu et al., 2013a). Other helminths were diagnosed with one Kato-Katz 41.7 mg thick smear on two samples and protozoa were screened with the formalin-ether concentration technique (FECT) on one sample (Allen and Ridley, 1970, Katz et al., 1972). *S. stercoralis* and hookworm larvae, helminths eggs and protozoa were identified through examination under a microscope and based on morphology.

For the national survey, a different diagnostic technique was used. The coprological combined approach described above was not an option at national scale due to the absence of local adequately equipped laboratories, and the lack of trained staff to perform microscopy-based parasite identification. An ELISA-based diagnostic method detecting *S. stercoralis* directed antibodies in urine and recently developed in Thailand was employed for the national survey. This method has a sensitivity of 84% on one sample and has no cross-reactivity with other STH (Eamudomkarn et al., 2015).

In all studies, all *S. stercoralis* cases were treated with a single oral dose of ivermectin (200 µg/kg BW), while other helminths and protozoa were treated according to national guidelines of the respective countries (CNM, 2004, MOH, 2004).

3.4 Research approach and study design

3.4.1 Community-effectiveness of annual targeted ivermectin treatment against *S. stercoralis*

a. Definition of infection status

Parasites were diagnosed on two stool samples collected over consecutive days and the Kato-Katz, Baermann and KAP techniques were performed.

To ensure high sensitivity and maximize specificity, the following infection status definitions were adopted. For *S. stercoralis*, a participant was considered positive if at least one larva was found in any of the four samples or *S. stercoralis* negative if no larva was detected in all four samples. Participants with only negative results but with fewer than four analysed samples were not included in the analysis. For the other helminths, positive status was identified on at least one egg found in any sample analysed with any method (Kato-Katz, Baermann or KAP) but only negatives with two negative Kato-Katz results were included. There was no particular definition for protozoa, which infection status was based on the single available FECT diagnosis result.

b. Rates of, and risk factors for, *S. stercoralis* incident infection

A two-year prospective intervention study (cohort study) was conducted in the Rovieng district of Preah Vihear province to estimate the rates of incident *S. stercoralis* infection (i) 1 year after treatment among participants previously infected with *S. stercoralis* (re-infection) and (ii) 1 year after screening among *S. stercoralis* participants (new infection), and investigate risk factors associated with the risk of incident infection.

Eight villages that had never been exposed to *S. stercoralis* treatment were chosen. All households were included and all individuals aged two years and more were eligible. This study consisted in a baseline survey conducted between February and June 2012 and two surveys following-up enrolled participants after 12 (January-March 2013) and 24 months

(February-March 2014).The cohort consisted in all *S. stercoralis* cases detected at baseline and a random sample of 300 *S. stercoralis* negative participants living in two of the study villages, who consented to participate.

At each survey, participants were asked to provide two stool samples over two consecutive days and to answer individual-level questionnaires pertaining to demographics, hygienic behaviour and knowledge about worms. Helminth and protozoa diagnosis was performed as described in Section 3.4. All participants underwent a basic medical exam conducted by a medical doctor and were asked about symptoms experienced within two weeks before the interview. At baseline and at the second follow-up, heads of households answered an additional questionnaire about water and sanitation (WASH) and household assets ownership.

Individual-, household-, and village-level risk factors for incident infection were investigated using mixed logistic regression models accounting for repeated measures (individual-level random effect) and heterogeneity between communities (village-level random effect).

Among all participants included at baseline, the association between each reported symptom and *S. stercoralis* infection was assessed using multivariate logistic regressions adjusting for other infections and confounders.

The association between stunting, i.e. moderate malnutrition among children and adolescents aged between 5 and 19 years was assessed using the z scores for height-for-age (HAZ) calculated based on the WHO Growth Reference Standard to calculate anthropometric indicators for school-aged children and adolescents (de Onis M, 2007, Group, 2006). Multivariate logistic regressions were employed to investigate the association between stunting, i.e. a HAZ smaller than -2 and *S. stercoralis* parasite load while adjusting for hookworm infection and other important confounders.

All regression models were built based on the Likelihood Ratio Test (LRT) and the Akaike's information criterion (AIC). Variable selection was conducted using (simple or mixed, as appropriate) bivariate logistic regressions and a 15% significance level of the LRT. In case of correlated variables, the variable resulting in the model with the smallest AIC was selected. Relevant interactions were checked for all models.

c. Cure rate and symptom resolution achieved by ivermectin

Within the baseline survey, a "before-after" treatment survey was conducted among a subsample of about 300 participants who were followed-up three weeks after treatment to assess (i) the cure rate achieved by a single oral dose of ivermectin (200 µg/kg BW) and (ii) the symptoms resolved by this treatment regimen. The "after treatment" assessment consisted in (i) a second parasitological exam, also conducted on two stool samples, to assess post-treatment infection status and (ii) a medical exam and interview about symptoms experienced in the past three days to assess the extent of morbidity.

The McNemar's test was employed to assess symptom resolution achieved my ivermectin treatment among participants with *S. stercoralis* mono-infection, and conditional logistic regressions were used to assess symptoms before and after treatment in all participants and accounting for co-infection with any other helminth or with pathogenic protozoa, i.e. either *Giardia lamblia* or *Entamoeba histolytica/dispar*.

3.4.2 Cost-effectiveness of control options

This work aimed at estimating the cost-effectiveness of three alternative control options for *S. stercoralis*, i.e. of targeted ivermectin treatment *vs.* MDA to pre-school-aged children, school-aged children and women of child-bearing age.

Effectiveness measures for the targeted treatment alternative, i.e. number of treated cases based on observed cure rate and screening coverage, as well as prevalence data were obtained from the baseline survey of the cohort study described in section 3.4.1 while diagnostic sensitivity was estimated based on literature data (Khieu et al., 2013a).

For the MDA approaches, population and treatment coverage rates of the ongoing STH control programme (for STH control risk groups) and of the community-based lymphatic filariasis (for additional groups) control programme were provided by the National Centre for Parasitology, Entomology and Malaria Control (CNM), Ministry of Health, Cambodia.

There are no DALYs or QALYs estimated for *S. stercoralis* infection so outcomes were expressed in natural units, i.e. a treated case or a cured case. Cost-effectiveness was assessed by calculating the incremental cost-effectiveness ratio (ICER) for a treated or cured *S. stercoralis* case of each intervention compared to the next best alternative.

We estimated financial costs for each alternative, which aimed at appraising how much the MoH would have to pay to implement each intervention. Costs included fixed and variable programme start-up and recurrent costs. For the implemented intervention (targeted treatment), diagnosis costs were estimated using a micro-costing approach with direct measurement: diagnosis processes, material quantities and labour time were directly observed from the baseline survey of the cohort study and all prices were directly provided by the CNM in USD. Other costs were based on CNM expert interview or were retrieved from the USAID NTD cost and Funding Gap Analysis (Chu and Project, 2011).

One-way sensitivity analysis was conducted on important parameters, including prevalence, coverage rates, cure rate, and ivermectin price.

3.4.3 National *S. stercoralis* distribution and case number in Cambodia

A nation-wide cross-sectional survey was conducted in 250 villages across all 25 provinces of Cambodia between May and August 2016. First, ten villages per province were randomly selected, thenhouseholds were randomly selected in each village until 35 participants were enrolled. All villagers aged 6 years and above were eligible.

Participants were asked to provide one urine sample, one stool sample and to answer a questionnaire about basic demographics, sanitation and knowledge about worms.

Environmental data including land surface temperature, rainfall, altitude, vegetation coverage anddistance to water were downloaded from freely available remote sensing sources. Further details are provided in the specific chapter.

The extent of spatial correlation of *S. stercoralis* infection risk was explored using Bayesian geostatistical logistic regression, i.e. including a random effect that integrates spatial structure. Alternatively a model without any explicit spatial structure was run for result and predictive ability comparison. The model with the best predictive ability was used to predict, using environmental covariates, *S. stercoralis* infection risk at non-surveyed locations across the entire country using Bayesian Kriging (Diggle et al., 1998).

3.4.4 Distribution of hookworm - *S. stercoralis co-infection* in North Cambodia

A cross-sectional survey was conducted in 60 villages of Preah Vihear Province North Cambodia, between February and June 2010. Fifteen households per village were randomly selected and all household members aged over two years were eligible. Household coordinates were recorded using a global positioning system (GPS) device (Garmin Ltd.; Olathe, United States of America).

Parasites were diagnosed on 2 stool samples collected over consecutive days and the Kato-Katz, Baermann and KAP techniques were performed. The same infection status definition as in section 3.5.1 was used. In brief, participants were *S. stercoralis* positive if at least one larva was found in any of the four samples analysed with the Baermann or the KAP technique and were positive and only negatives with 4 negative results (2 methods, 2 samples, no missing result) were included.

For any other helminth, a participant was positive if at least one egg was found in any of the 6 samples (3 methods, 2 samples). Negatives with two negative Kato-Katz slides only were included.

Participants were asked to answer an individual questionnaire about demographic information, hygienic behaviour and knowledge about worms. In addition, heads of household answered a questionnaire about WASH and household assets that were used to build a socioeconomic index using Multiple Correspondence Analysis (MCA) (Asselin and Tuan Anh, 2008).

Environmental data including altitude, rainfall, land surface temperature, vegetation coverage, land cover and soil type data were downloaded from free remote sensing sources for a period of 1 year, i.e. the 12 months preceding the end of the survey. Further details are available in the respective chapter.

Environmental data were linked to parasitological and questionnaire data by household unique location.

Analysis and spatial prediction were performed in a Bayesian framework. Mixed multinomial models accounting for village-level clustering either integrating a spatial structure (geostatistical) random effect or not (exchangeable random effect) were used to explore the co-distribution of *S. stercoralis* and hookworm infections and to assess risk factors for co-infection. After having identified the model with the best predictive ability, *S. stercoralis*-hookworm co- and mono-infection risks were predicted at 1x1 km resolution across the entire surface of the Preah Vihear province, based on their association with environmental factors and using Bayesian Kriging (Diggle et al., 1998).

3.4.5 Distribution of, and risk factors for, hookworm infection risk and intensity, Southern Lao PDR

Between January and May 2007, a community-based cross-sectional survey was conducted in all nine rural districts of Champasak province, southern Lao PDR. The tenth district, urban, was excluded. Champasack Province is endemic for STH, *Opisthorchis viverrini*, *Schistosoma mekongi*, and minute intestinal flukes (MIF) (Rim et al., 2003, Chai et al., 2005, Chai et al., 2007, Muth et al., 2010, Sayasone et al., 2011, Forrer et al., 2012, Chai et al., 2013).

Participants were selected using a two-stage sampling method. First 51 villages, then 10-15 households per village were randomly selected and all household members aged over 6 months were eligible. A global positioning system (GPS) device (Garmin Ltd.; Olathe, United States of America) was used to record household coordinates.

Helminths were screened on a single stool sample using a duplicate Kato-Katz thick smear. Both hookworm infection risk and intensity were investigated. A participant was considered positive for a given helminth species if at least 1 egg was identified under the microscope in any of the slides. Infection intensity was categorized in the World Health Organization (WHO): light (1-1,999 EPG), moderate (2,000-3,999 EPG), and heavy (≥4,000 EPG) (WHO, 2002).

Participants were asked to answer an individual pre-tested questionnaire to collect data on demography and personal hygiene. Heads of household answered an additional questionnaire about WASH and household assets.

Environmental data, which included altitude, rainfall, land surface temperature, vegetation coverage, land cover and soil type, were downloaded from free remote sensing sources for a period of 1 year starting from the end of the survey. Further details are available in the respective chapter. Environmental data were then linked to parasitological and questionnaire data by household unique location.

All data analysis was conducted in a Bayesian framework, including variable selection. Details are provided in the respective chapter.

For both outcomes, i.e. hookworm infection risk and intensity, a risk factor analysis including individual-, household- and village-level (environmental variables) factors was conducted and spatial distribution was predicted at non-surveyed locations at a 1x1 km resolution based on environmental factors only and using Bayesian Kriging (Diggle et al., 1998).

For each outcome, the model with the best predictive ability was selected for the risk factor analysis as well as the prediction and mapping of each outcome. For infection risk, alternative options were mixed logistic regression models accounting for village-level clustering either integrating a spatial structure (geostatistical) random effect or not (exchangeable random effect). For infection intensity, model selection tested alternatively the spatial (geostatistical) and non-spatial variants of various distributions accounting for the over-dispersion of egg count data (negative binomial, zero-inflated Poisson and zero-inflated negative binomial), which contains many zeros as many individuals are non-infected and few individuals harbour heavy intensity infections.

Chapter 4

Ivermectin treatment and sanitation effectively reduce *Strongyloides stercoralis* infection risk in rural communities in Cambodia

Armelle Forrer[1,2], Virak Khieu[3], Christian Schindler[1,2], Fabian Schär[1,2], Hanspeter Marti[1,2], Meng Chuor Char[3], Sinuon Muth[3], Peter Odermatt[1,2*]

1 Swiss Tropical and Public Health Institute, Basel, Switzerland; **2** University of Basel, Basel, Switzerland; **3** National Centre for Parasitology, Entomology and Malaria Control, Ministry of Health, Phnom Penh, Cambodia;

This paper has been published in PLoS Neglected Tropical Diseases 2016, 10(8):e0004909.

Abstract

Background

Strongyloides stercoralis is the only soil-transmitted helminth with the ability to replicate within its host, leading to long-lasting and potentially fatal infections. It is ubiquitous and its worldwide prevalence has recently been estimated to be at least half that of hookworm. Information on the epidemiology of *S. stercoralis* remains scarce and modalities for its large-scale control are yet to be determined.

Methodology / Principal Findings

A community-based two-year cohort study was conducted among the general population in a rural province in North Cambodia. At each survey, participants infected with *S. stercoralis* were treated with a single oral dose of ivermectin (200µg/kg BW). Diagnosis was performed using a combination of the Baermann method and Koga agar plate culture on two stool samples. The cohort included participants from eight villages who were either positive or negative for *S. stercoralis* at baseline. Mixed logistic regression models were employed to assess risk factors for *S. stercoralis* infection at baseline and re-infection at follow-up. A total of 3,096 participants were examined at baseline, revealing a *S. stercoralis* prevalence of 33.1%. Of these participants, 1,269 were followed-up over two years. Re-infection and infection rates among positive and negative participants at baseline were 14.4% and 9.6% at the first and 11.0% and 11.5% at the second follow-up, respectively. At follow-up, all age groups were at similar risk of acquiring an infection, while infection risk significantly decreased with increasing village sanitation coverage.

Conclusions / Significance

Chemotherapy-based control of *S. stercoralis* is feasible and highly beneficial, particularly in combination with improved sanitation. The impact of community-based ivermectin treatment on *S. stercoralis* was high, with over 85% of villagers remaining negative one year after treatment. The integration of *S. stercoralis* into existing STH control programmes should be considered without further delay.

4.1 Introduction

Infection with *Strongyloides stercoralis*, a soil-transmitted helminth (STH) that occurs worldwide, is a highly neglected tropical disease (NTD) (Olsen et al., 2009, Montes et al., 2010, Krolewiecki et al., 2013, Bisoffi et al., 2013, Schär et al., 2013).

S. stercoralis is endemic in humid and warm regions where sanitation and hygiene conditions are poor (Schär et al., 2013, Schär et al., 2015, Montes et al., 2010). Its global prevalence is largely underestimated due to the use of inadequate diagnostic techniques. A rough (and probably still conservative) estimate of 220-370 million infection cases has recently been put forward, corresponding to half of the number of hookworm cases (Bisoffi et al., 2013, Pullan et al., 2014). Many epidemiological aspects of *S. stercoralis* infection are unknown or poorly documented, but data from recent studies suggest prevalence rates between 10% and 40%, and possibly up to 60%, in the tropics and subtropics (Schär et al., 2013).

S. stercoralis larvae living in soil contaminated by human faeces penetrate the intact human skin and eventually reach the small intestine. Walking barefoot or prolonged contact with contaminated soil when farming are known risk factors for infection in areas with poor sanitation facilities (Grove, 1996). Although about half of all infections remain asymptomatic, chronic symptomatic strongyloidiasis commonly involves diarrhoea, abdominal pain, urticaria and the so-called “larva currens” (Grove, 1996, Becker et al., 2011, Khieu et al., 2013b). A particularity of *S. stercoralis* is its ability to replicate within its host, known as “autoinfection”, which may lead to potentially long-lasting and perpetual infections and to potentially fatal dissemination of the parasite (Keiser and Nutman, 2004, Vadlamudi et al., 2006, Fardet et al., 2007, Marcos et al., 2008).

Control programmes against soil-transmitted helminths target infections with *Ascaris lumbricoides*, *Trichuris trichiura* and hookworms. The mainstay of the WHO's "preventive chemotherapy" strategy is regular chemotherapy with albendazole or mebendazole, either through targeted treatment of specific at-risk groups or by mass-drug administration to entire populations (WHO and Crompton, 2006, WHO, 2010). Health education and sanitation improvement are also recommended because they contribute to reducing transmission and are necessary for sustainable control (World-Health-Assembly, 2001).

Mebendazole and albendazole have a suboptimal effect on *S. stercoralis* and require a long-term treatment schedule, which precludes their use for large-scale control of this parasite (Keiser and Utzinger, 2010, Suputtamongkol et al., 2011). Ivermectin is the drug of choice against *S. stercoralis* and a single oral dose has been shown to be highly efficacious (Suputtamongkol et al., 2011, Igual-Adell et al., 2004, Gann et al., 1994).

To date, there is no control strategy against *S. stercoralis*. Current evidence calls for action, but key aspects of *S. stercoralis* infections need to be documented to assess the effectiveness of preventive chemotherapy and to adequately target control measures (Olsen et al., 2009, Montes et al., 2010, Krolewiecki et al., 2013, Bisoffi et al., 2013, Khieu et al., 2014b). A major question is whether *S. stercoralis* control could be integrated into existing STH control programmes.

The purpose of this large two-year cohort study was to assess the impact of ivermectin for chemotherapy-based control, to determine which age groups should be targeted and to identify risk factors for *S. stercoralis* incident infections among the general population in an endemic setting.

4.2 Methods

4.2.1 Ethics statement

Ethical approval was obtained from the National Ethics Committee for Health Research, Ministry of Health, Cambodia (NECHR, reference number 192, dated December 19, 2011) and from the Ethics Committee of Basel and Baselland, Switzerland (EKBB, reference number 18/12, dated February 23, 2012). All participants received an explanation of the study goals and procedures prior to enrolment. Written informed consent was obtained from all participating adults, while consent from participants aged 2–18 years was obtained from the parents or legal guardians.

4.2.2 Study setting and population

The study was conducted among the general population of Preah Vihear, a rural province in Northern Cambodia with an estimated population of 234,370 in 2013 (National Institute of Statistics, 2013b). STH, including *S. stercoralis*, are highly endemic (Khieu et al., 2014b). Eight villages that had not been previously exposed to *S. stercoralis* treatment were selected in the Rovieng district. In each village, all households were included and all household members over two years old were eligible.

4.2.3 Study design and participants

This study was a two-year prospective intervention (cohort) study, consisting of a baseline survey conducted between February and June 2012 and two follow-up surveys of enrolled individuals, conducted after 12 months (January-March 2013) and 24 months (February-March 2014).

The study cohort included participants who submitted at least one stool sample and were either positive or negative at baseline. All *S. stercoralis* positive participants at baseline

(2012) were enrolled. A subset of negative participants at baseline was asked to participate in the cohort and was selected as follows: in each of the eight villages, 18 to 26 households were randomly selected and visited again in 2013. Household members who were present and *S. stercoralis* negative at baseline (2012) were asked to participate in the two follow-up surveys. A study diagram is presented in Figure 4.1.

The intervention consisted of face-to-face health education on worm infections and hygiene and of administering a single oral dose of ivermectin (200µg/kg BW) to all *S. stercoralis* cases at baseline and follow-up. A sample of 290 *S. stercoralis*-infected participants at baseline was followed-up 21 days after treatment to estimate the cure rate. Other parasitic infections were treated according to the national guidelines (CNM, 2004).

4.2.4 Demographic, socioeconomic, knowledge and behavioural data

Data on demographic features (age, sex, main occupation, level of education), knowledge about worms (sources of infection, health problems caused by worms) and hygiene practices (hand washing, shoe wearing, main defecation place) were obtained from each participant with a pre-tested questionnaire. Heads of households were interviewed about the size of the household, water and sanitation conditions, house construction material and ownership of household assets.

4.2.5 Parasitological data

At each survey, two stool samples were collected on consecutive days from each participant. *S. stercoralis* was diagnosed using the Koga agar plate (KAP) culture (Koga et al., 1991) and the Baermann technique (Baermann, 1917), performed on each sample. *S. stercoralis* larvae were identified through examination under a microscope and based on

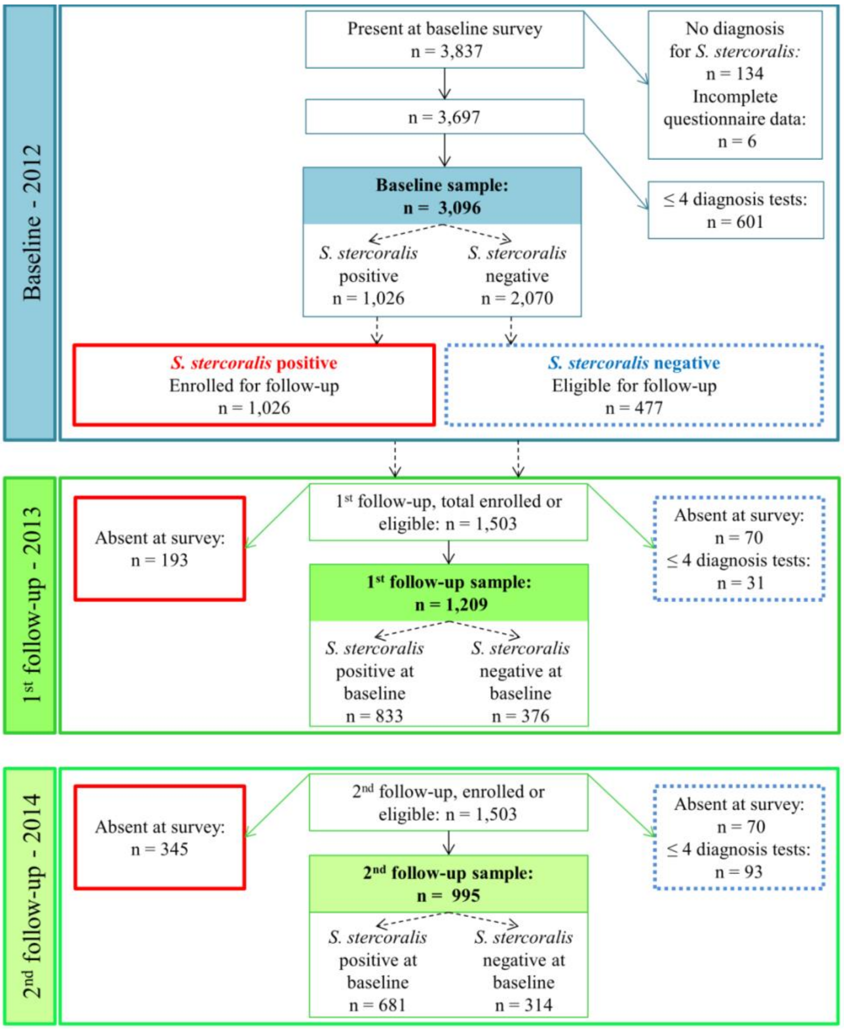


Figure 4.1.: Study diagram

Flowchart detailing compliance levels and the number of participants included at baseline (2012) and at follow-up (2013 & 2014) among community members from eight villages in Preah Vihear province, Cambodia. Negative participants with one (out of four) or more missing diagnostic examinations were excluded from the analysis. Numbers in bold correspond to the size of the analysed samples.

morphology. Combining those two methods on two samples has a 92.8% sensitivity rate (Khieu et al., 2013a). A detailed description of this laboratory procedure is given elsewhere (Khieu et al., 2014b).

4.2.6 Quality control

Technicians were specifically trained to identify *S. stercoralis* larvae. Throughout the study period, technicians were rigorously supervised by a qualified microscopist from the Swiss Tropical and Public Health Institute (Swiss TPH), Basel, Switzerland. Any unclear diagnosis was immediately discussed with both the qualified microscopist and the study supervisor.

4.2.7 Statistical analysis

All (questionnaire and laboratory) data were double-entered and validated in EpiData version 3.1 (EpiData Association; Odense, Denmark). Data management and statistical analyses were performed in STATA version 13.0 (StataCorp LP; College Station, United States of America).

Two risk definitions of *S. stercoralis* infection were considered: the risk of *S. stercoralis* infection at baseline (prevalence) and the risk of *S. stercoralis* infection at follow-up (incidence). The latter included all cases occurring at any follow-up survey, either among participants found positive at baseline, treated and considered negative after treatment ("re-infection") or participants diagnosed negative at baseline (”new-infection”).

A participant was considered *S. stercoralis* positive if at least one larva was found in any of the four samples or *S. stercoralis* negative if no larva was detected in the four samples. Participants with only negative results but with fewer than four analysed samples were not included in the analysis. The risk of infection at follow-up was defined as a binary outcome, which took the value of one if a participant was infected at any of the follow-up surveys, regardless of their infection status at baseline, and zero otherwise. All reported results in this paper use the definitions described above.

Age was centred at the mean of each sample (i.e. baseline or follow-up) and both squared and cubic terms were calculated. A socioeconomic index was built based on house construction material and asset ownership variables, using Principal Component Analysis (PCA) (Vyas and Kumaranayake, 2006, Houweling et al., 2003). Households were classified into wealth tertiles, with the first tertile corresponding to the least poor and the third tertile to the poorest. To ensure the comparability of household socio-economic status (SES) across time, the same asset weights were used to calculate the index of each year. Therefore, differences in SES between 2012 and 2014 relate to changes in the ownership of assets. For some of the subjects, age and education variables were inconsistent across the surveys. To resolve inconsistencies, the following procedure was used: if two of the three values were consistent then the third one was corrected so as to achieve consistency across all three values. Pearson's chi-squared (χ^2) test was used to compare proportions. The cure rate achieved by ivermectin was assumed to be the proportion of *S. stercoralis* patients who had four negative diagnosis results (Baermann method and Koga agar plate tests on two consecutive days) 21 days after treatment.

Mixed-effects logistic regression models were used to investigate the association of each risk definition with explanatory demographic, socioeconomic and behavioural variables, i.e. *S. stercoralis* infection risk at baseline ("baseline model") and infection risk at follow-up ("follow-up model"). Village-level correlation was taken into account with a village-level random effect in both models. For the follow-up model, within-individual correlation was accounted for using an individual-level random effect.

The model building process was similar for the two models. Age and sex were not submitted for variable selection. The same set of demographic, socioeconomic, water and sanitation, and behavioural variables was subjected to selection for each outcome, with the exception of village-level prevalence at baseline and an indicator for baseline infection status that were used only in the follow-up model. Those variables are presented in Appendix 4.6.1. For the follow-up model, age, occupation and level of education as of the previous year were used as explanatory variables. For behaviour and knowledge, current year values were used because health education was always delivered after administering questionnaires and current values were also deemed more representative of the knowledge and behaviour of the past months than the values of the previous survey.

Variable selection was first performed using mixed-effects bivariate logistic regressions, including the appropriate random effects as described above. Variables exhibiting an association at a significance level of 15% in the likelihood ratio test (LRT) were included in the multivariate logistic regression models. In case two explanatory variables were strongly correlated, only one of them was included in the model and this variable was selected based on the Akaike Information Criterion (AIC) of the resulting model.

Additionally, sex was checked to determine if it could be an effect modifier of any other variable in the baseline and follow-up models. For the follow-up model, the interaction between defecation place and occupation was checked, as well as whether infection status at baseline could be an effect modifier of any other variable in the model, in case infection status at baseline involved different risk factors at follow-up. Finally, the significance of the village-level random effect was assessed and the random effect was removed if the AIC indicated a better fit in absence of the random effect. We also conducted a mixed Poisson regression analysis providing incidence rate ratios instead of odds ratios (Miettinen, 1976).

To illustrate the impact of village-level sanitation coverage, the STATA command “margins” was used to predict the risk of infection at follow-up as a function of village-level sanitation coverage, while adjusting for all other covariates of the underlying risk factor model.

4.3 Results

4.3.1 Study population and compliance

At baseline, 3,837 participants were present and 3,697 had complete questionnaire data and at least one available diagnostic result for *S. stercoralis*, i.e. with either Baermann and/or KAP results available for one day (Figure 4.1).

All 1,026 *S. stercoralis* positive participants were enrolled and 477 *S. stercoralis* negative participants were eligible for enrolment. The compliance rate was 86.7% among the 1,503 eligible cohort participants. There were no differences in the proportions of males and females or in reported defecation place between compliant and non-compliant participants at baseline. The proportion of preschool-aged children and of adults who declared staying at home, having a small business or working in the tertiary sector were higher in the non-compliant group, while participants who attained primary school were better represented in the compliant group. Therefore, these variables were adjusted for in the risk factor analysis.

According to the outcome definition adopted in this work, samples from 3,096 participants at baseline were analysed, while the study cohort included 1,269 participants, of whom 873 were positive and 396 were negative at baseline. For the follow-up, 935 cohort participants were present at both surveys, 274 were present only at the first follow-up and 60 were present only at the second follow-up. Hence, 1,269 participants were included in the analysis of infection risk at follow-up, i.e. all participants who were present at one or both follow-up surveys. Figure 4.1 displays compliance levels and the number of participants excluded from the analysis at each survey. The characteristics of the baseline and cohort participants are given in Appendix 4.6.1. Overall, the cohort appeared to be representative of the baseline sample, with similar distributions of covariates. The only notable difference between the baseline and cohort samples was the frequency of females, which was higher at baseline (n=1,702, 55.0%) while more males were represented in the cohort (n=653, 51.4%). This difference is due to the higher risk of infection among males and was statistically significant (χ^2=14.9, p<0.001). Cohort participants were older than non-participants (median = 23 vs. 25 years) at baseline. The most frequent occupation was rice farmer (baseline: 50.5%; cohort: 54.4%). About half (baseline: 55.9%; cohort: 55.1%) of participants did not have access to sanitation and 57.9% of participants declared regularly defecating in the open at baseline. Almost all existing sanitation facilities were latrines types recognized as efficient in preventing from exposure to excreta, with 93% of them being ventilated improved pit latrines (96%) or pour-flush toilets. No village had full sanitation coverage and the village-level proportion of households owning a latrine ranged from 4.2% to 77.6%.

4.3.2 *S. stercoralis* infection risk at baseline and at follow-up

The prevalence of *S. stercoralis* infection at baseline was 33.1%, (95% Confidence Interval (CI): 31.5 – 34.8). Prevalence was similar across the eight study villages (χ^2=10.62, p=0.16) and ranged from 24.2% (95%CI: 19.7 – 29.2) to 33.8% (95%CI: 27.6 – 40.4). Among the cohort participants who were infected at baseline, 120/833 and 75/681 participants were found to have been re-infected, yielding re-infection rates of 14.4% (95%CI: 12.1 – 17.0) and 11.0% (95%CI: 8.6 – 13.6) at the first (2013) and second (2014) follow-up, respectively. Among the cohort participants who were infection-free at baseline, 36/376 and 36/314 were found to have been infected at the first and second follow-up, respectively, resulting in new infection rates of 9.6% (95%CI: 6.8 – 13.0) and 11.5% (95%CI: 8.1 – 15.5) in 2013 and 2014, respectively. Re-infection rates significantly varied across villages (χ^2=32.2, p<0.001) and ranged from 8.3% (95%CI: 3.1 –17.3) to 20.1% (15.5 – 25.3). Compared to the positive cohort, the rate of infection was significantly lower among the negative cohort at the first follow-up, but there was no difference at the second follow-up. Figure 4.2 displays the rates of *S. stercoralis* infection among participants testing positive or negative at baseline, at each follow-up.

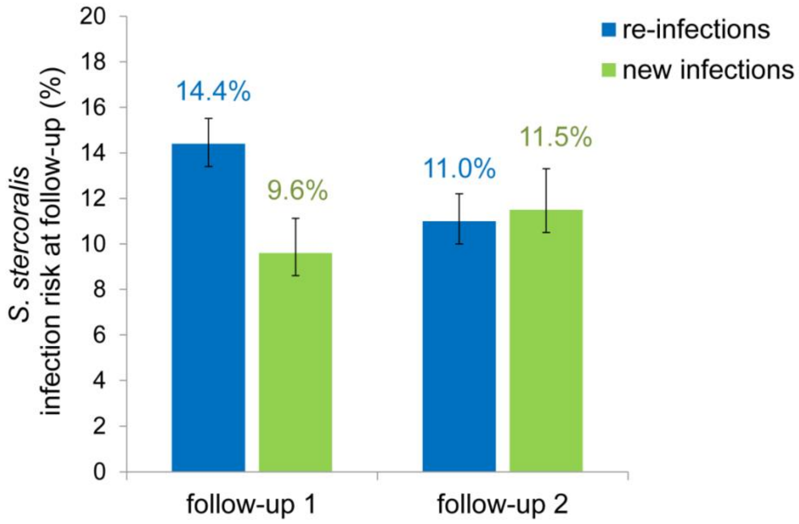


Figure 4.2.: Rates of *S. stercoralis* infection at follow-up surveys among participants who tested positive or negative at baseline.

Re-infections: *S. stercoralis* infection at follow-up among participants who tested positive at baseline. New infections: *S. stercoralis* infection at follow-up among participants who tested negative at baseline. Error bars indicate 95% confidence interval. Data were obtained from repeated surveys carried out among 1,269 participants at follow-up in eight villages of Preah Vihear province, Cambodia, in 2013 and 2014.

4.3.3 Cure rate of ivermectin

Of the 290 participants who were enrolled for follow-up 21 days after treatment, 261 (90.0%) were present at follow-up and of those, 206 (78.9%) had completed diagnostic testing (i.e. four results). 7/206 patients had not been cured, so the cure rate achieved by ivermectin was 96.6% (95%CI: 93.1 – 98.6).

4.3.4 Risk factors for *S. stercoralis* infection at baseline and at follow-up

The results of the bivariate mixed-effects logistic regression models at baseline (prevalent cases) and follow-up (incident cases) are presented in Appendix 4.6.2. At baseline, the village-level random effect was not significant in any bivariate model, so risk factors for infection at baseline were explored using a simple logistic regression model. At follow-up, the village-level random effect lost significance upon introduction of the proportion of households owning a latrine in each village, which suggests that this variable accounted for most of the between-village differences in infection risk.

The results of the multivariate logistic regression models built for baseline and follow-up are presented in Table 4.1. No interaction was found either in the baseline or follow-up model. At baseline, females had lower odds of being infected and infection risk increased with age. The poorest were at a higher risk for infection, as were participants with a higher education level. At follow-up, the risk of acquiring a new infection decreased with increasing sanitation coverage at village level. Neither age nor sex was significantly associated with the risk of acquiring an *S. stercoralis* infection at follow-up. Rice farmers had higher odds of acquiring an infection, as did participants who reported regularly defecating in rice fields or water. Another behavioural risk factor was not wearing shoes, both at home and when going to the toilet. The odds ratios obtained with this multivariate model were similar to risk ratios produced by the mixed Poisson multivariate model. Incidence rate ratios obtained from this model are presented in Appendix 4.6.3.

Table 4.1: Risk factors for infection with *S. stercoralis* at baseline and follow-up

		Infection risk at baseline			**Infection risk at follow-up**		
Variable	**Category**	OR	95% CI	p-value	OR	95% CI	p-value
Sex	Male	1.00	-	-	1.00	-	-
	Female	**0.46**	0.40-0.54	< 0.001	0.80	0.61-1.04	0.096
Age (years)	Linear term	**1.02**	1.01-1.03	< 0.001	0.99	0.98-1.00	0.094
	Quadratic term	0.99	0.99-1.00	0.060	n.a.	n.a.	n.a.
Level of education	Primary	1.00	-	-	1.00	-	-
	No schooling	0.85	0.64-1.14	0.281	**0.47**	0.27-0.84	0.010
	Secondary or higher	**1.29**	1.07-1.56	0.007	1.15	0.86-1.55	0.335
Socioeconomic level	Least poor				n.a.	n.a.	n.a.
	Poor	1.14	0.94-1.37	0.184	n.a.	n.a.	n.a.
	Poorest	**1.37**	1.12-1.68	0.003	n.a.	n.a.	n.a.
Occupation	School, at home, other	1.00	-	-	1.00	-	-
	Rice farmer	1.12	0.86-1.45	0.403	**1.60**	1.10-2.31	0.013
Reported regular place of defecation	Toilet	1.00	-	-	1.00	-	-
	Forest	1.14	0.93-1.40	0.207	1.11	0.79-1.56	0.559
	Rice field or water	0.95	0.78-1.16	0.605	**1.53**	1.09-2.14	0.014
	Behind the house	0.88	0.62-1.26	0.486	1.11	0.60-2.08	0.738
Wearing shoes, frequency	Often	1.00	-	-	n.a.	n.a.	n.a.
	Always	1.08	0.90-1.28	0.409	n.a.	n.a.	n.a.
	Sometimes or never	1.14	0.83-1.56	0.430	n.a.	n.a.	n.a.
Wearing shoes, at home and/or to toilets	Any other case	n.a.	n.a.	n.a.	1.00	-	-
	No at home, yes to toilets	n.a.	n.a.	n.a.	2.14	0.69-6.63	0.189
	No at home, no to toilets				**3.20**	1.38-7.42	0.007
Washing hands after defecating	Yes	n.a.	n.a.	n.a.	1.00		
	No	n.a.	n.a.	n.a.	1.42	0.94-2.14	0.094
Do you know anything about worms?	No	1.00	-	-	n.a.	n.a.	n.a.
	Yes	0.94	0.78-1.14	0.552	n.a.	n.a.	n.a.
Positive for *S. stercoralis* at baseline	No	n.a.	n.a.	n.a.	1.00	-	-
	Yes	n.a.	n.a.	n.a.	1.17	0.87-1.57	0.301
Proportion of houses with latrines in the village (%)		n.a.	n.a.	n.a.	**0.989**	0.982-0.996	0.003

OR: odds ratio; CI: confidence interval; OR in bold are significant at 5% level.

Data were obtained from a two-year cohort survey carried out among 3,096 participants at baseline (2012) and 1,269 participants at follow-up (2013 & 2014), in eight villages of Preah Vihear province, Cambodia.

Figure 4.3 shows the predicted risk of *S. stercoralis* infection at follow-up as a function of the village-level proportion of households owning a latrine and reported individual defecation place. The risk of *S. stercoralis* infection at follow-up decreases with increasing sanitation coverage as expressed by the village-level proportion of households owning improved latrines. While the risk of individuals regularly defecating in rice fields or water is the highest, the risk of acquiring a *S. stercoralis* infection at follow-up for individuals usually defecating in toilets *vs.* in the forest or behind the house appear similar and reflect the absence of a significant protective effect of defecation in latrines, at individual level.

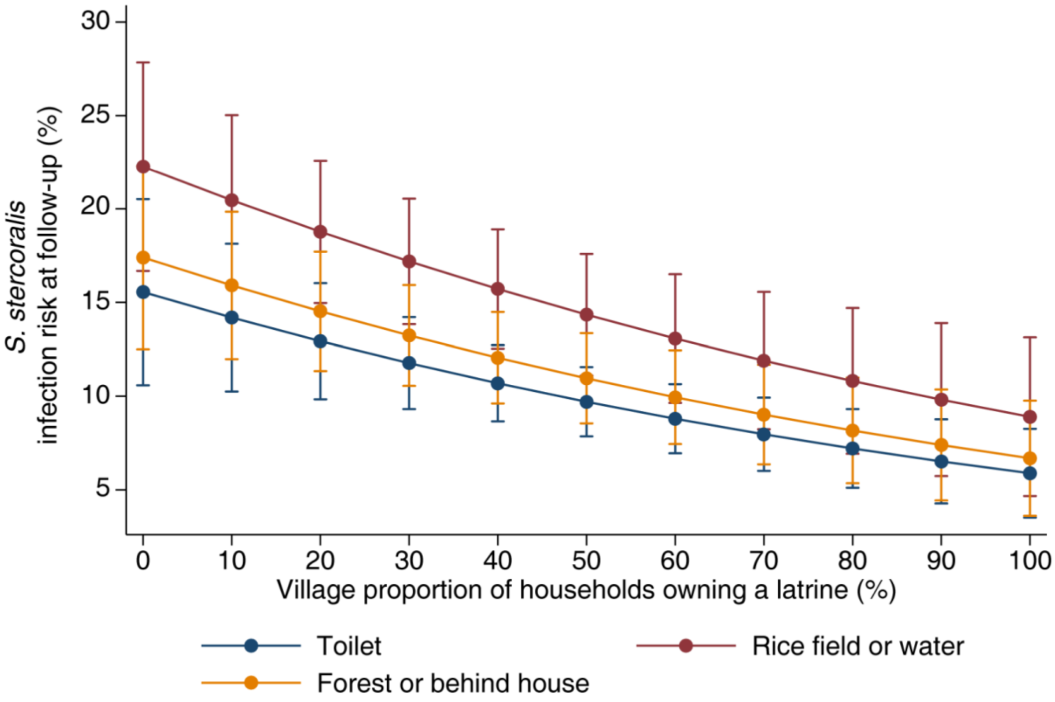


Figure 4.3.: Predicted risk of *S. stercoralis* infection risk at follow-up by village sanitation coverage level and defecation place.

Data were obtained from longitudinal surveys carried out among 1,269 participants at follow-up in eight villages of Preah Vihear province, Cambodia, between 2013 and 2014. The prediction was adjusted for sex, age, level of education, occupation, shoe wearing, hand washing after defecating and infection status at baseline.

4.4 Discussion

This two-year community-based cohort study documents, for the first time to our knowledge, incidence rates and risk factors for incidence of *S. stercoralis* among the general population and provides essential information to guide control efforts: ivermectin chemotherapy against *S. stercoralis* infections is highly beneficial, and its impact is enhanced by community-level improved sanitation coverage.

About one in three of the 3,000 participants present at baseline were infected with *S. stercoralis.* A cohort of 1,269 participants was followed-up over two years and *S. stercoralis* re-infection rates were 14% and 11% at the first and second follow-up, respectively. The rates of newly acquired infections among participants who were negative at baseline were 10% and 11% in 2013 and 2014, respectively. The re-infection rates estimated here confirm results by Khieu and colleagues who found a re-infection rate of 31% in a 2-year cohort study of 300 schoolchildren in semi-rural Cambodia (Khieu et al., 2014a). Since only eight individuals were positive at both follow-ups, a rate of similar magnitude might have been observed after two years if cases found at the first follow-up had not been treated.

Most importantly, the *S. stercoralis* infection risk at follow-up was low, with almost 90% of cohort participants testing negative one year after treatment or after having been diagnosed negative at baseline, indicating that populations strongly benefited from ivermectin treatment. Indeed, an incidence rate of around 13% is particularly low, even compared to that of hookworm, which has the lowest re-infection rates of the three other STH (Jia et al., 2012).

A striking finding of the present work was the corresponding decrease of *S. stercoralis* infection risk at follow-up with increasing sanitation coverage measured at community level. While the rationale behind improving sanitation for STH control is to prevent re-infection by decreasing transmission, evidence supporting this fact remains rare and mostly arises from cross-sectional studies using latrine availability or use at individual level.

We did not find any protective effect of defecating in latrines on *S. stercoralis* infection risk at baseline or follow-up. The association between *S. stercoralis* infections and sanitation has rarely been studied so far. Varying results were obtained mostly from cross-sectional studies, which either found no association or a decreased risk associated with defecation in or access to improved latrines (Khieu et al., 2014b, Echazú et al., 2015, Conlan et al., 2012, Khieu et al., 2014c). In our study, we found a strong impact of sanitation coverage in combination with treatment which explained the differences in infection rates at follow-up across villages. Two key aspects underlie this finding. First, coverage was strongly correlated with use in this setting, with almost all participants living in a house equipped with latrines declaring regularly defecating in them. Second, more than 90% of existing sanitation facilities were improved latrines, which effectively prevent environmental contamination (Asaolu and Ofoezie, 2003, UNICEF, 2015). This result is in line with another study that also found a protective effect of 75% sanitation coverage and above on STH infection (Nikolay et al., 2015).

Unlike infection risk at follow-up, baseline prevalence was similar across villages and was not associated with sanitation coverage. This suggests that, in absence of treatment, the protective effect of sanitation coverage levels-off with time, with parasites steadily accumulating in the environment. In that case, the association between infection risk and sanitation coverage and use may not be observable in the absence of a longitudinal approach. Additionally, participants who declared not wearing shoes, both at home and when going to defecate, had a three-fold higher risk of acquiring an infection by the time of follow-up. This result adequately reflects that infection occurs due to exposure of bare feet to larvae and is in line with meta-analysis findings showing that the risk of infection with *S. stercoralis* was almost halved by footwear use (OR: 0.56, 95%CI: 0.38 – 0.83) (Tomczyk et al., 2014).

It is widely accepted by the STH control community that water, sanitation and hygiene (WASH) improvement is important for controlling and preventing those infections (Freeman et al., 2013b, Campbell et al., 2014, Gazzinelli et al., 2012). However, sanitation measures have not concretely been included in STH intervention packages because implementing them is particularly challenging due to their high cost, complexity, need for cross-sectorial collaboration, and lack of perceived need by communities (Campbell et al., 2014, Freeman et al., 2013b). Regarding the latter, promoting behaviour change and/or triggering demand for sanitation through initiatives like community-led total sanitation (CLTS) and targeted social marketing or social networking appears to be useful but actual evidence of their impact is scarce (Kim et al., 2015a, Pickering et al., 2015, Patil et al., 2014, Campbell et al., 2014). There is still a need to assess and quantify which sanitation improvement measures are effective in reducing *S. stercoralis* (as well as other STH) transmission, including combinations with chemotherapy, and according to which ecological and socio-cultural settings.

Infection at follow-up was not associated with age, so no age risk group could be identified as a target for control. Given the implications of this result, various additional models were run throughout the model building process to test the relationship between infection risk at follow-up and age. None provided evidence in favor of a significant role of age, indicating that the result presented here is robust. Yet, *S. stercoralis* prevalence at baseline did increase with age, a result consistent with other findings from all continents (Steinmann et al., 2007a, Becker et al., 2011, Conlan et al., 2012, Khieu et al., 2014b, Schär et al., 2015). Combined, the relationships between age and prevalence or incidence indicate that *S. stercoralis* infections are permanently acquired through life and that higher prevalence rates observed among adults result from infections that are maintained and accumulated over time (Khieu et al., 2014b).

Finally, regarding other risk factors, farmers were found to have higher odds of acquiring an infection, which was reflected by the positive association between infection risk at follow-up and both occupation and defecating in rice fields. This result is consistent with farmers' frequent and intense contact with soil and is in line with the fact that *S. stercoralis* infection is known to be an occupational disease of farmers and miners, even in temperate climates (Schär et al., 2013).

Our study has some limitations. First, we restricted the inclusion of *S. stercoralis* negative participants to those who had four negative diagnostic results to ensure a high specificity while keeping the maximum number of positive observations in the sample. This approach resulted in a slight overestimation of prevalence at baseline, i.e. 33.1% *vs.* 29.3% with a complete case analysis, but it did not significantly affect the incidence rates. Moreover, because *S. stercoralis* can replicate within its host, an undetected infection can reappear through auto-infection (Keiser and Nutman, 2004). This may have led to overestimation of the ivermectin cure rate and infection risk at follow-up. Given the high cure rate achieved by ivermectin and the rapid clearance of parasites after a single oral dose, the number of re-emerging infections and uncured patients should be low. Nonetheless, the genotyping of parasites before and after treatment is needed to assess the proportion of re-emerging *vs.* new infections, which is an important aspect of *S. stercoralis* infection (Khieu et al., 2014a, Schär et al., 2014). The cure rate achieved by ivermectin has likely been overestimated due to the use of coprological methods, but this issue might have been mitigated by the highly sensitive diagnostic approach used in this study (Krolewiecki et al., 2013, Bisoffi et al., 2011). An alternative way to confirm cure would be to use serological tests which would involve measuring antibody titers 6-12 months after treatment but which would be inappropriate in a high risk setting where reinfection occurs (Buonfrate et al., 2015b, Levenhagen and Costa-Cruz, 2014). Second, one village had a very low proportion of households owning a latrine

(4%), which could have biased the association with follow-up infection risk. However, the association remained significant after excluding this village from the analysis (the results from this model are available in Appendix 4.6.4). Additionally, a more flexible model (i.e. including a quadratic term for sanitation coverage) was run to assess the potential non-linearity of this effect. Although this term was not significant, the resulting dose-response curve suggested that the effect of sanitation coverage of *S. stercoralis* might level off at around 60%. The existence of such a threshold would be consistent with other findings (Albonico et al., 2006, Nikolay et al., 2015). This aspect might be of importance when setting goals for sanitation improvement measures. While we are confident in our results about sanitation coverage, additional studies including a larger number of villages could help to assess whether there is indeed a threshold. The possibility that sanitation acted in a fashion similar to herd immunity as suggested by our results, would have far-reaching implications in terms of equity, since even partial sanitation coverage would benefit entire communities, including those who cannot afford latrines. Finally, the generalization of findings presented here will necessitate additional studies and modelling across different settings to account for variation in geographic and living conditions, including access to improved sanitation across regions, when assessing the impact of both treatment and sanitation on *S. stercoralis* infections (Freeman et al., 2015).

The high efficacy of ivermectin and the low incidence rates estimated in the present work suggest that mass ivermectin chemotherapy as a control measure against *S. stercoralis* would have a strong impact. Our results actually confirm recent findings from a retrospective study conducted in Ecuador which found that mass drug administration of ivermectin targeting onchocerciasis had a significant impact on *S. stercoralis* prevalence (Anselmi et al., 2015). Whether treatment should be delivered annually or more or less frequently cannot be addressed by the present study design, but a cost-effectiveness analysis could help to assess this component of control. In the absence of age-specific morbidity data for chronic infections and because the risk of developing hyper-infection is similar at any age, the similarity of incident infection risk across ages and the highest prevalence rates among adults in populations naïve to treatment suggest a community-wide approach.

Reaching entire communities may raise feasibility and treatment availability issues, although the experience of lymphatic filariasis and onchocerciasis show that community-wide mass treatment is feasible (Anderson et al., 2015). For Cambodia, this should not be a major issue given its effective and well-established delivery systems of treatment against other STH, working through health centres with community health workers and, lately, even in factories (Montresor et al., 2008, Sinuon et al., 2005, National Center for Parasitology, 2014). The affordability of treatment is of greater concern. Ivermectin is not subsidized or donated in Cambodia, where a tablet produced by a certified good manufacturing practice company is available for Cambodia at 10 USD and two to four tablets are needed to treat one individual. Unfortunately, and whatever the target population, such a cost currently precludes the implementation of large-scale *S. stercoralis* control. Although the difficulties of improving sanitation are widely acknowledged, cost-effectiveness studies for sanitation measures might be of interest in the framework of *S. stercoralis* control in Cambodia, given the current high cost of ivermectin. Additional research on sanitation impact should include assessments of latrine promotion and behaviour change measures.

The findings presented here indicate that *S. stercoralis* should be integrated into existing STH control programmes and include adults. In addition, improved sanitation enhances the effect of successful treatment by reducing *S. stercoralis* transmission, so sanitation improvement measures using new participatory approaches such as CLTS or social marketing should be considered (Evans et al., 2014, Kar and Chambers, 2008). However controlling *S. stercoralis* will not be achievable unless funds are made available or generic ivermectin is produced at an affordable price so that low- and middle-income countries, which are the most affected, can start tackling the *S. stercoralis* problem.

4.5 Acknowledgments

We are grateful to all of the study participants. We sincerely thank the local authorities of Preah Vihear. We are grateful to the laboratory technicians and staff from the Helminth Control Program of the National Centre for Parasitology, Entomology and Malaria Control in Phnom Penh and from the Preah Vihear Provincial Health Department for their excellent laboratory and field work.

4.6 Appendix

4.6.1 Baseline characteristics of participants included in the analysis of *S. stercoralis* infection at baseline and at follow-up

Variable		**Baseline** (N = 3,096) **Median; IQR**	**Cohort** (N=1,269) **Median; IQR**
Age (years) [a]		23 ; 26	25; 28
Variable	**Category**	**n (%)**	**n (%)**
Sex	Male	1,394 (45.0)	653 (51.5)
	Female	1,702 (55.0)	616 (48.5)
Level of education [b]	No schooling	449 (14.5)	169 (13.3)
	Primary	1,700 (54.9)	718 (56.6)
	Secondary and higher	947 (30.6)	382 (30.1)
Occupation	Rice farmer	1,564 (50.5)	690 (54.4)
	At home	331 (10.7)	123 (9.7)
	School	1,085 (35.0)	407 (32.1)
	Tertiary, business, other	116 (3.8)	49 (3.8)
Reported regular place of defecation	Toilet	1,326 (42.8)	539 (42.5)
	Forest	685 (22.1)	306 (24.1)
	Rice field or water	857 (27.7)	247 (27.3)
	Behind house	228 (7.4)	77 (6.1)
Availability of toilets at home	No	1,730 (55.9)	700 (55.2)
	Yes	1,366 (44.1)	569 (44.8)

		Baseline (N = 3,096)	**Cohort** (N=1,269)
Variable	**Category**	**n (%)**	**n (%)**
Wearing shoes, frequency	Always	1,522 (49.2)	459 (36.2)
	Often	1,174 (37.9)	661 (52.1)
	Sometimes or never	400 (12.9)	149 (11.7)
Wearing shoes at work or school	Yes	2,874 (92.8)	1,187 (93.5)
	No	222 (7.2)	82 (6.5)
Wearing shoes at home	Yes	2,861 (92.4)	1,170 (92.2)
	No	235 (7.6)	99 (7.8)
Wearing shoes when go defecating/to toilets	Yes	2,748 (88.8)	1,130 (89.0)
	No	348 (11.2)	139 (11.0)
Washing hands after defecating	Yes	2,830 (91.4)	1,160 (91.4)
	No or don't know	266 (8.6)	109 (8.6)
Washing hands before eating	Yes	2,907 (93.9)	1,195 (94.2)
	No or don't know	189 (6.1)	74 (5.8)
Use of soap or ashes when washing hands	No	1,658 (53.6)	703 (55.4)
	Yes	1,438 (46.4)	566 (44.6)
Do you know anything about worms?	No	1,112 (35.9)	448 (35.3)
	Yes	1,984 (64.1)	821 (64.7)
Sources of infection with worms, number of correct answers	0	1269 (41.0)	502 (39.5)
	< 3	349 (11.3)	139 (11.0)
	3-5	868 (28.0)	357 (28.1)
	6-8	610 (19.7)	271 (21.4)
Own dog	Yes	2,461 (79.5)	1,001 (78.9)
	No	635 (20.5)	268 (21.1)
Own farm animals	Yes	2,992 (96.6)	1,240 (97.7)
	No	104 (3.4)	29 (2.3)

IQR: interquartile range;

The baseline sample (left column) includes both participants and non-participants in the cohort; the cohort sample includes all cohort participants regardless of whether they were present at one or two follow-up survey(s).

[a] For age, in case of inconsistencies in the age across years, if two of the three values were consistent then the third one was corrected so as to achieve consistency across all three values.

[b] For education attainment, the same procedure as for age was used in case of inconsistencies reported by individuals over 20 years old and not attending school anymore.

Data were obtained from a two-year cohort survey carried out among 3,096 participants at baseline (2012) and 1,269 participants at follow-up (2013 & 2014), in eight villages of Preah Vihear province, Cambodia.

4.6.2 Bivariate associations between explanatory variables submitted for variable selection and *S. stercoralis* infection risk at baseline and at follow-up

		Prevalence at baseline			Incidence at follow-up		
Variable	**Category**	**OR**	**95%CI**	**LRT p-value**	**OR**	**95%CI**	**LRT p-value**
Level of education (a)	Primary	1.00		<0.0001	1.00		0.028
	No schooling	0.72	0.57-0.91		0.55	0.33-0.93	
Occupation (a)	School, at home, other	1.00		<0.0001	1.00		0.099
	Rice farmer	1.53	1.32-1.78		1.25	0.96-1.62	
	Secondary or higher	1.27	1.08-1.50		1.11	0.83-1.48	
Socioeconomic level (b)	Least poor	1.00		0.103	1.00		0.220
	Poor	1.08	0.90-1.29		0.91	0.66-1.25	
	Poorest	1.22	1.02-1.47		1.2	0.88-1.63	
Reported regular place of defecation	Toilet	1.00		0.006	1.00		0.047
	Forest	1.15	0.95-1.40		1.20	0.86-1.67	
	Rice field or water	0.98	0.81-1.17		1.61	1.15-2.24	
	Behind the house	0.67	0.49-0.93		1.07	0.60-1.93	
Availability of toilets at home	No	1.00		0.256	1.00		0.094
	Yes	1.09	0.94-1.27		0.78	0.59-1.04	
Wearing shoes, frequency	Often	1.00		< 0.001	1.00		0.596
	Always	1.18	1.01-1.39		0.78	0.47-1.28	
	Sometimes or never	0.72	0.56-0.92		0.99	0.57-1.71	
Wearing shoes at work or school	Yes	1.00		0.009	1.00		0.876
	No	0.66	0.49-0.91		1.03	0.68-1.56	
Wearing shoes at home	Yes	1.00			1.00		0.876
	No	0.9	0.68-1.21	0.491	1.03	0.68-1.56	
Wearing shoes to go defecating/to toilets	Yes	1.00			1.00		0.035
	No	0.71	0.55-0.91	0.007	2.07	1.10-3.91	
Wearing shoes at home and/or to toilets	Any other case	n.a.	n.a.	n.a.	1.00		0.102
	No at home, yes to toilets	n.a.	n.a.	n.a.	1.81	0.59-5.49	
	No at home, not to toilets	n.a.	n.a.	n.a.	2.22	1.04-4.76	
Wearing shoes at work/school and/or to toilets	Any other case	1.00		0.014	n.a.	n.a.	n.a.
	No at work/school, yes to toilets	0.91	0.52-1.60		n.a.	n.a.	n.a.
	No at work/school, not to toilets	0.59	0.41-0.85		n.a.	n.a.	n.a.
Washing hands after defecating	Yes	1.00		0.500	1.00		0.040
	No	1.10	0.84-1.43		1.54	1.04-2.28	
Washing hands before eating	Yes	1.00		0.920	1.00		0.910
	No	1.02	0.74-1.39		0.97	0.54-1.72	
Use of soap or ashes when washing hands	Yes	1.00		0.600	1.00		0.639
	No	1.04	0.90-1.21		1.07	0.81-1.41	
Do you know anything about worms?	No	1.00		0.017	1.00		0.933
	Yes	1.22	1.04-1.43		1.02	0.70-1.47	
Sources of infection with worms, number of	0	1.00		0.030	1.00		0.569

		Prevalence at baseline			Incidence at follow-up		
Variable	**Category**	**OR**	**95%CI**	**LRT p-value**	**OR**	**95%CI**	**LRT p-value**
correct answers							
	< 3	1.23	0.95-1.58		1.00	0.60-1.67	
	3-5	1.22	1.02-1.47		1.22	0.84-1.79	
	6-8	1.33	1.08-1.63		1.02	0.68-1.53	
Own a dog	No	1.00		0.600	1.00		0.269
	Yes	0.95	0.79-1.14		1.20	0.86-1.67	
Own farm animals	Yes	1.00		0.340	1.00		0.988
	No	0.81	0.53-1.25		0.99	0.39-2.56	
Infected at baseline	No	n.a.	n.a.	n.a.	1.00		0.124
	Yes	n.a.	n.a.	n.a.	1.25	0.94-1.67	
Number of family members at baseline	-	1.00	0.96-1.04	0.940	1.03	0.97-1.10	0.372
Proportion of houses with latrines in the village at baseline (%)		1.00	1.00-1.01	0.270	0.99	0.980-0.996	0.045
Village prevalence at baseline	-	n.a.	n.a.	n.a.	0.95	0.88-1.03	0.269

OR: odds ratio; CI: confidence interval; LRT: likelihood ratio test;

[a]: For infection risk at follow-up, the values are that of the previous year, i.e. 2012 for the first follow-up and 2013 for the second follow-up.

[b] For infection risk at follow-up, the baseline values of socioeconomic level were used.

Data were obtained from a two-year cohort survey carried out among 3,096 participants at baseline (2012) and 1,269 participants at follow-up (2013 & 2014), in eight villages of Preah Vihear province, Cambodia.

4.6.3 Incidence rate ratios for risk factors of *S. stercoralis* infection risk at follow-up

Variable	**Category**	**IRR**	**95% CI**	**p-value**
Sex	Male	1.00	-	
	Female	0.82	0.65-1.04	0.120
Age (years)	Linear term	0.99	0.98-1.00	0.115
Level of education	Primary	1.00	-	
	No schooling	**0.52**	0.31-0.86	0.016
	Secondary or higher	1.13	0.88-1.45	0.372
Occupation	School, at home, other	1.00	-	
	Rice farmer	**1.50**	1.09-2.06	0.021
Reported regular place of defecation	Toilet	1.00	-	
	Forest	1.10	0.80-1.50	0.569
	Rice field or water	**1.43**	1.07-1.91	0.024
	Behind the house	1.10	0.62-1.96	0.741
Wearing shoes at home and/or to toilets	Any other case	1.00	-	
	No at home, yes to toilets	1.88	0.73-4.80	0.217
	No at home, no to toilets	**2.59**	1.30-5.17	0.010
Washing hands after defecating	Yes	1.00	-	
	No	1.33	0.95-1.87	0.126
Positive for *S. stercoralis* at baseline	No	1.00	-	
	Yes	1.14	0.89-1.48	0.338
		1.00	-	
Proportion of houses with latrines in the village (%)		**0.99**	0.980-0.997	0.006

IRR: incidence rate ratio; CI: confidence interval; IRR in bold are significant at 5% level.

As Poisson regression provides biased standard errors when being applied to binary data, confidence intervals were computed using Miettinen's formula IRR^(1 +/- 1.96/t) where t is the valid t-value of the respective parameter estimate in the corresponding mixed logistic regression model.

4.6.4 Results of the multivariate model for *S. stercoralis* infection risk at follow-up, excluding the village with 4% sanitation coverage.

Variable	Category	OR	95% CI	p-value
Sex	Male			
	Female	0.79	0.59-1.05	0.100
Age (years)	Linear term	0.99	0.98-1.01	0.280
Level of education	Primary	1.00		
	No schooling	**0.45**	0.23-0.85	0.015
	Secondary or higher	1.10	0.81-1.50	0.549
Occupation	School at home, other	1.00		
	Rice farmer	**1.51**	1.02-2.24	0.038
Reported usual place of defecation	Toilet	1.00		
	Forest	1.05	0.74-1.50	0.782
	Rice field or water	**1.51**	1.05-2.16	0.025
	Behind the house	1.00	0.48-2.06	0.995
Wearing shoes at home and/or to toilets	Any other case	1.00		
	No at home, yes to toilets	2.58	0.82-8.13	0.107
	No at home, not to toilets	**2.61**	0.98-6.94	0.054
Washing hands after defecating	Yes	1.00		
	No	**1.58**	1.01-2.47	0.044
Positive for *S. stercoralis* at baseline	No	1.00		
	Yes	1.13	0.82-1.56	0.457
Proportion of houses with latrines in the village (%)		**0.990**	0.980-0.999	0.033

OR: odds ratio; CI: confidence interval; OR in bold are significant at 5% level.

Data were obtained from a two-year cohort survey carried out among 1,128 participants at follow-up (2013 & 2014), in seven villages of Preah Vihear province, Cambodia.

Chapter 5

Strongyloides stercoralis is associated with significant morbidity in rural Cambodia, including stunting in children

Armelle Forrer[1,2], Virak Khieu[3] , Fabian Schär[1,2], Jan Hattendorf[1,2], Hanspeter Marti[2,4], Andreas Neumayr[1,2], Meng Chuor Char[3], Christoph Hatz[1,2], Sinuon Muth[3], Peter Odermatt[1,2*]

1 Department of Epidemiology and Public Health, Swiss Tropical and Public Health Institute, Basel, Switzerland; **2** University of Basel, Basel, Switzerland; **3** National Centre for Parasitology, Entomology and Malaria Control, Ministry of Health, Phnom Penh, Cambodia; **4** Medical Department and Diagnostics, Swiss Tropical and Public Health Institute, Basel, Switzerland.

This paper has been published in PLoS Neglected Tropical Diseases 2017, 11(10)**:** e0005685.

Abstract

Background: *Strongyloides stercoralis* is a soil-transmitted nematode that can replicate within its host, leading to long-lasting and potentially fatal infections. It is ubiquitous and highly prevalent in Cambodia. The extent of morbidity associated with *S. stercoralis* infection is difficult to assess due to the non-specificity of symptoms and, thus, remains uncertain.

Methodology / Principal Findings: Clinical signs were compared among *S. stercoralis* infected *vs.* non-infected participants in a cross-sectional survey conducted in 2012 in eight villages of Northern Cambodia, and before and after treatment with a single oral dose of ivermectin (200µg/kg BW) among participants harboring *S. stercoralis*. Growth retardation among schoolchildren and adolescents was assessed using height-for-age and thinness using body mass index-for-age. *S. stercoralis* prevalence was 31.1% among 2,744 participants. Urticaria (55% vs. 47%, OR: 1.4, 95% CI: 1.1-1.6) and itching (52% vs. 48%, OR: 1.2, 95% CI: 1.0-1.4) were more frequently reported by infected participants. Gastrointestinal, dermatological, and respiratory symptoms were less prevalent in 103 mono-infected participants after treatment. Urticaria (66% vs. 11%, OR: 0.03, 95% CI: 0.01 - 0.1) and abdominal pain (81 vs. 27%, OR: 0.07, 95% CI: 0.02 - 0.2) were mostly resolved by treatment. *S. stercoralis* infection was associated with stunting, with 2.5-fold higher odds in case of heavy infection.

Conclusions / Significance: The morbidity associated with *S. stercoralis* included gastrointestinal and dermatological symptoms unrelated to infection intensity, and long-term chronic effects when associated with malnutrition. The combination of high prevalence and morbidity calls for the integration of *S. stercoralis* into ongoing STH control measures in Cambodia.

Author summary

Strongyloides stercoralis is an intestinal parasite that infects humans by penetrating intact skin. It thrives particularly in tropical countries with poor sanitation. Because it can replicate within its host, it causes long-lasting infections and is potentially fatal in patients with a disseminated infection. *S. stercoralis* is largely neglected due to the difficulty in detecting it with standard field diagnostic techniques but has recently been found to be very common in Cambodia, with prevalence rates exceeding 40%. It is difficult to identify symptoms associated with infection in endemic areas because co-infections with other helminths or protozoan parasites, which cause similar health problems, are common. We compared clinical signs in infected *vs.* non-infected participants living in eight villages in Northern Cambodia, and before and after treatment with ivermectin, the drug of choice against *S. stercoralis*, among 103 patients infected with *S. stercoralis* only. We also assessed the association between infection and growth retardation among children and adolescents. Of the participants, 31.1% were infected with *S. stercoralis*. Infected participants were more likely to report itching and urticaria. After treatment, fewer participants reported urticaria, abdominal pain, vomiting and, to a lesser extent, nausea, diarrhea, cough, and tiredness. *S. stercoralis* infection was associated with growth retardation as expressed by stunting.

5.1 Introduction

Strongyloides stercoralis, one of the most neglected helminths among neglected tropical diseases, is an intestinal soil-transmitted parasitic nematode that occurs worldwide and is highly prevalent in warm regions with poor sanitation (Schär et al., 2013, Olsen et al., 2009). Its prevalence is largely underestimated due to the inability of simple coprological diagnostic techniques to detect *S. stercoralis* larvae (Bisoffi et al., 2013, Krolewiecki et al., 2013). Although numerous aspects of the epidemiology of *S. stercoralis* remain poorly documented, the parasite is very common, with prevalence rates in the tropics and subtropics exceeding 40% (Schär et al., 2013). A rough estimate of 200–370 million cases worldwide has recently been put forward (Bisoffi et al., 2013).

Because it can be life-threatening in immunocompromised patients, *S. stercoralis* is also known throughout the developed countries, with most literature originating from hospital-based case reports of severe strongyloidiasis among transplant recipients, travelers, and migrants. Commonly reported symptoms of uncomplicated strongyloidiasis include diarrhea, vomiting, abdominal pain, urticaria, and “larva currens”, while half of the cases are asymptomatic (Grove, 1996, Becker et al., 2011, Khieu et al., 2013b). Larva currens is an intermittent urticarial linear, serpiginous eruption due to the migration of larvae under the skin. The high speed at which larvae travel (5 to 10 centimetres per hour) and the location of lesions (lower trunk, bottom and thighs) make larva currens a highly specific symptom of *S. stercoralis* infection (Grove, 1996, Toledo et al., 2015, Nutman, 2016).

S. stercoralis can replicate within its host, permitting ongoing “autoinfection”. This leads to long-lasting infections and potential fatalities among immunosuppressed patients, such as those undergoing corticosteroid therapy or suffering certain concomitant diseases or malnutrition (Olsen et al., 2009, Grove, 1996, Keiser and Nutman, 2004). There is a paucity of studies investigating the health impacts of *S. stercoralis* in low-income tropical and sub-tropical countries, including its associations with malnutrition and growth retardation (Olsen et al., 2009, Krolewiecki et al., 2013). It is challenging to document the clinical signs and symptoms associated with specific soil-transmitted helminth (STH) infections in endemic poly-parasitic settings because of their non-specific clinical presentations and because of the difficulty accounting for co-infections (due to the sub-optimal sensitivity of most direct diagnostic approaches) (Krolewiecki et al., 2013).

Preventive chemotherapy, the strategy for controlling STH, would be feasible for *S. stercoralis* using a single oral dose of ivermectin (200 µg / kg body weight (BW)), which achieves high cure rates, comparable to that of a two dose regimen (Henriquez-Camacho et al., 2016, Barda et al., 2017, Forrer et al., 2016, Igual-Adell et al., 2004, Suputtamongkol et al., 2011, Gann et al., 1994).

The evidence base for morbidity in endemic areas is small and must be improved in order to have *S. stercoralis* integrated into the WHO control strategy for reducing the global burden of soil-transmitted helminths (Krolewiecki et al., 2013, Bisoffi et al., 2013, Olsen et al., 2009).The objective of this work was to quantify the morbidity associated with chronic *S. stercoralis* infection in a highly endemic setting in Cambodia. We compared infected *vs.* non-infected participants and assessed the degree of symptom resolution achieved with a single oral dose of ivermectin (200 µg / kg BW), while excluding or adjusting for co-infection with other helminth species or pathological protozoan parasites. Additionally, we investigated the association between growth retardation and *S. stercoralis* infection risk and intensity among schoolchildren and adolescents.

5.2 Methods

5.2.1 Ethics statement

The study protocol was approved by the National Ethics Committee for Health Research, Ministry of Health, Cambodia; and by the Ethics Committee of Northeast and Central Switzerland. All participants were informed of the study purpose and procedures and written informed consent was obtained before enrollment.

5.2.2 Study area, design and population

Morbidity associated with *S. stercoralis* infection was assessed in three sub-studies. First, symptoms associated with *S. stercoralis* infection were identified in a community-based cross-sectional study. Second, symptom resolution by standard treatment was quantified in a before-and-after treatment approach. Third, the association between malnutrition and *S. stercoralis* infection was assessed for children participating in the cross-sectional study.

A community-based, cross-sectional survey was conducted between February and June 2012 in Rovieng district, Preah Vihear Province, Northern Cambodia, where *S. stercoralis* is highly endemic (Khieu et al., 2014b). The survey was part of a larger two-year intervention study, described in detail elsewhere (Forrer et al., 2016). In brief, we included all households in the eight villages that had never received ivermectin treatment; all household members over the age of two were eligible. The resulting sample was used to assess the symptoms associated with *S. stercoralis* infection while adjusting for co-infection with any other diagnosed helminths or pathological protozoan parasites. All *S. stercoralis* cases were treated with a single oral dose of ivermectin (200µg/kg BW) and all other diagnosed parasitic infections were treated in accordance with the national guidelines (CNM, 2004).

Symptom resolution after ivermectin treatment was investigated among *S. stercoralis* infected patients from two villages who were followed-up 21 days after treatment with a single oral dose of ivermectin (200µg/kg BW). Assessment was conducted both among patients with *S. stercoralis* mono-infection (i.e. excluding co-infection with any other diagnosed helminths or pathological protozoan parasites) and among all *S. stercoralis* infected patients (i.e. all *S. stercoralis* cases whether they were co-infected with other parasites or not). Adjustments were made for those with co-infections. A time span of 21 days is long enough to maximize cure time and short enough to avoid reinfection. The single oral dose was chosen because it achieves a high cure rate and is appropriate in the framework of control efforts

(Forrer et al., 2016). Post-treatment data were collected through parasitological assessment and medical interviews about symptoms experienced in the three days preceding follow-up.

The growth retardation assessment was performed among children 5 –19 years of age, residing in the eight villages.

5.2.3 Clinical assessment

Demographic data (sex, age, occupation and education level) and history of anthelmintic treatment were collected from all participants using a pre-tested questionnaire. A clinical assessment of all participants was conducted by a medical doctor and included anthropometric measures, physical examination, and an assessment of signs and symptoms experienced in the two-weeks prior. Information on ownership of household-assets was obtained from heads of households.

5.2.4 Assessment of parasitological infection

S. stercoralis was diagnosed using Koga Agar plate (KAP) culture (Koga et al., 1991) and the Baermann technique (Baermann, 1917) on two samples collected on consecutive days (Khieu et al., 2014b). KAP and Baermann centrifuged eluents were checked for species to prevent the misclassification of hookworm vs. *S. stercoralis* larvae. Other STH were diagnosed using a Kato-Katz thick smear on each of the two fecal samples and formalin-ether concentration technique (FECT) on one sample (Katz et al., 1972, Marti and Escher, 1990). Protozoa were diagnosed with FECT performed on one stool sample (Marti and Escher, 1990).

S. stercoralis parasite load was estimated based on larvae counts from the Baermann test, using the following thresholds: low parasite load — a positive count, but fewer than one larva per gram of stool (LPG); moderate parasite load —two to nine LPG; and high parasite load —

more than 10 LPG (Khieu et al., 2013a, Schär et al., 2014). Participants with positive KAP and negative Baermann tests were assumed to have low parasite loads.

To keep all the positives in the sample and to maximize the specificity, the infection status of participants in any of the three assessments was determined using the following case definition: a *S. stercoralis* patient had at least one *S. stercoralis* positive fecal sample. A *S. stercoralis*-free participant had negative results in all four available diagnostic results (one Baermann and one KAP on each of two samples). The same approach was used for the other helminths, i.e. all positives (determined by at least one positive diagnosis result by any method) and only negatives with two Kato-Katz results were included. Since there was only one FECT result per participant, the case definition does not apply to protozoa.

5.2.5 Data management and statistical analysis

Clinical and laboratory data were managed and analyzed in STATA version 13.0 (StataCorp LP; College Station, United States of America). Anthropometric measures and summaries were calculated using the WHO code (http://www.who.int/growthref/tools/en/) in R (RCoreTeam, 2016). In the cross-sectional study, age of participants was categorized into four groups, as follows: (i) < 6 years, (ii) 6–18 years, (iii) 19–59 years, and (iv) ≥ 60 years. The age of children included in the growth retardation analysis was categorized into three groups, corresponding to school level (i.e. primary, secondary, high school), as follows: (i) 5–9 years, (ii) 10–13 years and (iii) 14–19 years.

First, the association between each symptom, *S. stercoralis* infection status, and parasite load was investigated using multivariate logistic regression models (with the symptom as outcome), adjusting for any other helminthic or protozoan infection, sex, age, and treatment uptake in the past year.

Second, the symptom resolution achieved by ivermectin treatment was assessed with McNemar’s exact test among patients harboring *S. stercoralis* mono-infections, by comparing the proportions of participants with a particular symptom before and after treatment. An additional analysis including co-infections with other parasites used conditional logistic regression models with each symptom as outcome, and survey (before *vs.* after treatment) and presence of any other infection as explanatory variables.

Third, for assessing growth retardation and thinness in children, the z-scores for height-for-age (HAZ) and body mass index (BMI)-for-age (BAZ) were calculated for children between 5 and 19 years old, participating in the cross-sectional survey. The WHO Growth Reference Standard was used to calculate anthropometric indicators for school-aged children and adolescents (de Onis M, 2007, Group, 2006).

Children with HAZ and BAZ values lower than -2 were classified as “stunted” or “thin”, respectively, as opposed to “normal” for values larger than -2. The association between stunting, thinness and *S. stercoralis* infection status and parasite load was assessed using logistic regression with the nutritional variable as outcome. The final multivariate model was built based on Akaike Information Criterion (AIC). Interactions between sex and *S. stercoralis* parasite load, age, and socioeconomic status, as well as between age and *S. stercoralis* parasite load and socioeconomic status were checked using the Likelihood Ratio Test (LRT).

5.3 Results

5.3.1 Study population

Among the 3,837 participants enrolled in the study, diagnostics could not be performed for *S. stercoralis* or for protozoan infections for 134 and 320 individuals, respectively, due to absent samples (participants did not return any sample) or an insufficient amount of stool to perform the FECT. The remaining 3,377 participants all completed the interview and the clinical assessment and were included in the study. Females and children under six years old were significantly more prone to missing diagnostics and exclusion from the sample. All regression models were adjusted for sex and age, which controlled for this potential bias.

The association between *S. stercoralis* infection and symptoms was assessed for 2,744 participants with confirmed infection status for all investigated parasites (Figure 5.1). This final sample size results from excluding all participants with uncertain negative status for helminth infection, i.e. those with fewer than four negative results (two methods applied to two samples) for *S. stercoralis* and fewer than two negative results (Kato Katz on two samples) for other helminths, to maximize diagnosis specificity. Basic characteristics of this sample are presented in Table 5.1. The prevalence of *S. stercoralis* infection was 31.1% (95%CI: 29.4 – 32.9). A quarter (699/2,744; 25.3%) of participants were infected with hookworm, which was the only other common parasite. Among *S. stercoralis* infected patients, 325/853 (38.1%) were co-infected with hookworm. Other helminths species were rare, with prevalence rates below 3%, while 8% of participants were infected with pathogenic protozoa, namely *Giardia lamblia* or *Entamoeba histolytica/dispar*. Species specific prevalences are available in Appendix 5.7.1.

For the before-after treatment assessment, 208 of the 316 *S. stercoralis* positive participants had confirmed infection status for all parasites at both assessments. About half (103/208) of the participants in that sub-sample were not infected with any other helminth or pathogenic protozoa either before or after treatment, while the other 105 harbored other intestinal parasites before and/or after treatment. Basic characteristics of the sample analyzed for the before-after assessment are presented in Table 5.1.

Table 5.1: Characteristics of participants included in the three analysed samples

Variable	Category	n (%)
Sample "positives *vs.* negatives"		**N = 2,744**
Sex	Male	1,240 (45.2)
	Female	1,504 (54.8)
Age (years)	< 6	203 (7.4)
	6-18	982 (35.8)
	19-59	1,379 (50.3)
	≥ 60	180 (6.5)
Anthelmintic treatment in the past year	No or don’t know	1,015 (37.0)
	Yes	1,729 (63.0)
Occupation	Rice farmer	1,397 (50.9)
	At home	296 (10.8)
	School	945 (34.4)
	Tertiary, business, other	106 (3.9)
Sample "before-after"		**N = 103**
Sex	Male	55 (53.4)
	Female	48 (46.6)
Age (years)	< 6	3 (2.9)
	6-18	22 (21.4)
	19-59	68 (66.0)
	≥ 60	10 (9.7)
Anthelmintic treatment in the past year	No or don’t know	50 (48.5)
	Yes	52 (51.5)
Occupation	Rice farmer	64 (62.1)
	At home	7 (6.8)
	School	21 (20.4)
	Tertiary, business, other	11 (10.7)
Sample "childhood malnutrition"		**N=1,057**
Sex	Male	535 (50.6)
	Female	522 (49.4)
Age (years)	5-9	339 (32.1)
	10-13	364 (34.4)
	14-19	354 (33.5)
Anthelmintic treatment in the past year	No or don’t know	394 (37.3)
	Yes	663 (62.7)
Occupation	School	904 (85.5)
	At home, other	90 (8.5)
	Rice farmer	63 (6.0)

Sample "positives vs. negatives": cross-sectional sample for assessing the association between *S. stercoralis* infection and clinical signs

Sample "before-after": sample for assessing clinical signs present before and after a single oral dose of ivermectin (200 µg/kg BW) in patients infected with *S. stercoralis* only

Sample "children malnutrition": sample for assessing the association between stunting and *S. stercoralis* infection

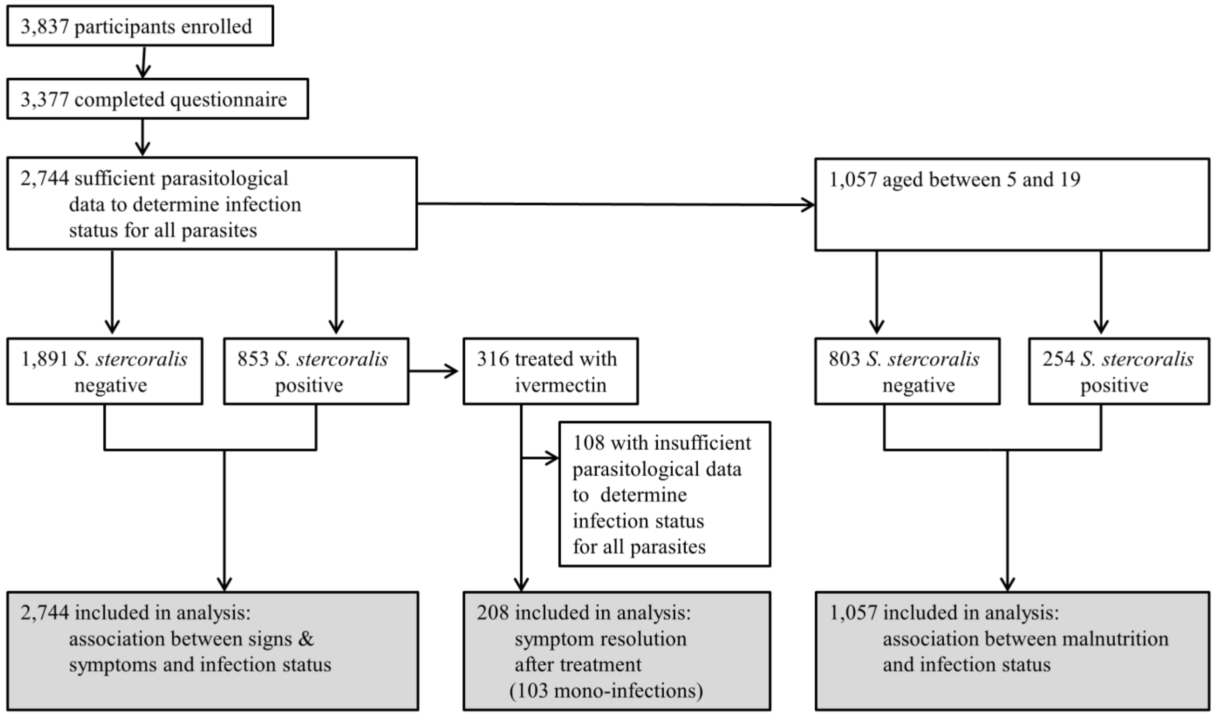


Figure 5.1.: Study flow chart

Flowchart detailing the number of participants included in the three sub-studies. Insufficient parasitological data corresponds to one (out of four) or more missing diagnostic examinations for *S. stercoralis,* to one (out of two) or more missing diagnostic examinations for other helminths, and to any missing diagnostic examination for protozoa.

The growth retardation assessment was conducted among 1,057 children aged 5–19 years, with confirmed infection status for *S. stercoralis* and hookworm, and who had *S. stercoralis* parasite load assessed. Of the 1,338 children aged 6–19 years participating in the study, 229 were excluded from the final analysis due to incomplete diagnostics for *S. stercoralis*, seven were excluded because of missing diagnosis results for hookworm, and 47 were excluded due to the absence of parasite load information. Basic characteristics of the sample are presented in Table 5.1. Among this group, the prevalence of *S. stercoralis* was 24.0% (95%CI: 21.5 – 26.7). Most cases (160/254; 63.0%) had low parasite loads, whereas 20.1% and 16.9% had moderate and heavy parasite loads, respectively.

5.3.2 Symptoms associated with *S. stercoralis* infection

Overall, only five (1.1%) participants with *S. stercoralis* mono-infection and 18 (1.4%) infection-free participants were reportedly without symptoms. The most frequently reported symptoms by *S. stercoralis*-infected patients were abdominal pain, cough, epigastric pain, diarrhea, and urticaria, ranging from 83.1% to 55.3%. However, in most cases they were not significantly associated with *S. stercoralis* infection. The symptoms with the strongest association were urticaria (55.3% *vs.* 46.5%, OR: 1.35, 95% CI: 1.13 - 1.60) and itching (52.4% *vs*. 47.7%, OR: 1.19, 95% CI: 1.00 - 1.41). The frequency of symptoms and the strength of their association with *S. stercoralis* infection status are presented in Table 5.2.

When accounting for *S. stercoralis* parasite load instead of infection status, urticaria was also found to be significantly associated, but not itching (Appendix 5.7.2). Of note, age was associated with most of the reported symptoms (Appendix 5.7.3).

5.3.3 Symptom resolution after treatment

Table 5.3 presents the extent of symptom resolution following single-dose ivermectin treatment among patients with *S. stercoralis* mono-infection (i.e. excluding co-infection with any other helminth or pathogenic protozoan parasite). All participants reported at least one symptom before treatment. The symptoms that declined most after treatment were urticaria (66.0% *vs.* 10.7%, OR: 0.03, 95% CI: 0.00 - 0.13), abdominal pain (80.6% *vs.* 27.2%, OR: 0.07, 95% CI: 0.02 - 0.18), and vomiting (23.3% *vs.* 1.0%, OR: <0.1, 95% CI: 0.00 - 0.17). Other symptoms that declined significantly after treatment were nausea, diarrhea, tiredness, and cough. With the addition of 105 patients co-infected with other helminth or pathogenic protozoan parasites, the same symptoms were found to significantly recede post-treatment. Loss of appetite was also less frequently reported in this group (Appendix 5.7.4). Figure 5.2 displays the proportions, stratified by parasite load, of the six symptoms that were

Table 5.2: Association between symptoms and *S. stercoralis* infection among 853 positive and 1,891 negative participants in Cambodia

			Association with *S. stercoralis* infection		
Symptom	*S. stercoralis* positive n (%)	*S. stercoralis* negative n (%)	OR$^{(a)}$	95% CI	LRT p-value
Loss of appetite / Anorexia	277 (32.5)	598 (31.6)	0.97	0.80 - 1.17	0.73
Nausea	291 (34.1)	611 (32.3)	1.02	0.85 - 1.22	0.86
Vomiting	181 (21.2)	380 (20.1)	1.05	0.85 - 1.29	0.67
Abdominal pain	709 (83.1)	1479 (78.2)	1.11	0.88 - 1.41	0.36
Epigastric pain	531 (62.3)	1085 (57.4)	0.98	0.81 - 1.20	0.85
Diarrhea	513 (60.1)	1086 (57.4)	1.10	0.93 - 1.31	0.28
Constipation	128 (15.0)	293 (15.5)	0.91	0.71 - 1.15	0.42
Cough	533 (62.5)	1208 (63.9)	1.00	0.84 - 1.19	0.98
Wheezing	72 (8.4)	147 (7.8)	1.03	0.76 - 1.41	0.85
Itching	447 (52.4)	902 (47.7)	**1.19**	1.00 - 1.41	0.05
Urticaria	472 (55.3)	880 (46.5)	**1.35**	1.13 - 1.60	0.001
Generalized rash	186 (21.8)	345 (18.2)	1.20	0.97 - 1.48	0.09
Fever	360 (42.2)	855 (45.2)	0.98	0.83 - 1.17	0.85
Tiredness	205 (24.0)	421 (22.3)	1.04	0.84 - 1.28	0.75
Muscle pain	381 (44.7)	688 (36.4)	1.17	0.97 - 1.40	0.10

(a) Odds ratios were adjusted for age, sex, medication (uptake of anthelmintic tablets within the past year), and infection with any diagnosed helminth or pathogenic protozoa.
OR in bold were significant at 5% level.
OR: odds ratio.
CI: confidence interval.
LRT: likelihood ratio test

significantly less frequently reported by the *S. stercoralis* patients who were free of any other diagnosed parasite, following treatment. The decreases appear to be of a similar magnitude among participants with light *vs.* moderate or heavy infections.

5.3.4 Growth retardation in children and *S. stercoralis* infection

More than half of the children were either moderately (40.4%) or severely (11.3%) stunted. The proportion of stunting was 46.6% in *S. stercoralis*-infected children and 37.8%

Table 5.3: Symptoms before and after ivermectin treatment in 103 patients with *S. stercoralis* mono-infection

Symptom	Before treatment	After treatment			
	n (%)	n (%)	OR	95% CI	p-value (a)
Loss of appetite / anorexia	42 (40.8)	30 (29.1)	0.56	0.27 - 1.08	0.09
Abdominal pain	83 (80.6)	28 (27.2)	**0.07**	0.02 - 0.18	< 0.001
Nausea	34 (33.0)	13 (12.6)	**0.30**	0.13 - 0.65	< 0.001
Vomiting	24 (23.3)	1 (1.0)	**< 0.01**	0.00 - 0.17	< 0.001
Diarrhea	61 (59.2)	35 (34.0)	**0.33**	0.16 - 0.64	< 0.001
Constipation	12 (11.7)	11 (10.7)	0.89	0.30 - 2.60	> 0.99
Itching	56 (54.4)	52 (50.5)	0.86	0.48 - 1.53	0.68
Urticaria	68 (66.0)	11 (10.7)	**0.03**	0.00 - 0.13	< 0.001
Cough	71 (68.9)	44 (42.7)	**0.29**	0.13 - 0.58	< 0.001
Wheezing	10 (9.7)	11 (10.7)	1.13	0.39 - 3.35	> 0.99
Fever	36 (35.0)	45 (43.7)	1.60	0.81 - 3.28	0.20
Tiredness	35 (34.0)	21 (20.4)	**0.44**	0.20 - 0.93	0.03
Muscle pain	40 (38.8)	44 (42.7)	1.22	0.63 - 2.42	0.64

(a) p-values and odds ratios were obtained from McNemar's exact test.
Treatment: ivermectin 200 µg/kg BW.
OR in bold were significant at 5% level.
Data were obtained from a before-after treatment study conducted in 2012 in Preah Vihear province, Cambodia, among 103 patients with *S. stercoralis* mono-infection.
OR: odds ratio.
CI: confidence interval.
LRT: likelihood ratio test.

in non-infected children. Stunting was associated with *S. stercoralis* infection status, with a higher risk of being stunted when infected with *S. stercoralis* (OR: 1.35, 95%CI: 1.00 – 1.81) or when heavily infected compared to uninfected (OR: 2.49, 96%CI: 1.31 – 4.71). No interactions were found. Results of the association between stunting and *S. stercoralis* parasite load are presented in Table 5.4. Thinness was also common, with one in five children (19.9%) being underweight, but thinness was not associated with either *S. stercoralis* infection status (χ^2=0.47, LRT p-value=0.5) or parasite load (χ^2=1.21, LRT p-value=0.75).

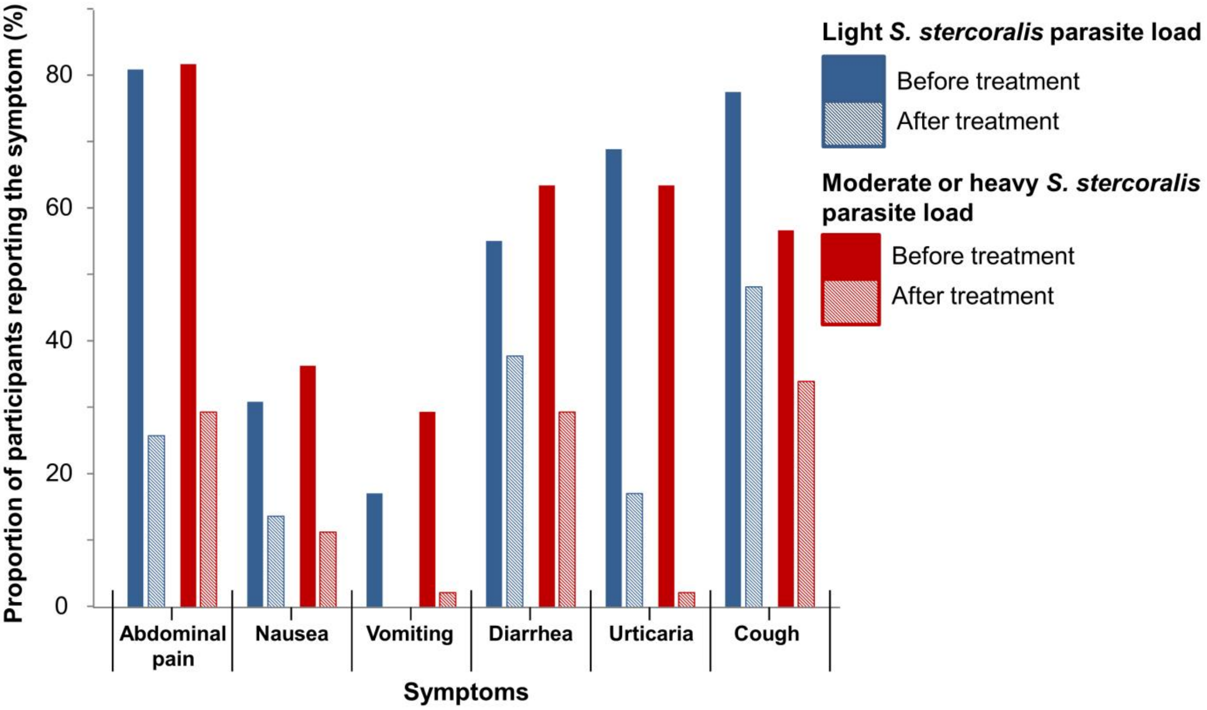


Figure 5.2: Proportion of participants harboring *S. stercoralis* mono-infections and reporting abdominal pain, nausea, vomiting, diarrhea, urticaria, and cough before and 21 days after ivermectin (200 µg/kg BW) treatment

The decreases in the proportion of participants reporting any of the symptoms in the figure was significant at 5% level, as assessed by the McNemar’s test.

S. stercoralis low parasite load: positive count and ≤ 1 larvae per gram (LPG).

S. stercoralis moderate or high parasite load: > 1 LPG.

Data were collected in 2012 in Preah Vihear Province, North Cambodia, from 103 participants in the post-treatment survey who harbored *S. stercoralis* mono-infection at both surveys and met the case definitions used in this work for all parasites.

Table 5.4: Results of the multivariate logistic model assessing the association between stunting and *S. stercoralis* parasite load

		Stunted no	**Stunted yes**			
Variable	Category	n (%)	n (%)	OR	95%CI	p-value
S. stercoralis infection intensity	No infection	499 (78.6)	304 (72.0)	1.00		
	Light	88 (13.8)	72 (17.1)	1.26	0.89 - 1.79	0.20
	Moderate	31 (4.9)	20 (4.7)	0.97	0.53 - 1.76	0.91
	Heavy	17 (2.7)	26 (6.2)	**2.48**	1.31 - 4.71	0.01
Infected with hookworm	No	495 (77.9)	330 (78.2)	1.00		
	Yes	140 (22.1)	92 (21.8)	0.84	0.61 - 1.15	0.28
Sex	Male	302 (47.6)	233 (55.2)	1.00		
	Female	333 (52.4)	189 (44.8)	**0.73**	0.57 - 0.95	0.02
Age (years)	5-9	223 (35.1)	116 (27.5)	1.00		
	10-13	211 (33.2)	153 (36.3)	**1.66**	1.18 - 2.33	0.003
	14-19	201 (31.7)	153 (36.2)	**1.88**	1.32 - 2.69	< 0.001
Socioeconomic status	Least poor	267 (42.1)	141 (33.4)	1.00		
	Poor	214 (33.7)	156 (37.0)	**1.44**	1.07 - 1.94	0.02
	Most Poor	154 (24.2)	125 (29.6)	**1.63**	1.18 - 2.25	0.003
Occupation	School	548 (86.3)	356 (84.4)	1.00		
	At home	49 (7.7)	41 (9.7)	**1.72**	1.06 - 2.81	0.03
	Farmer, other	635 (6.0)	422 (5.9)	0.84	0.48 - 1.46	0.53

Data were obtained from a cross-sectional study conducted in 2012 among 1,057 children aged 5–19 years.

OR: odds ratio.

CI: confidence interval.

OR in bold were significant at 5% level.

5.4 Discussion

This is the first report, to our knowledge, showing symptoms specifically associated with *S. stercoralis,* i.e. excluding other helminth infections and pathogenic protozoa, in a multi-parasitic setting, where it is particularly difficult to assess the morbidity associated with specific STH infections. Both the sensitivity and specificity of helminth diagnosis were maximized by combining all available methods (Baermann, KAP, and Kato-Katz on two stool samples, and FECT on one stool sample), and by including only negatives with the highest "certainty" of negative status, i.e. participants with all four (Baermann and KAP each on two samples) available results for *S. stercoralis* and with two (Kato Katz on two samples) results for other helminths. Women and children under six years were more likely to have incomplete diagnostic results for helminths, but this aspect did not bias the analysis as all models were adjusted to account for those factors.

Among the 103 participants infected with *S. stercoralis* only, dermatological, gastrointestinal, and respiratory symptoms, such as urticaria, abdominal pain, nausea, vomiting, diarrhea, and cough, were found to be significantly less frequent after treatment. A previous study conducted in the same province also found that abdominal pain, diarrhea, and urticaria were resolved by ivermectin treatment among 21 heavily infected patients (Khieu et al., 2013b). All of those symptoms are consistent with various phases of the infection (Becker et al., 2011, Grove, 1996).

The most prominent symptom of *S. stercoralis* infection was urticaria, also called "hives", which was both mostly resolved by ivermectin treatment and associated with infection status. However, the association was weak given the high proportion of *S. stercoralis* negative patients also reporting those symptoms and results should be interpreted with caution. The lack of association between reported symptoms and infection status could be due to misclassification of *S. stercoralis* cases, however we used a diagnosis approach with sensitivity exceeding 92%, even for light infections, so the number of false negative *S.*

stercoralis cases would be low (Schär et al., 2014). The weak or absent associations between symptoms and *S. stercoralis* infection status mostly reflect the difficulty of assessing the relationship between nonspecific clinical signs and STH or protozoan infections in poly-parasite endemic settings (Becker et al., 2011). The challenges faced in such assessments tend to include diagnostic approaches with imperfect sensitivity, reported symptoms being subject to recall and reporting biases, and the non-specificity of symptoms that could be due to other pathologies including viral infections.

Treatment had no effect on itching, indicating that itching could be the result of numerous other conditions and reasons, particularly in tropical settings, including allergies and insect bites, and not necessarily disease-specific (Caraballo et al., 2016).

Urticaria is a well-known symptom of chronic strongyloidiasis and has already been identified as such in Cambodia through studies that did not, however, exclude cases of co-infection with other helminth species or pathogenic protozoa (Khieu et al., 2014c, Khieu et al., 2013b, Kolkhir et al., 2016, Krolewiecki et al., 2013, Nutman, 2016). Acute urticaria may occur at the penetration site for hookworms and *S. stercoralis* and is mostly located on the feet (Toledo et al., 2015, Nutman, 2016). Urticaria occurring during the chronic phase of infection is accepted as a systemic reaction due to the parasite-induced immunologic anti-inflammatory response, which results in increased eosinophil and IgE levels, similar to an allergic response (Rampur et al., 2016, Sanders and Mishra, 2016, Bakiri and Mingomataj, 2010). However, the actual mechanisms explaining the relationship between skin reactions and helminths remain unclear (Bakiri and Mingomataj, 2010, Nahshoni et al., 2016). Some authors suggest that urticaria is induced by the parasite to ease migration under the skin, in the lymphatic ways and in some parenchimatous organs (Bakiri and Mingomataj, 2010). Therefore, urticaria would relate to the larval stage of infection or to the parasite migration phase, rather than to the mere presence of parasites in the body (Bakiri and Mingomataj, 2010). This statement is of particular interest to the etiology of urticaria in strongyloidiasis and would be in line with *S. stercoralis'* autoinfection ability, whereby the parasite continuously replicates and produces larvae that re-infect the host.

Another striking effect of ivermectin treatment was the resolution of abdominal pain among most of the patients. The impact on other clinical signs — with the exception of vomiting, which was reported by only one patient after treatment — was more modest, as indicated by the substantial proportion of participants still reporting symptoms three weeks after treatment. These symptoms also declined significantly among participants co-infected with *S. stercoralis* and other parasites (75% of which were hookworm). Morbidity was not associated with hookworm infection in this study, probably because almost all cases (97.7%) were light intensity infections, as defined by the WHO thresholds, or because of a high prevalence of *Ancylostoma ceylanicum*, a hookworm common to dogs and cats that often infects humans in the region (Inpankaew et al., 2014, Traub, 2013, WHO, 2002). Interestingly, self-reported morbidity was higher among individuals infected with *S. stercoralis* than among those with hookworm in a setting endemic for both parasites (Becker et al., 2011).

Surprisingly, while STH morbidity is known to increase with worm load, the clinical manifestations associated with *S. stercoralis* were not associated with parasite loads in this setting. This result could be due to the irregular larval output of *S. stercoralis* that might have affected the estimated parasite load. It is also possible that the thresholds used inadequately reflected the impact of parasite load on health or that other undiagnosed pathogenic parasites were effectively treated by ivermectin, thereby having a confounding effect (Uparanukraw et al., 1999, Schär et al., 2014, Sato et al., 1995, Siddiqui and Berk, 2001). However, confusion between larval output thresholds used for low (1 LPG) vs. high (≥ 10 LPG) parasite load appears unlikely (Schär et al., 2014, Uparanukraw et al., 1999, Sato et al., 1995). Yet, it cannot be excluded that *S. stercoralis* is pathogenic even in cases of low parasite load, which would largely affect any indicator of parasite burden or treatment effect, including cost-effectiveness assessments.

An important finding was the association between growth retardation and both *S. stercoralis* infection risk and parasite load. The risk of being stunted increased with age, indicating the accumulation of growth retardation through time. This association may also reflect greater exposure to malnutrition in the past, among older children. Stunting may be due to a number of causes, including suffering from heavy STH infections before STH controls were implemented. However, in this setting, where about half of the children suffered from growth retardation, the cross-sectional design of this study could not address causality. Further studies accounting for potential confounders of the relationship between malnutrition and *S. stercoralis* infection, such as medical history, quantitative and qualitative food intake, and social aspects, are needed to determine specific factors and the strength and direction of the association.

Moderate to heavy infections with any of the three other STH, *A. lumbricoides*, *T. trichiura*, and hookworm, are widely recognized as causes of stunting, which make STH one of the most important causes of physical and intellectual growth impairment (Hotez, 2003, Yap et al., 2014, Bethony et al., 2006, Stephenson et al., 2000b). Yet, current evidence supporting the association between STH and childhood growth is currently of low quality and warrants further research, which should also include *S. stercoralis.* Confirming that *S. stercoralis* infection plays a role in growth retardation due to its contribution to chronic malnutrition in childhood would have an important impact on estimating the disease burden.

Protein-calorie malnutrition is a known cause of immunodeficiency in resource-poor countries and may be a pivotal aspect of *S. stercoralis* morbidity (Olsen et al., 2009). First, chronic strongyloidiasis causes gastro-intestinal symptoms that potentially lead to malnutrition through lower food intake and nutrient loss (Stephenson et al., 2000b). Second, there is evidence from animal studies that nematode infections in malnourished hosts induce a decrease in the T-Helper Type 2 (Th2) mediated immune response, including eosinophil counts, which are known to be an important part of the immune response against *S. stercoralis* (Koski and Scott, 2001, Concha et al., 2005). Finally, immunodeficiency may increase the risk of complicated strongyloidiasis in malnourished populations, and malnutrition unrelated to known causes of immunosuppression might be responsible for severe strongyloidiasis cases in developing countries (Cimino and Krolewiecki, 2014, Olsen et al., 2009, Concha et al., 2005). The risk of developing severe strongyloidiasis could be high in settings with widespread malnutrition such as Cambodia, while an additional issue is the increased availability of over-the-counter drugs containing corticoids (Olsen et al., 2009, Greffeuille et al., 2016).

Our study has several limitations. The difference in durations considered for symptom reporting before and after treatment may have overestimated pre-treatment symptom reporting frequencies as well as treatment effects, particularly in the case of vomiting. Additionally, in the absence of a control group, our study did not account for placebo effects, which could have influenced symptom reporting.

Finally, some co-infections with pathogenic protozoa may have been missed due to the limited sensitivity of FECT performed on one stool sample, which might explain the moderate impact of ivermectin treatment on diarrhea and nausea. However, this limitation would not apply to helminths, for which the diagnostic approach used in the present study has been assessed several times and has shown high sensitivity and specificity (Khieu et al., 2013a, Schär et al., 2014, Khieu et al., 2014b, Khieu et al., 2014c). Nor would it apply to urticaria, which is commonly associated with other helminths including *Ascaris lumbricoides*, *Hymenolepis nana*, *and Fasciola hepatica*, as well as with protozoans including *Giardia lamblia* and *Blastocystis hominis* (Lepczynska et al., 2015, Caraballo et al., 2016, Bakiri and Mingomataj, 2010, Kolkhir et al., 2016, Giacometti et al., 2003). While we cannot exclude that some protozoan infections were missed, ivermectin is not effective against *G. lamblia* or *B. hominis*, and so the resolution of urticaria, a widely recognized symptom of chronic strongyloidiasis, in 84% of participants in the before-after study would appear to arise from clearance of *S. stercoralis*. Scabies is another important cause of itching/urticaria in developing countries that would be resolved by ivermectin treatment (Hay et al., 2012). However, the basic medical examination that was conducted during data collection included a skin check, which would have led to scabies diagnosis if present.

Our combined results demonstrate that the burden of strongyloidiasis, which encompasses all health states from mild symptoms to severe, life-threatening infection, might be much higher in endemic settings than previously thought. Chronic strongyloidiasis appears to cause both acute gastrointestinal symptoms and urticaria, all bothersome symptoms that are experienced whatever the age of the individual and intensity of infection. It also causes subtle long-term health effects through its association with malnutrition.

Next steps towards estimating the *S. stercoralis* burden include assessing the extent of strongyloidiasis morbidity, including growth retardation and malnutrition, with regard to infection intensity — for which standards have yet to be established, and estimating the risk of severe strongyloidiasis and hyperinfection in endemic settings. *S. stercoralis* is not currently addressed by the WHO control strategy against STH that relies on “preventive chemotherapy”, i.e. regular mebendazole or albendazole treatment of specific at-risk groups or mass-drug administration (MDA) (WHO and Crompton, 2006, WHO, 2010). Single dose benzimidazoles have suboptimal effects on *S. stercoralis,* for which ivermectin is the drug of choice. This drug is highly efficacious at a single oral dose of 200 µg/kg body weight (BW) and is well tolerated. Moreover, ivermectin is also efficacious against *Ascaris lumbricoides*. In combination with benzimidazoles, it improves therapeutic outcomes against *Trichuris trichiura,* while in combination with albendazole, it improves therapeutic performances against hookworm (Gann et al., 1994, Igual-Adell et al., 2004, Keiser and Utzinger, 2010, Knopp et al., 2010a, Suputtamongkol et al., 2011).

In the absence of infection intensity figures, the similarity of reinfection rates and morbidity across age groups support arguments for community-wide controls (Forrer et al., 2016). However, the long-term impact of malnutrition on childhood development could justify integrating *S. stercoralis* control into ongoing school-based STH control programmes, which are well established throughout the country and currently target children from infancy to high school. Monitoring the impact of controls on infection levels in various transmission settings would help to assess whether, where and how control measures should be extended, while optimizing cost-effectiveness. The cost of ivermectin poses a challenge to expanding its use. In Cambodia, the drug is neither donated or subsidized and treating one individual with quality tablets produced by a certified manufacturing company costs 20 to 40 USD, depending on the patient’s weight. The high prevalence of the parasite and its significant morbidity clearly advocate for increased donations or the production of generic ivermectin so *S. stercoralis* control can be implemented without further delay in Cambodia.

5.5 Acknowledgements

We are grateful to all of the study participants. Our sincere thanks go to the laboratory technicians and staff at the Helminth Control Program of the National Centre for Parasitology, Entomology and Malaria Control in Phnom Penh and from the Preah Vihear Provincial Health Department for their excellent laboratory and field work. We thank Dr Saomony Chhay and the local authorities of Preah Vihear for their support. We are very grateful to Mr Hae PROM, Chief of Rovieng Health Centre, for his support. We thank Mrs. Amena Briet for her efficient English editing.

5.6 Appendix

5.6.1 Prevalences and number of cases of all diagnosed helminths and protozoa

Helminths	n	Prevalence (%)	95% CI
Strongyloides stercoralis	853	31.09	29.36-32.86
Hookworm	699	25.47	23.85-27.15
Fasciola/Fasciolopsis	85	3.10	2.48-3.82
Opisthorchis viverrini	43	1.57	1.14-2.11
Taenia spp.	22	0.80	0.50-1.21
Dicrocoelium dendriticum	10	0.36	0.17-0.67
Enterobius vermicularis	8	0.29	0.13-0.57
Hymenolepis nana	6	0.22	0.08-0.48
Trichuris trichiura	2	0.07	0.01-0.26
Clonorchis sinensis	1	0.04	<0.01-0.20
Protozoa	n	Prevalence (%)	95% CI
Entoamoeba coli [a]	632	23.03	21.47-24.65
Blastocystis hominis [a]	504	18.37	16.93-19.87
Giardia lamblia [b]	198	7.22	6.28-8.25
Iodamoeba bütschlii [a]	135	4.92	4.14-5.80
Endolimax nana [a]	112	4.08	3.37-4.89
Entamoeba histolytica/dispar [b]	32	1.17	0.80-1.64
Entamoeba hartmanni [a]	12	0.44	0.23-0.76
Chilomastix mesnili [a]	2	0.07	0.01-0.26
Sarcocystis spp. [b]	1	0.04	0.0004-0.20

Data were obtained from a cross-sectional survey carried out 2012 in eight villages of Preah Vihear province, Cambodia, among 2,744 participants meeting the study case definition for all diagnosed parasites.

[a] non-pathogenic; [b] pathogenic; n: number of cases; CI: confidence interval.

5.6.2 Association between symptoms and *S. stercoralis* parasite load

Symptom	*S. stercoralis* infection intensity	Symptom present n (%)	OR	95% CI	LRT p-value
Loss of appetite / Anorexia	No infection	598 (31.6)	1.00		
	Light	149 (34.3)	1.08	0.85 - 1.38	0.381
	Moderate or Heavy	87 (30.3)	0.85	0.63 - 1.14	
Nausea	No infection	611 (32.3)	1.00		
	Light	147 (33.9)	1.02	0.81 - 1.28	0.595
	Moderate or Heavy	89 (31.0)	0.88	0.66 - 1.15	
Vomiting	No infection	380 (20.1)	1.00		
	Light	94 (21.7)	1.09	0.84 - 1.42	0.805
	Moderate or Heavy	60 (20.9)	1.04	0.76 - 1.43	
Abdominal pain	No infection	1,479 (78.2)	1.00		
	Light	358 (82.5)	1.10	0.82 - 1.49	0.632
	Moderate or Heavy	242 (84.3)	1.16	0.80 - 1.67	
Epigastric pain	No infection	1,085 (57.4)	1.00		
	Light	264 (60.8)	0.94	0.74 - 1.21	0.867
	Moderate or Heavy	185 (64.5)	1.03	0.76 - 1.39	
Diarrhea	No infection	1,086 (57.4)	1.00		
	Light	260 (59.9)	1.08	0.86 - 1.34	0.647
	Moderate or Heavy	172 (59.9)	1.11	0.85 - 1.44	
Constipation	No infection	293 (15.5)	1.00		
	Light	60 (13.8)	0.84	0.62 - 1.15	0.531
	Moderate or Heavy	44 (15.3)	0.91	0.64 - 1.31	
Cough	No infection	1,208 (63.9)	1.00		
	Light	277 (63.8)	1.05	0.84 - 1.31	0.563
	Moderate or Heavy	170 (59.2)	0.89	0.69 - 1.16	
Wheezing	No infection	147 (7.8)	1.00		
	Light	38 (8.8)	1.07	0.73 - 1.57	0.879
	Moderate or Heavy	23 (8.0)	0.93	0.58 - 1.49	
Itching	No infection	902 (47.7)	1.00		
	Light	220 (50.7)	1.11	0.90 - 1.38	0.149
	Moderate or Heavy	155 (54.0)	1.27	0.98 - 1.64	
Urticaria	No infection	880 (46.5)	1.00		
	Light	240 (55.3)	1.35	1.08 - 1.68	0.003
	Moderate or Heavy	163 (56.8)	1.41	1.09 - 1.83	
Generalized rash	No infection	345 (18.2)	1.00		
	Light	104 (24.0)	1.36	1.05 - 1.76	0.075
	Moderate or Heavy	58 (19.4)	1.06	0.77 - 1.46	
Fever	No infection	855 (45.2)	1.00		
	Light	192 (44.2)	1.06	0.85 - 1.32	0.499
	Moderate or Heavy	111 (38.7)	0.88	0.68 - 1.15	
Tiredness	No infection	421 (22.3)	1.00		
	Light	109 (25.1)	1.13	0.86 - 1.48	0.644
	Moderate or Heavy	67 (23.3)	0.98	0.71 - 1.35	
Muscle pain	No infection	688 (36.4)	1.00		
	Light	195 (44.9)	1.21	0.96 - 1.53	0.268

Symptom	*S. stercoralis* infection intensity	Symptom present n (%)	OR	95% CI	LRT p-value
	Moderate or Heavy	125 (43.6)	1.06	0.81 - 1.40	

OR: Odds ratio; CI: confidence interval; LRT: likelihood ratio test.

(a) Odds ratios were adjusted for sex, age, treatment and infection with any diagnosed helminth or pathogenic protozoa.

S. stercoralis light intensity: positive count and ≤ 1 larvae per gram (LPG). *S. stercoralis* moderate or heavy intensity: > 1 LPG.

Treatment corresponds to uptake of anthelmintic tablets within the past year.

Data were obtained from a cross-sectional survey carried out 2012 in eight villages of Preah Vihear province, Cambodia, among 2,612 participants with *S. stercoralis* infection intensity data.

5.6.3 Complete results of multivariate logistic regressions assessing the association between each reported symptom and *S. stercoralis*

Nausea

Variable	Category	OR	95%CI	p-value
S. stercoralis infection	No	1.00		
	Yes	1.02	0.85 - 1.22	0.855
Hookworm infection	No	1.00		
	Yes	1.08	0.90 - 1.31	0.409
Other helminth infection	No	1.00		
	Yes	1.05	0.76 - 1.46	0.756
Pathogenic protozoa infection	No	1.00		
	Yes	0.90	0.67 - 1.22	0.512
Sex	Male	1.00		
	Female	0.97	0.82 - 1.14	0.693
Age (years)	6 - 18	1.00		
	< 6	0.94	0.67 - 1.33	0.728
	19 - 59	1.73	1.44 - 2.07	<0.0001
	≥ 60	1.05	0.73 - 1.51	0.795
Anthelmintic treatment, past year	No	1.00		
	Yes	1.32	1.11 - 1.58	0.002

Vomiting

Variable	Category	OR	95%CI	p-value
S. stercoralis infection	No	1.00		
	Yes	1.05	0.85 - 1.29	0.67
Hookworm infection	No	1.00		
	Yes	1.10	0.89 - 1.37	0.384
Other helminth infection	No	1.00		
	Yes	1.00	0.68 - 1.47	0.992
Pathogenic protozoa infection	No	1.00		
	Yes	1.01	0.72 - 1.41	0.962

Sex	Male	1.00		
	Female	0.90	0.74 - 1.10	0.301
Age (years)	6 - 18	1.00		
	< 6	1.00	0.69 - 1.45	0.988
	19 - 59	1.10	0.89 - 1.35	0.379
	≥ 60	0.47	0.28 - 0.79	0.004
Anthelmintic treatment, past year	No	1.00		
	Yes	1.33	1.08 - 1.63	0.006

Abdominal pain

Variable	Category	OR	95%CI	p-value
S. stercoralis infection	No	1.00		
	Yes	1.11	0.88 - 1.41	0.364
Hookworm infection	No	1.00		
	Yes	0.95	0.74 - 1.21	0.669
Other helminth infection	No	1.00		
	Yes	1.42	0.92 - 2.21	0.116
Pathogenic protozoa infection	No	1.00		
	Yes	0.97	0.69 - 1.35	0.844
Sex	Male	1.00		
	Female	0.92	0.75 - 1.13	0.44
Age (years)	6 - 18	1.00		
	< 6	0.28	0.20 - 0.38	< 0.001
	19 - 59	5.32	4.17 - 6.79	< 0.001
	≥ 60	3.41	2.15 - 5.42	< 0.001
Anthelmintic treatment, past year	No	1.00		
	Yes	1.70	1.37 - 2.11	< 0.001

Epigastric pain

Variable	Category	OR	95%CI	p-value
S. stercoralis infection	No	1.00		
	Yes	0.98	0.81 - 1.20	0.85
Hookworm infection	No	1.00		
	Yes	0.97	0.79 - 1.20	0.8
Other helminth infection	No	1.00		
	Yes	1.30	0.91 - 1.85	0.154
Pathogenic protozoa infection	No	1.00		
	Yes	0.93	0.68 - 1.27	0.629
Sex	Male	1.00		
	Female	1.08	0.90 - 1.30	0.401
Age (years)	6 - 18	1.00		
	< 6	0.18	0.11 - 0.29	< 0.001
	19 - 59	7.11	5.85 - 8.63	< 0.001
	≥ 60	7.09	4.81 - 10.44	< 0.001
Anthelmintic treatment, past year	No	1.00		
	Yes	1.63	1.34 - 1.97	< 0.001

Diarrhea

Variable	Category	OR	95%CI	p-value
S. stercoralis infection	No	1.00		
	Yes	1.10	0.93 - 1.31	0.276
Hookworm infection	No	1.00		
	Yes	0.96	0.80 - 1.15	0.673
Other helminth infection	No	1.00		
	Yes	0.96	0.70 - 1.32	0.816
Pathogenic protozoa infection	No	1.00		
	Yes	1.11	0.83 - 1.47	0.479
Sex	Male	1.00		
	Female	0.69	0.59 - 0.82	< 0.001
Age (years)	6 - 18	1.00		
	< 6	1.09	0.79 - 1.49	0.613
	19 - 59	1.07	0.90 - 1.27	0.468
	≥ 60	0.69	0.50 - 0.96	0.027
Anthelmintic treatment, past year	No	1.00		
	Yes	1.82	1.54 - 2.15	< 0.0001

Constipation

Variable	Category	OR	95%CI	p-value
S. stercoralis infection	No	1.00		
	Yes	0.91	0.71 - 1.15	0.422
Hookworm infection	No	1.00		
	Yes	0.99	0.77 - 1.27	0.93
Other helminth infection	No	1.00		
	Yes	1.33	0.89 - 2.00	0.168
Pathogenic protozoa infection	No	1.00		
	Yes	1.03	0.67 - 1.57	0.899
Sex	Male	1.00		
	Female	1.37	1.09 - 1.72	0.007
Age (years)	6 - 18	1.00		
	< 6	0.68	0.37 - 1.28	0.233
	19 - 59	2.48	1.90 - 3.24	<0.0001
	≥ 60	4.36	2.93 - 6.50	<0.0001
Anthelmintic treatment, past year	No	1.00		
	Yes	0.83	0.66 - 1.04	0.102

Cough

Variable	Category	OR	95%CI	p-value
S. stercoralis infection	No	1.00		
	Yes	1.00	0.84 - 1.19	0.983
Hookworm infection	No	1.00		
	Yes	0.87	0.72 - 1.05	0.138
Other helminth infection	No	1.00		
	Yes	0.85	0.62 - 1.17	0.327
Pathogenic protozoa infection	No	1.00		
	Yes	0.73	0.55 - 0.96	0.024
Sex	Male	1.00		
	Female	0.99	0.84 - 1.17	0.931
Age (years)	6 - 18	1.00		
	< 6	1.06	0.77 - 1.48	0.708
	19 - 59	0.75	0.62 - 0.89	0.001
	≥ 60	0.85	0.60 - 1.19	0.345

Anthelmintic treatment, past year	No	1.00		
	Yes	1.00	0.84 - 1.18	0.974

Wheezing

Variable	Category	OR	95%CI	p-value
S. stercoralis infection	No	1.00		
	Yes	1.03	0.76 - 1.41	0.848
Hookworm infection	No	1.00		
	Yes	0.99	0.71 - 1.37	0.937
Other helminth infection	No	1.00		
	Yes	1.05	0.59 - 1.86	0.869
Pathogenic protozoa infection	No	1.00		
	Yes	0.61	0.30 - 1.21	0.156
Sex	Male	1.00		
	Female	1.28	0.95 - 1.73	0.111
Age (years)	6 - 18	1.00		
	< 6	0.77	0.32 - 1.86	0.566
	19 - 59	2.78	1.91 - 4.04	<0.0001
	≥ 60	4.17	2.46 - 7.09	<0.0001
Anthelmintic treatment, past year	No	1.00		
	Yes	0.92	0.68 - 1.23	0.559

Itching

Variable	Category	OR	95%CI	p-value
S. stercoralis infection	No	1.00		
	Yes	1.19	1.00 - 1.41	0.046
Hookworm infection	No	1.00		
	Yes	0.84	0.70 - 1.01	0.061
Other helminth infection	No	1.00		
	Yes	1.30	0.95 - 1.77	0.096
Pathogenic protozoa infection	No	1.00		
	Yes	0.89	0.68 - 1.18	0.416
Sex	Male	1.00		
	Female	0.91	0.78 - 1.06	0.234
Age (years)	6 - 18	1.00		
	< 6	0.75	0.55 - 1.03	0.075
	19 - 59	1.35	1.14 - 1.60	0.001
	≥ 60	1.62	1.17 - 2.26	0.004
Anthelmintic treatment, past year	No	1.00		
	Yes	1.22	1.03 - 1.43	0.018

Urticaria

Variable	Category	OR	95%CI	p-value
S. stercoralis infection	No	1.00		
	Yes	1.35	1.13 - 1.60	0.001
Hookworm infection	No	1.00		
	Yes	0.93	0.78 - 1.12	0.439
Other helminth infection	No	1.00		
	Yes	1.10	0.80 - 1.50	0.565
Pathogenic protozoa infection	No	1.00		
	Yes	0.94	0.71 - 1.25	0.681
Sex	Male	1.00		
	Female	0.93	0.79 - 1.09	0.38
Age (years)	6 - 18	1.00		

	< 6	0.57	0.41 - 0.80	0.001
	19 - 59	1.95	1.64 - 2.31	<0.0001
	≥ 60	1.64	1.18 - 2.28	0.003
Anthelmintic treatment, past year	No	1.00		
	Yes	1.50	1.27 - 1.77	<0.0001

Generalized rash

Variable	Category	OR	95%CI	p-value
S. stercoralis infection	No	1.00		
	Yes	1.20	0.97 - 1.48	0.089
Hookworm infection	No	1.00		
	Yes	0.74	0.59 - 0.94	0.014
Other helminth infection	No	1.00		
	Yes	1.12	0.76 - 1.65	0.562
Pathogenic protozoa infection	No	1.00		
	Yes	1.13	0.79 - 1.62	0.506
Sex	Male	1.00		
	Female	0.90	0.74 - 1.10	0.313
Age (years)	6 - 18	1.00		
	< 6	0.99	0.63 - 1.54	0.953
	19 - 59	2.28	1.81 - 2.87	<0.0001
	≥ 60	2.10	1.40 - 3.16	<0.0001
Anthelmintic treatment, past year	No	1.00		
	Yes	1.29	1.05 - 1.59	0.018

Fever

Variable	Category	OR	95%CI	p-value
S. stercoralis infection	No	1.00		
	Yes	0.98	0.83 - 1.17	0.851
Hookworm infection	No	1.00		
	Yes	0.94	0.78 - 1.13	0.507
Other helminth infection	No	1.00		
	Yes	0.91	0.66 - 1.24	0.536
Pathogenic protozoa infection	No	1.00		
	Yes	0.88	0.66 - 1.16	0.365
Sex	Male	1.00		
	Female	1.07	0.91 - 1.25	0.436
Age (years)	6 - 18	1.00		
	< 6	1.31	0.97 - 1.79	0.082
	19 - 59	0.76	0.64 - 0.90	0.002
	≥ 60	0.67	0.48 - 0.94	0.02
Anthelmintic treatment, past year	No	1.00		
	Yes	1.69	1.43 - 1.99	<0.0001

Tiredness

Variable	Category	OR	95%CI	p-value
S. stercoralis infection	No	1.00		
	Yes	1.04	0.84 - 1.28	0.75
Hookworm infection	No	1.00		
	Yes	0.79	0.63 - 0.99	0.041
Other helminth infection	No	1.00		
	Yes	1.54	1.06 - 2.24	0.023
Pathogenic protozoa infection	No	1.00		
	Yes	0.62	0.40 - 0.98	0.039

Sex	Male	1.00		
	Female	1.41	1.15 - 1.74	0.001
Age (years)	6 - 18	1.00		
	< 6	0.54	0.26 - 1.09	0.085
	19 - 59	4.71	3.63 - 6.12	<0.0001
	≥ 60	19.22	13.00 - 28.41	<0.0001
Anthelmintic treatment, past year	No	1.00		
	Yes	0.84	0.69 - 1.04	0.105

Muscle pain

Variable	Category	OR	95%CI	p-value
S. stercoralis infection	No	1.00		
	Yes	1.17	0.97 - 1.40	0.099
Hookworm infection	No	1.00		
	Yes	1.03	0.85 - 1.25	0.772
Other helminth infection	No	1.00		
	Yes	1.09	0.78 - 1.53	0.627
Pathogenic protozoa infection	No	1.00		
	Yes	1.05	0.76 - 1.45	0.76
Sex	Male	1.00		
	Female	0.79	0.66 - 0.94	0.009
Age (years)	6 - 18	1.00		
	< 6	0.22	0.12 - 0.41	<0.0001
	19 - 59	4.72	3.89 - 5.73	<0.0001
	≥ 60	4.86	3.45 - 6.84	<0.0001
Anthelmintic treatment, past year	No	1.00		
	Yes	1.56	1.30 - 1.86	<0.0001

5.6.4 Symptoms reported before and after ivermectin treatment by *S. stercoralis* infected patients including co-infection with other parasites (208 patients)

Symptom		**OR**	**95% CI**	**LRT p-value**
Loss of appetite / anorexia	before treatment	1.00		
	after treatment	**0.61**	0.40 - 0.95	0.027
	any other infection	1.69	0.70 - 4.07	
Abdominal pain	before treatment	1.00		
	after treatment	**0.07**	0.04 - 0.15	<0.0001
	any other infection	0.91	0.25 - 3.25	
Nausea	before treatment	1.00		
	after treatment	**0.27**	0.15 - 0.48	<0.0001
	any other infection	1.56	0.52 - 4.64	
Vomiting	before treatment	1.00		
	after treatment	**0.08**	0.03 - 0.27	<0.0001
	any other infection	2.66	0.23 - 30.71	

Symptom		OR	95% CI	LRT p-value
Diarrhea	before treatment	1.00		
	after treatment	**0.42**	0.27 - 0.65	<0.0001
	any other infection	0.77	0.35 - 1.70	
Constipation	before treatment	1.00		
	after treatment	0.80	0.39 - 1.63	0.536
	any other infection	2.15	0.54 - 8.54	
Itching	before treatment	1.00		
	after treatment	1.04	0.70 - 1.55	0.841
	any other infection	1.55	0.72 - 3.30	
Urticaria	before treatment	1.00		<0.0001
	after treatment	**0.03**	0.01 - 0.08	
	any other infection	1.22	0.16 - 9.56	
Cough	before treatment	1.00		
	after treatment	**0.23**	0.14 - 0.38	<0.0001
	any other infection	1.26	0.51 - 3.11	
Wheezing	before treatment	1.00		
	after treatment	1.48	0.78 - 2.80	0.227
	any other infection	1.51	0.50 - 4.53	
Fever	before treatment	1.00		
	after treatment	1.16	0.76 - 1.78	0.486
	any other infection	1.57	0.70 - 3.49	
Tiredness	before treatment	1.00		
	after treatment	**0.49**	0.30 - 0.81	0.004
	any other infection	0.84	0.31 - 2.27	
Muscle pain	before treatment	1.00		
	after treatment	1.07	0.70 - 1.62	0.755
	any other infection	1.62	0.73 - 3.58	

Treatment: ivermectin 200 µg/kg BW. OR in bold were significant at 95% level. LRT p-values were obtained from conditional logistic regressions.

Data were collected in 2012 in 2 villages of Preah Vihear Province, North Cambodia, from 208 *S. stercoralis* patients regardless of infection with any other diagnosed helminth or pathogenic protozoa and met the cases definitions used in this work for all parasites. The total of 208 patients is constituted of 103 patients free of any infection other than *S. stercoralis* and 105 patients co-infected by any other parasite at any survey.

OR: odds ratio; CI: confidence interval; LRT: likelihood ratio test

Chapter 6

Cost-effectiveness analysis for potential control strategies for *Strongyloides stercoralis* in Cambodia

Armelle Forrer[1,2*], C. Simone Sutherland[3*], Virak Khieu[4], Fabian Schär[1,2], Hanspeter Marti[1,2], Sinuon Muth[4], Peter Odermatt[1,2]

1 Swiss Tropical and Public Health Institute, Basel, Switzerland; **2** Universität Basel, Basel, Switzerland; **3** Institute of Pharmaceutical Medicine, Universität Basel, Basel, Switzerland; **4** National Centre for Parasitology, Entomology and Malaria Control, Ministry of Health, Phnom Penh, Cambodia.

*Those authors contributed equally to this work.

This paper has been prepared for PLoS Neglected Tropical Diseases

Abstract

Background

Strongyloides stercoralis is a neglected soil-transmitted helminth that can replicate within its host, leading to long-lasting and potentially fatal infections. Chronic infections are associated with significant gastrointestinal and dermatological morbidity. *S. stercoralis* thrives in areas with warm climate and poor sanitation. Almost a third (30.5%) of the Cambodian population is infected. Control programmes delivering ivermectin are needed to address infection but there is currently no defined control strategy for this parasite.

Methods/Principal findings

Based on data from an intervention study consisting in the targeted (i.e. following diagnosis) treatment with a single oral dose of ivermectin (200 µg/kg BW) of *S. stercoralis* cases in endemic villages of North Cambodia, the cost-effectiveness of three alternative control interventions, i.e. targeted treatment, *vs.* mass drug administration targeting either children and women or entire communities was estimated. Financial costs were estimated from the study, from the USAID NTD cost and funding gap analysis and on interviews of experts from the National Centre for Parasitology, Entomology and Malaria Control, responsible for control activities in Cambodia. The analysis was conducted from the viewpoint of the Ministry of Health.

All interventions were cost effective compared to Cambodia Gross Domestic Product. MDA interventions were the most cost-effective a few exceptions aside, at $108 and $107 per case cured, for the interventions targeting children and women or entire communities, respectively. The targeted treatment alternative was more cost-effective when prevalence was below 20%. The cost to treat one person was $29 with any MDA option.

Conclusions/Significance

All interventions for *S. stercoralis* control are cost-effective but the costs of any option are high due to the current price of $10 per ivermectin tablet and cannot be supported by the Cambodian Ministry of Health. Subsidizations or donations are needed to start control in Cambodia where a third of the population is infected.

6.1 Introduction

The threadworm *Strongyloides stercoralis* is an intestinal nematode whose larvae, living in faecally-polluted soil, enter the human body transcutaneously in a manner similar to hookworms. This soil-transmitted helminth (STH), although not listed as “major” with its peers, causes strongyloidiasis, a highly neglected tropical disease (Krolewiecki et al., 2013, Bisoffi et al., 2013, Olsen et al., 2009, Schär et al., 2013). Occurring worldwide, *S. stercoralis* thrives in tropical and sub-tropical regions with humid and warm climate where poor sanitation conditions prevail (Olsen et al., 2009, Bisoffi et al., 2013, Krolewiecki et al., 2013, Schär et al., 2013). However, due to use of standard field diagnostic techniques that fail to detect it, the parasite has been underreported and overlooked for decades. While since the late eighties, reported figures claim 30-100 million cases worldwide, current estimates lie closer to 220-370 million cases globally (Genta, 1989, Bisoffi et al., 2013). Epidemiological data on *S. stercoralis* is scarce but recent data suggest rates between 10% and 40% in the tropics and subtropics (Schär et al., 2013).

Using a combined Baermann technique and Koga Agar plate (KAP) method on two stool samples, *S. stercoralis* has been found to be highly prevalent in Cambodia, with rates up to 45% in the northern regions (Khieu et al., 2014b, Khieu et al., 2013a, Khieu et al., 2014c). A national survey recently conducted in Cambodia in all 25 provinces of the country found a prevalence rate as high as 30.5% (REF). In endemic settings of Cambodia, chronic strongyloidiasis has also been found to cause bothersome gastrointestinal and dermatological symptoms, including abdominal pain, diarrhea, urticaria, and, a highly specific symptom of strongyloidiasis, larva currens (Grove, 1996, Becker et al., 2011, Khieu et al., 2013b, Toledo et al., 2015, Nutman, 2016, Concha et al., 2005, Forrer et al., 2017). An important distinctive characteristic of *S. stercoralis* is its capacity to permanently re-infect its host without leaving it. This “auto-infection” ability causes long-term infections that can last for decades, but can also potentially lead to hyperinfection due to uncontrolled multiplication of the parasite, a condition inevitably fatal if untreated (Keiser and Nutman, 2004, Marcos et al., 2008, Fardet et al., 2007, Nutman, 2016).

Due to its high prevalence and the significant morbidity associated with chronic infections recently identified in Cambodia, the Ministry of Health has recognized the parasite as a major public health problem that needs to be addressed by a control programme.

The backbone of STH control strategy as defined by the WHO is “preventive chemotherapy” (PC), i.e. mass drug administration (MDA) to risk groups with mebendazole or albendazole in areas with prevalence of 20% and above. For the three major STH, those groups have been defined as preschool-, school-aged children and women of child bearing age (WHO and Crompton, 2006, WHO, 2010). However, single doses of benzimidazoles have suboptimal efficacy against *S. stercoralis*, against which the drug of choice Ivermectin. A single oral dose of 200 µg/kg body weight (BW) has been found to be highly efficacious against chronic uncomplicated infections (Igual-Adell et al., 2004, Gann et al., 1994, Henriquez-Camacho et al., 2016).

In addition, due to the overall underestimate of *S. stercoralis* prevalence and absence of burden estimates, there is no defined control strategy for this parasite since it has not yet been acknowledged as a public health problem by the public health community.

Regarding potential modalities for *S. stercoralis* control in Cambodia, recent studies conducted in the country’s endemic settings estimated that all age groups were at similar risk of post-treatment re-infection, and that they experienced similar symptoms, although children might have an additional risk of long-term impacts due to the potential association between *S. stercoralis* infection and stunting (Forrer et al., 2016, Forrer et al., 2017). In absence of more detailed burden figures, a community-wide approach is recommended; while the potential additional burden undergone by children, might justify integrating *S. stercoralis* control into ongoing control measures targeting children and women of child-bearing age alone.

A major issue in the control of *S. stercoralis* is the high cost of ivermectin since this drug is not subsidized or donated like other deworming drugs in Cambodia. The price of a tablet produced by a certified good manufacturing practice company is $10 so the cost to treat an individual may range from $10 to $40 (e.g. number of tablets needed per treatment depends on weight). . In light of the public health problem that *S. stercoralis* poses in Cambodia and the limited resources available to health authorities, it is crucial to assess the cost-effectiveness of various control options to identify the optimal approach.

The aim of this study was to assess the cost-effectiveness of three alternative PC control options with a single oral dose of ivermectin 200 µg/kg body weight (BW) to the current standard of care. The three alternatives are as follows: (i) targeted control using combined Baermann technique and the KAP on two samples, (ii) MDA to preschool-, school-aged children and women of child bearing age, i.e. risk groups currently targeted for STH control, and (iii) community-based MDA targeting the entire population. This is, to our knowledge, the first attempt to assess the cost-effectiveness of *S. stercoralis* control options.

6.2 Methods

6.2.1 Study setting & population

A hypothetical population of 10,000 patients was modelled based on the data from a study in Preah Vihear Province, Northern Cambodia. The study was a community-based cross-sectional survey conducted between February and June 2012 in 8 villages of the Rovieng district, where *S. stercoralis* was highly endemic at a prevalence rate of 44.7%

(Range: 10.5% - 83.4%) (Khieu et al., 2014b). Additional details related to the study protocol and design have been previously published(Forrer et al., 2016).

6.2.2 Study perspective and outcomes

The analysis considers a the perspective of the National Centre for Parasitology, Entomology and Malaria Control (CNM, Ministry of Health) which is in charge of deworming programmes, including associated direct costs from the public sector of financing relating to helminth control, and effectiveness calculated as the number of individuals treated or cases cured. Cost-effectiveness was assessed by calculating the incremental cost-effectiveness ratio (ICER) for a treated or cured *S. stercoralis* case of each intervention compared to the next best alternative. We used the country gross domestic product (GDP), which was $1,089 in 2017 as cost-effectiveness threshold (WHO, 2003, Worldbank, 2015).

6.2.3 Modelling

A decision tree model in Microsoft Excel 2016® (Redmond, WA) was developed to take the current paradigm in combination with several different interventional approaches for *Strongyloides* from a health care provider perspective. Decision trees for control approaches assessed in this study are presented in Figure 6.1.

The time horizon of the decision tree is one year, hence long-term discounting of the total costs and effects was not necessary.

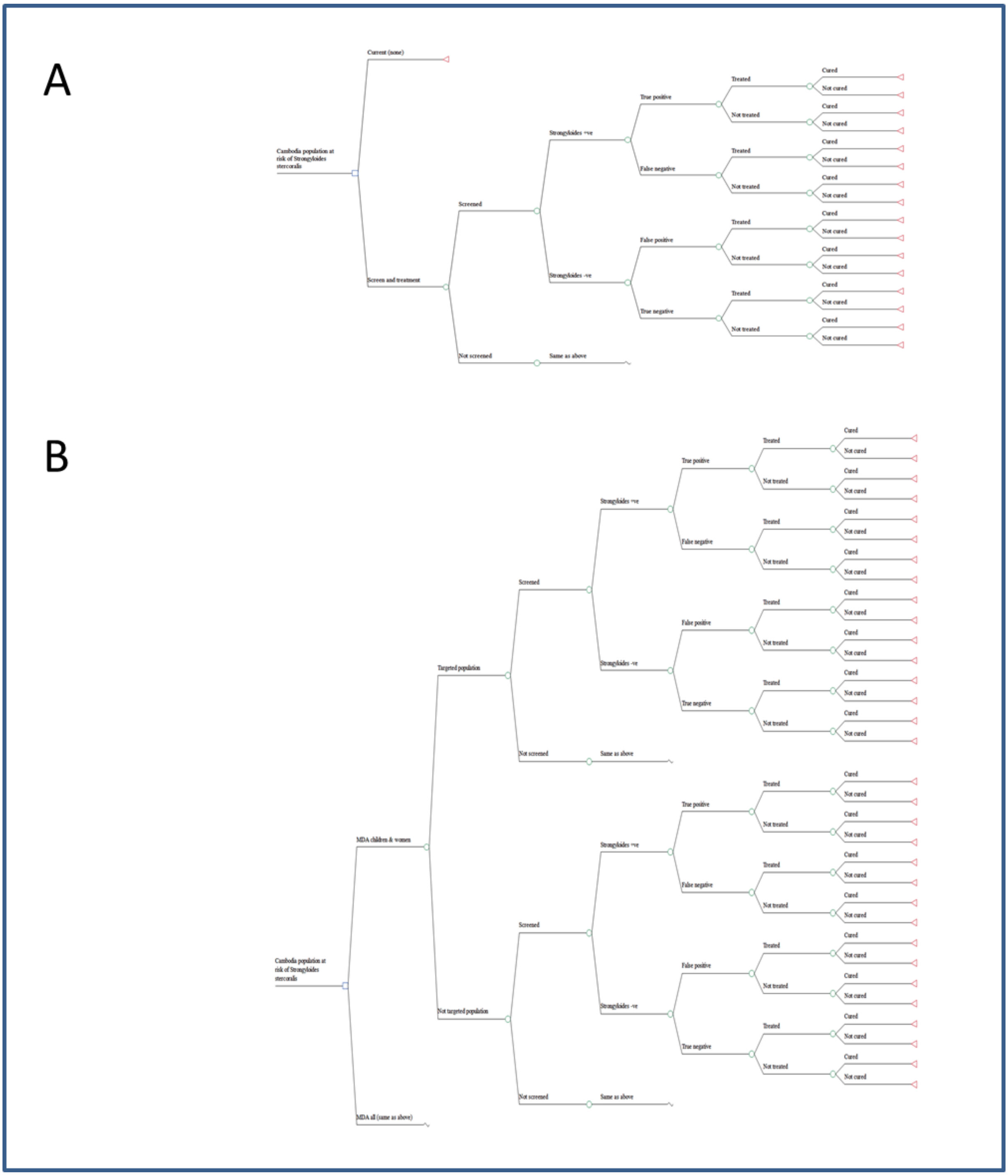


Figure 6.1: Decision tree for control approaches of *S. stercoralis* in Cambodia

Decision tree for targeted control (A) and MDA control (B).

6.2.4 Interventions

Although primary health care is provided by health centres covering approximately villages within a 40 km radius, *S. stercoralis* is not addressed by the Cambodian health system because health centres and helminth control programmes deliver mebendazole, of which long treatment schedules, which are not used, are needed to reach a cure rate below 70% (Keiser and Utzinger, 2010). Therefore, the current standard of care was assumed to correspond to "do nothing", and is referred to as the "Current" intervention hereafter in this analysis.

The first intervention is the one implemented in the study (hereafter named *"Screen & Treat")* which is a targeted-treatment intervention (Forrer et al., 2016). It consisted of an annual delivery of a single dose of ivermectin (200 µg/kg of body weight) to villagers diagnosed with *S. stercoralis* infection. The diagnosis was performed using combined Baermann method and Koga agar plate (KAP) culture on two stool samples, an approach that has 92% diagnostic sensitivity (Khieu et al., 2013a) .

The second and third interventions were mass drug administration (MDA) of the same treatment, i.e. a single dose of ivermectin (200 µg/kg BW), to all individuals belonging to the target groups. The second intervention, hereafter named *"MDA children & women"* targets all children, and women of child-bearing age. Those groups are already targeted for STH infection. Preschool-aged children (and their mothers) are reached in communities through primary health care interventions such as immunization campaigns and Vitamin A distribution. School-based deworming reaches school-aged children in primary, secondary and high schools. Women of child bearing age are reached either with preschool-aged children or factories (National Center for Parasitology, 2014).

The third intervention targets the entire community and is referred to thereafter as *"MDA all"*. This intervention adds, to the "*MDA children and women"* intervention, by additionally targeting population groups not reached through the *“MDA children & women”* intervention, and would be community-based.

6.2.5 Clinical

Diagnostic sensitivity and cure rate were retrieved from the literature (Khieu et al., 2013a, Forrer et al., 2016). Specificity was estimated based on negative predictive value based on literature data (Khieu et al., 2013a). Prevalence was estimated from the study data.

For the *“screen & treat”* intervention, the screen and treat population coverage was the proportion of individuals that would be reached by the intervention. This coverage was set to be the same as the “*MDA all*” coverage. The “*screen & treat”* screening coverage was the proportion of screened individuals who provided samples, i.e. the proportion of diagnosed participants. The treatment coverage was estimated from the study and corresponded to the proportion of *S. stercoralis* positives who were treated (some individuals do not show up for diagnosis results and treatment).

For the “*MDA children & women*” approach, the population coverage corresponded to the proportion of the total population represented by the target groups. Treatment coverage rates were provided by CNM data for the ongoing STH control programmes targeting pre-SAC, SAC and WCBA. We estimated the number of treated based on the coverage achieved by the STH programme in each risk group, diagnostic sensitivity, and the proportions of each risk group in the sample.

For the “*MDA all*” population coverage, we used the same rates for the risk groups described above and we used the coverage rate achieved by the first year of the lymphatic filariasis control programme for the remaining population (men, women aged 50 years and above).

6.2.6 Costs

We estimated financial costs for each alternative, which aimed at appraising how much the CNM would have to pay to implement each intervention. Costs included programme fixed and variable programme start-up and recurrent costs. All prices were directly provided by the CNM in US dollars. Costs from 2015 of capital assets were discounted at a 3% rate, as recommended by the WHO (WHO, 2003). Capital assets and training refer to material bought and training investments that only occur once at the beginning of the study and which can be used over time, e.g. vehicles, laboratory machines, training of staff for *S. stercoralis* diagnosis.

Costs of the *"Screen & Treat"* intervention were based on activities and quantities used in the cross-sectional study relating to the intervention only (excluding research costs) with the exception of social mobilization costs that did not occur in the study. Capital training costs were based on CNM expert knowledge. Social mobilization costs estimated in the USAID NTD cost and Funding Gap Analysis for the STH MDA were used (Chu and Project, 2011).

"Screen and treat" diagnosis costs included laboratory building and material for diagnosis, vehicles, staff capital training. *"Screen & treat"* other start-up costs were social mobilization material creation and production. Capital costs (expect training) were annuitized over their useful lives using a 3% discount rate, corresponding to a yearly cost of owning and using a capital asset over its life span.

"Screen and treat" recurrent costs included screening material (consumables), staff per diems, and staff refreshment training, transport (insurances, gasoline, and maintenance) and overhead.

Diagnosis costs were estimated using a micro-costing approach with direct measurement: diagnosis processes, material quantities and labour time were directly observed from the study. Labour costs were estimated based on the study laboratory capacity which was limited to 75 samples per day due to the number of Baermann devices present in the lab and the time needed for this diagnosis approach. We excluded the workforce and time needed for research purposes. Staff member numbers and corresponding wages by position (i.e. per diem for nurses, doctors, drivers, cleaner, etc.) were multiplied with the number of workers needed per position type and the number of days needed for the screening to obtain a total labour cost. This cost was then divided by the number of study participants to obtain a labour cost per person. Variables costs were grouped according to the unit at which they occurred (individual (stool container, diagnostic consumables), staff (gloves, etc.), or study round (cleaning material, etc.)). All costs were broken down at the unit of cost per person per year.

For costs not occurring at individual level, including for capital costs, the total cost (unit cost*quantity needed) was divided by the number of screened or diagnosed persons in the observed study round. Social mobilization costs were available for an average province, so this cost was divided by the average province size to get a cost per person.

Costs were then classified into treatment costs, and start-up *vs.* ongoing or recurring costs for one year. For both the *“MDA children and women”* and *“MDA all”* interventions start-up costs included social mobilization material creation, production costs and capital training. Recurrent costs included treatment, drug importation, storage and distribution costs and overhead.

Distribution costs included the costs of storage (in Central Medical Stores, Phnom Penh) and transport of products used in the programme. However, only part of transport costs incur to the MoH, i.e. from central storage to districts and from districts authorities to health centres, as well as to particularly remote schools necessitating specific transport. In most cases, drug transport from health centres to schools is not borne by the MoH as school directors usually transport them from health centres to the villages without additional costs to the health care system.

Costs were also based on expert interview or were retrieved from the USAID NTD cost and Funding Gap Analysis (Chu and Project, 2011). Costs provided at national level, i.e. social mobilization, refreshment training, monitoring & evaluation costs, and overhead were divided by the number of provinces (25) and the average province size to get a per person cost. Based on expert opinion, the overhead share of the STH control programme was assumed to be a third of the overhead for the three CNM helminth control programmes (STH, Schistosomiasis, and LF). Costs provided at province level were divided by the average province size to obtain a cost per person.

For the three interventions, treatment cost was based on $10 for a 300 mg ivermectin tablet, price at which ivermectin can currently be purchased in Cambodia. Ivermectin is administrated according to the patient weight (200 µg/kg of body weight) so treatment costs were estimated based on Merck recommended posology, the sample age structure and the average weight per each control risk group (i.e. pre-SAC, SAC. WCBA, men, women aged 50 years and above). The average number of tablets per individual was calculated to be 2.855. Hence, the average treatment cost per person was $28.55. A full list of all the included model parameters is listed in Table 6.1.

Table 6.1: List of model input parameters

	Mean estimate	Lower Range	Upper Range	Source
Clinical events				
Disease prevalence	27.75%	10.5%	83.7%	Khieu et al 2013(Khieu et al., 2014b)
Coverage				
Screen and treat population coverage [(a)]	78.80%	71%	84%	Based on coverage of lymphatic filariasis community-based control, MoH, CNM report
Active, community, S&T screening coverage [(b)]	96.50%	60%	99%	Based on study data, Forrer et al (Forrer et al., 2016)
Active, community, S&T treatment coverage [(c)]	95.00%	80%	99%	Based on study data, Forrer et al (Forrer et al., 2016)
Active, MDA pre-SAC, SAC, WCBA, treatment coverage	79.36%	70%	90%	based on 2015 coverage preSAC, SAC, WCBA, CNM report
Active, MDA all treatment coverage	78.78%	69%	81%	based on 2015 coverage preSAC, SAC, WCBA CNM report and 70% for remaining groups = LF coverage 1st year
Diagnostics				
Baermann + KAP method - Sensitivity	91.20%	76%	95%	Khieu et al 2014 (Khieu et al., 2013a)
Baermann + KAP method - Specificity	99.00%	95%	100%	Assumption
Treatment				
Cure rate	96.00%	93.1%	98.6%	Forrer et al, 2016 (Forrer et al., 2016)
Costs (per person/per annum)				
Screening				
Screen & Treat, diagnosis start-up	$ 3.75	2.8116713	4.686119	

Table 6.1 (cont.)

	Mean estimate	Lower Range	Upper Range	Source
Screen & Treat, diagnosis ongoing	$ 17.03	12.771811	21.28635	
Screen & Treat, other start-up costs	$ 0.02	0.0141359	0.02356	
MDA community-based, start-up	$ 0.04	0.026868	0.04478	
MDA community-based, ongoing	$ 0.02	0.0129719	0.02162	
MDA pre-SAC, SAC, WCBA, start-up	$ 0.05	0.0401429	0.066905	
MDA pre-SAC, SAC, WCBA, ongoing	$ 0.03	0.0220956	0.036826	
Treatment cost per person				
"Screen & Treat", ivermectin	$28.55	$1.50	$ 60.00	
MDA community-based, ivermectin	$28.55	$1.50	$ 60.00	
MDA pre-SAC, SAC, WCBA, ivermectin	$28.55	$1.50	$ 60.00	

(a) The “*screen and treat*” population coverage was the proportion of individuals that would be reached by the intervention and was set to be the same as the “*MDA all*” coverage.

(b) The screening coverage was the proportion of screened individuals who provided samples, i.e. the proportion of diagnosed participants.

(c) The treatment coverage was estimated from the study and corresponded to the proportion of *S. stercoralis* positives who were treated.

6.2.7 Uncertainty

A one-way sensitivity analysis was conducted on prevalence, coverage, cure rates and cost parameters in the model. In addition, scenarios related to the donation of materials, costs of ivermectin combined with a lower cost diagnostic and the impact of an integrated control programme with STH were also evaluated.

6.3 Results

A summary of the cost-effectiveness results for the base case analysis are provided in Table 6.2. The current scenario incurred zero costs, but also resulted in no cases cured. Administering a MDA campaign targeted at children and women specifically *("MDA children & women")* led to an additional $147,745, but also cured an additional 1,374 cases and 5158 treated relative to the current approach. Implementing a "*Screen and Treat*" control programme resulted in a total cost of $202, 121, cured 1755 cases and treated 1888 individuals. While a MDA approach that includes the entire community (*"MDA (all)"*) incurred a total cost of $225,387, cured 2099 cases, and treated a total of 7878 individuals. When assessed for dominance (in order of increasing benefits), an MDA programme targeted specifically at women and children *("MDA children & women")* yielded an incremental cost-effectiveness ratio (ICER) of $108 per case cured and $29 per treated. "*Screen and Treat*" led to additional costs and patients cured, but fewer individuals treated, relative to *"MDA children & women",* however; resulted in a lower ICER in comparison to an *MDA all* intervention (extendedly dominated). Hence, in the end, *MDA all* relative to an *MDA children & women* led to additional costs, cases and treated individuals with ICERs of $108 per cured and $29 per treated. These results are further depicted in the incremental cost-effectiveness plane presented in Figure 3.2 as the ICER of the "*Screen and Treat*" approach lies above the efficiency frontier, while the MDA interventions rest upon the efficiency frontier.

Table 6.2: Cost-effectiveness results of control strategies for *S. stercoralis*

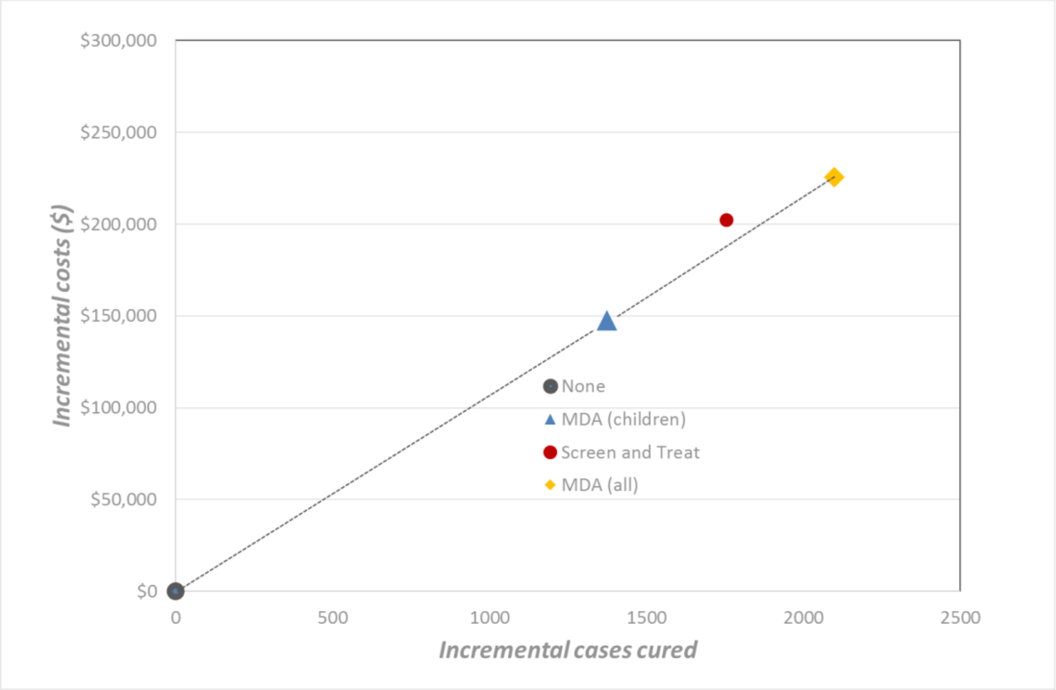
Intervention	**Total costs (USD)**	**Total cured**	**Total treated**	**ICER cured**	**ICER treated**
Current (none)	$0	0	0	-	-
MDA (children & women)	$147,745	1374	5158	$108*	$29*
Screen and Treat	$202,121	1755	1888	Extendedly dominated¥	Dominated+
MDA (all)	$225,387	2099	7878	$108+	$29+

*relative to Current, +relative to “MDA (children & women)”, ¥relative to “MDA (all)”,

Dominated = costs more, less effective

Extendedly dominated = ICER of next best comparator lower

Figure 6.2. Incremental cost-effectiveness plane for *S. stercoralis* ($ per case cured)



The results from the sensitivity analysis are presented in Table 6.3. They demonstrate the robustness of the findings, as MDA interventions dominate or extendedly dominate the "*Screen and Treat*" programme over the majority of variations of key input parameters in the model and scenario analyses conducted. However, when prevalence is equal to or less than 20%, (Figure 6.3) or if the cost of treatment per individual increases by 50% (6.3), the ICERs of "*Screen and Treat*" programme remain equivalent or lower than its MDA comparators. Figure 6.4 displays the change in ICER per individual treated across ivermectin cost ranging from zero to $10. Results from the sensitivity and scenario analysis also highlighted that the ICER of MDA programmes could be under $5 if the cost on an ivermectin tablet was less than $1.

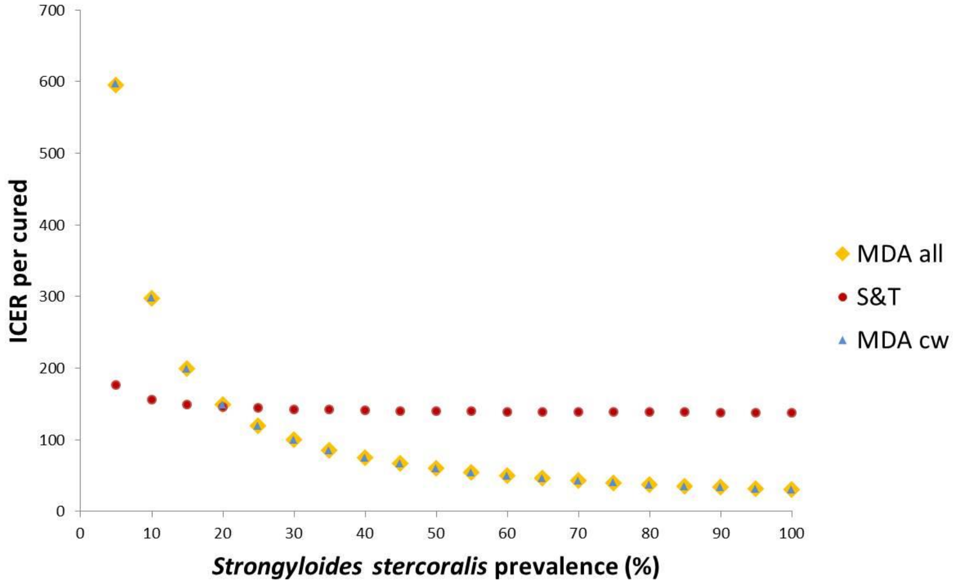


Figure 6.3: Changes in ICER related to *S. stercoralis* prevalence
The "*Screen and Treat*" intervention was dominated (higher costs, lower effects) when prevalence was equal to or above 45%.

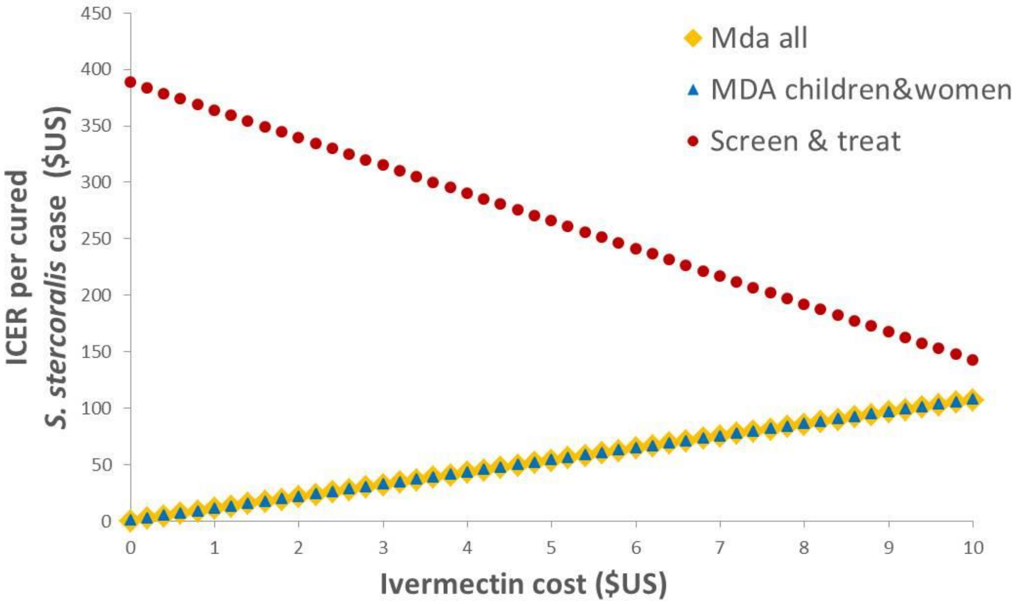


Figure 6.4: Changes in ICER related to ivermectin cost

The “*Screen and Treat*” intervention was dominated (higher costs, lower effects) when ivermectin cost ranged between zero and $8.6.

Table 6.3. One-way sensitivity analysis (OWSA) and scenario analyses

		ICER results ($ per case cured)	
Input parameter	**Low value, High value**	**Low value**	**High value**
1. Disease prevalence	11%, 84%	Current - *reference* Screen & Treat – $155 per case cured MDA (children&women) – ED MDA (all) – $283 per case cured	Current - *reference* Screen & Treat – ED MDA (children&women) – $36 per case cured MDA (all) – $36 per case cured
2. Community, S&T screening coverage	60%, 90%	Current - *reference* Screen & Treat – ED MDA (children&women) – $108 per case cured MDA (all) –$107 per case cured	Current - *reference* Screen & Treat – ED MDA (children&women) – $108 per case cured MDA (all) –$107 per case cured
3. Community, S&T treatment coverage	80%, 99%	Current - *reference* Screen & Treat – ED MDA (children&women) – $108 per case cured MDA (all) – $107 per case cured	Current - *reference* Screen & Treat – ED MDA (children&women) – $108 per case cured MDA (all) – $107 per case cured
4. Community, S&T population coverage	71%,84%	Current - *reference* Screen & Treat – ED MDA (children&women) – $108 per case cured MDA (all) – $107 per case cured	Current - *reference* Screen & Treat – ED MDA (children&women) – $108 per case cured MDA (all) – $107 per case cured
5. Community, MDA children & women treatment coverage	70%, 90%	Current - *reference* Screen & Treat – ED MDA (children&women) — $108 per case cured MDA (all) – $107 per case cured	Current - *reference* Screen & Treat – ED MDA (children&women) – $107 per case cured MDA (all) – $107 per case cured
6. Community, MDA all treatment coverage	69%, 81%	Current - *reference* Screen & Treat – ED MDA (children&women) – $108 per case cured MDA (all) – $107 per case cured	Current - *reference* Screen & Treat – ED MDA (children&women) – $108 per case cured MDA (all) – $107 per case cured
7. Cure rate	93%, 99%	Current - *reference* Screen & Treat – ED MDA (children&women) – $111 per case cured MDA (all) – $110 per case cured	Current - *reference* Screen & Treat – ED MDA (children&women) – $105 per case cured MDA (all) – $104 per case cured

Table 6.3 (cont.)

		ICER results ($ per case cured)	
Input parameter	**Low value, High value**	**Low value**	**High value**
8. Diagnostic sensitivity	76%, 95%	Current - *reference* MDA (children&women) – $108 per case cured Screen & Treat – ED MDA (all) – $107 per case cured	Current - *reference* MDA (children&women) – $108 per case cured Screen & Treat – ED MDA (all) – $107 per case cured
9. Diagnostic specificity	95%, 100%	Current - *reference* MDA (children&women) – $108 per case cured Screen & Treat – ED MDA (all) – $107 per case cured	Current - *reference* MDA (children&women) - $108 per case cured Screen & Treat – ED MDA (all) – $107 per case cured
10. Community, S&T diagnosis start-up costs	2.8; 4.7	Current - *reference* Screen & Treat – ED MDA (children&women) – $108 per case cured MDA (all) – $107 per case cured	Current - *reference* Screen & Treat – ED MDA (children&women) – $108 per case cured MDA (all) – $107 per case cured
11. Community, S&T diagnosis ongoing costs	12.8, 21.3	Current - *reference* Screen & Treat – $83 per case cured MDA (children&women) – $108 per case cured MDA (all) – $107 per case cured	Current - *reference* Screen & Treat –D MDA (children&women) – $108 per case cured MDA (all) – $107 per case cured
12. Community, S&T diagnosis other start-up costs	0.01, 0.02	Current - *reference* Screen & Treat – ED MDA (children&women) – $108 per case cured MDA (all) – $107 per case cured	Current - *reference* Screen & Treat – ED MDA (children&women) – $108 per case cured MDA (all) – $107 per case cured
13. Community, MDA all, start-up costs	0.03, 0.04	Current – *reference* Screen & Treat – ED MDA (children&women) – $108 per case cured MDA (all) – $107 per case cured	Current - *reference* Screen & Treat – ED MDA (children&women) – $108 per case cured MDA (all) – $107 per case cured
14. Community, MDA all, ongoing costs	0.01,0.02	Current - *reference* Screen & Treat – ED MDA (children&women) – $108 per case cured MDA (all) – $107 per case cured	Current - *reference* Screen & Treat – ED MDA (children&women) – $108 per case cured MDA (all) – $107 per case cured

Table 6.3 (cont.)

		ICER results ($ per case cured)	
Input parameter	**Low value, High value**	**Low value**	**High value**
15. Community, MDA children & women, start-up costs	0.04, 0.07	Current - *reference* Screen & Treat – ED MDA (children&women) – $107 per case cured MDA (all) – $107 per case cured	Current - *reference* Screen & Treat – ED MDA (children&women) – $108per case cured MDA (all) – $107 per case cured
16. Community, MDA children & women, ongoing costs	0.02, 0.04	Current - *reference* Screen & Treat – ED MDA (children&women) – $108 per case cured MDA (all) – $107 per case cured	Current - *reference* Screen & Treat – ED MDA (children&women) – $108per case cured MDA (all) – $107 per case cured
17. Ivermectin cost	1.50, 60.0	Current - *reference* Screen & Treat – dominated MDA (children&women) – $6 per case cured MDA (all) – $6 per case cured	Current - *reference* Screen & Treat – $149 per case cured MDA (children&women) – dominated MDA (all) – $617per case cured

Scenario analyses	
Donation scenario: capital diagnosis & vehicles only	Current - *reference* Screen & Treat – ED MDA (children) – $108 per case cured MDA (all) – $107 per case cured
Donation scenario: all capital costs except capital training	Current - *reference* Screen & Treat – ED MDA (children & woman) – $107 per case cured MDA (all) – $107 per case cured
IVM & RDT costs = $1.5 each	Current - *reference* Screen & Treat – D MDA (children) – $6 per case cured MDA (all) – $6 per case cured
MDA all scenario (Only add on programme costs necessary for integration with STH MDA programme)	Current - *reference* Screen & Treat – ED MDA (children) – $106 per case cured MDA (all) – $106 per case cured
Diagnosis (KAP+Baermann) on 1 sample	Current - *reference* Screen & Treat – $ 127 per cured case MDA (children) – D MDA (all) – $146 per case cured

D: dominated = costs more, less effective

ED: Extendedly dominated = ICER of next best comparator lower

6.4 Discussion

Overall, the current analysis demonstrates that when evaluating an annual programme for control of *S. stercoralis*, both targeted and MDA interventions are cost-effective with ICERs well below the GDP/GNI of Cambodia that was reported as $1,089 in 2017 (Worldbank, 2015). MDA strategies were, a few exceptions aside, consistently the most cost-effective options at $108 and $107 per case cured for the interventions targeting entire communities (“MDA all”) *vs.* children and women (“MDA children & women”), respectively. These results were consistent over a range of prevalences and costs for the programmes evaluated.

However the targeted treatment (*“Screen & treat”)* approach was more cost-effective than both MDA options if prevalence was below 20%. Yet, although no prevalence thresholds have been delineated for *S. stercoralis* control, the threshold to deliver STH PC is 20% so control would probably not be implemented in such areas until thresholds specific to S. stercoralis are delineated and might suggest otherwise..

The “*Screen and Treat*” intervention was the most cost-effective if ongoing costs were reduced by 20% in the one-way sensitivity analysis or in the scenario analysis with a less intensive diagnostic approach based on one stool samples for which the cost to diagnose one individual was $13 instead of $20 when diagnosing using two samples. Yet, the deployment of targeted treatment nationwide with Baermann and KAP methods for diagnosis might be hindered by the lack of trained laboratory technicians, as currently, *S. stercoralis* diagnosis relies solely on the laboratory personnel from the central CNM office in Phnom Penh. This finding further underlines that the absence of a cheap and readily available diagnostic approach for the detection of *S. stercoralis* in endemic settings is a major bottleneck in the parasite investigation and management (Albonico et al., 2016).

Rather than on diagnosis, the entire feasibility of *S. stercoralis* control relies on the cost of ivermectin, an aspect that was consistent throughout all our analysis. Looking at the two MDA interventions, the average cost per treatment (ACER), i.e. comparing each intervention cost to “do nothing”, was $28.6. Almost all (99.8%) of that cost consisted in ivermectin cost, with an average treatment being estimated to cost $28.55. The cost to treat an additional person with the *“MDA all”* option compared to *the “MDA children and women option”* was $28.55 (ICER), which is the cost of ivermectin. This reflects the fact that programme costs would be slightly lower for community-wide control, mostly due to scale economies, as most programme implementation costs borne by the CNM, i.e. social mobilization, staff capital training, and treatment delivery costs were fixed. The marginal contribution of programme costs to the total cost was also reflected in the scenario analysis where donation of capital assets, or integration (i.e. including only costs necessary for the integration of *S. stercoralis* control into the existing programme), did not significantly impact the cost-effectiveness of any intervention as both scenarios involved programme costs changes only.

A cost of $28.6 per treated person prevents the implementation of *S. stercoralis* control, even if intervention was restricted to specific high risk areas. The highest imaginable ACER to treat one person that would be affordable by the government would be maximum $2, which corresponds to one tablet costing $ 0.7, if control was implemented with either MDA approach. Yet, this estimation is an educated guess and does not rely on hard data. A budget analysis should be conducted to assess at which ivermectin cost strongyloidiasis control would be affordable for Cambodia. Interestingly, this $2 limit is of the same order of magnitude than the cost per treatment of $1.5 suggest by Merck for the control on onchocerciasis before donation started (Kim et al., 2015b). However this cost is still high, particularly if compared to the respective median prices of \$0.019 and \$0.018, for mebendazole and albendazole, respectively and would still probably constitute a major obstacle to control implementation (Chu and Project, 2011, Kabatereine et al., 2005, Lo et al., 2016).Our estimates of programme delivery costs are in range with that of other STH control programmes and remain below the high end of 50 cents found in the literature (Lo et al., 2015, Kabatereine et al., 2005). For STH control in Southeast Asia, the cost per treatment, excluding drug cost ranged between \$0.012 in Vietnam and \$0.06 in Lao PDR (Montresor et al., 2010). In Cambodia, it was estimated in 2005 that the cost to deworm one child attending primary school was 6 cents, of which 2 cents were mebendazole cost (Sinuon et al., 2005). Looking at ICERs if ivermectin was donated, we estimated that programme costs would be 10 and 7 cents for the *"MDA children and women"* and *"MDA all"* strategies, respectively. The higher costs for the programme targeting children and women is probably due to the fact that our estimates account for the extension of deworming to secondary schools, high schools, and factories that occurred in 2013 (National Center for Parasitology, 2014, Sinuon et al., 2005).

Looking at six NTDs - lymphatic filariasis, onchocerciasis, schistosomiasis, STH, trachoma and yaws - addressed by preventive chemotherapy, the financial cost of treatment delivery is usually below \$0.50 where the population treated is at least of 100,000 (Fitzpatrick et al., 2016). Those costs are highly sensitive to the size of the targeted population, i.e. to economies of scale and to economies of scope, i.e. costs are lower in integrated than independent vertical programmes (Fitzpatrick et al., 2016, Turner et al., 2016b, Leslie et al., 2013, Evans et al., 2011, Turner et al., 2015b).

Looking at costs per treatment in other STH programmes a modelling study using data from Côte d'Ivoire and estimating the cost of integrated delivery of albendazole and praziquantel for a 15-year programme estimated the cost per treatment to be $0.74 and $1.74 for school-based MDA and community-based MDA, respectively (Lo et al., 2015). As a comparison, the costs of an ivermectin treatment for lymphatic filariasis a zithromax treatment for trachoma were estimated at $5 and $4.5, respectively, although those drugs were donated for control in Niger (Leslie et al., 2013).

Our study has several limitations. First, we used data from a study based on 8 villages with similar prevalence rates, around 30%, which are not readily generalizable to other settings with different prevalence and/or transmission intensity. An important limitation of our approach is that estimating the cost effectiveness of a 1-year intervention is very short-term for a communicable disease control programme and further investigations would be needed over a longer time horizon, e.g. 10 years, so the impact of transmission can be accounted for, but transmission intensity estimates are not yet available for *S. stercoralis*. While a lifetime evaluation would allow estimating all potential health gains, assessing the long-term burden of STH infections, which mostly relate to the potential impact of cognitive and physical impairment on fitness and productivity at adults age is particularly challenging (Campbell et al., 2016).

We expressed effectiveness in terms of natural units. i.e., 1 treated person or 1 cured case, as there are no QALYs or DALYs available for strongyloidiasis, which precludes any comparison with the cost-effectiveness of other STH and NTDs control programmes. Yet, the burden of STH infections is challenging to appraise and both QALYs and DALYs have been claimed to largely underestimate STH burden. First, the non-specificity of STH infection symptoms usually results in STH cases mostly being considered as asymptomatic, and second because DALYs only account for –underestimated- disabilities but not for STH-related mortality, i.e. years of life lost (YLLs), which would be particularly important for hookworm infections but not only (King and Bertino, 2008, Brooker, 2010, Hotez et al., 2014).

Additionally, externalities such as increased school attendance are also not accounted for by such measures. Of note, the burden of *S. stercoralis* is at high risk of being largely underestimated as not only chronic infection with this parasite causes bothersome gastrointestinal and dermatological symptoms and is associated with stunting in children, but the parasite is potentially fatal in case of immunosuppression (Keiser and Nutman, 2004, Forrer et al., 2017).Mostly due to therapy with corticosteroids, the parasite uncontrolled replication resulting in hyperinfection is fatal if untreated (Keiser and Nutman, 2004, Fardet et al., 2007). Hyperinfection cases might not be rare in low- and middle income countries where over-the counter drug cocktails containing corticosteroids are widely available. Yet this aspect of *S. stercoralis* morbidity in endemic countries is totally unknown and must be investigated to properly assess the parasite burden (Olsen et al., 2009, Marcos et al., 2008).

Upon *S. stercoralis* control eventual implementation, ivermectin distribution would be integrated into ongoing STH control measures already in place for STH control and would be distributed together with mebendazole to children and adolescents in schools (primary, secondary and high schools), and through community-based Vitamin A distribution and immunization delivery campaigns for preschool-aged children (pre-SAC) and their mothers, and to women of child-bearing age (WCBA) through community-based distribution as well as in factories for women (National Center for Parasitology, 2014).

The cost-effectiveness of integrating ivermectin into ongoing STH programmes is potentially more favourable than the current estimates provided in this analysis, which did not account for any treatment effect on other STH than *S. stercoralis.* First, costs would decrease following economies of scope due to integration (Leslie et al., 2013, Evans et al., 2011, Turner et al., 2015b). Second, under the current practice, ivermectin would be combined to mebendazole, a combination that would result in better treatment performances on *T. trichiura* than mebendazole alone. Third, the short-term assessment conducted in the present work does not account for the impact of treatment on transmission (Knopp et al., 2010a, Keiser and Utzinger, 2010).

An even more effective approach might be to replace mebendazole by albendazole, which, in combination with ivermectin achieves better treatment performances both on hookworms and *T. trichiura*. Moreover, this combination has been distributed for years to millions of people for the control of onchocerciasis and lymphatic filariasis and is safe (Knopp et al., 2010a, Keiser and Utzinger, 2010, WHO and Crompton, 2006, Reddy et al., 2007, Lammie et al., 2006, Hotez, 2009, WHO, 2013, WHO, 2016b). The suboptimal efficacy of mebendazole against hookworms in Southeast Asia and elsewhere has been raising concerns (Flohr et al., 2007, Vercruysse et al., 2011, Soukhathammavong et al., 2012, Forrer et al., 2018). While the main reasons why hookworms remain high in Cambodia might include programme coverage, high reinfection rates and drug efficacy, distributing ineffective drugs would diminish the expected benefits. The efficacy of both benzimidazoles, alone or in combination with ivermectin should be assessed in Cambodia, and those investigations should include drug efficacy on *Ancylostoma ceylanicum*. This hookworm of cats and dogs causes patent infections in humans and is highly prevalent in the in Southeast Asian population, including Cambodia (Inpankaew et al., 2014, Traub, 2013).

A major aspect of deworming is its impact on transmission which magnitude depends on the targeted risk groups. As opposed to *A. lumbricoides* and *T. trichiura* infections that are concentrated in children, infection levels of both hookworm and *S. stercoralis* are higher among adults, a large proportion of who are untreated by the current control strategy. Mathematical models of hookworm transmission have shown that community-wide treatment has a stronger impact on the hookworm reservoir in the environment and is more cost-effective than treating children only (Anderson et al., 2013, Truscott et al., 2015, Turner et al., 2015a). Furthermore, those results obtained through modelling were recently found to be in line with a review and meta-analysis based on field observations (Clarke et al., 2017). This meta-analysis assessing the impact of deworming children *vs*. entire communities actually found that expanding deworming community-wide resulted in greater impacts on prevalence of both hookworm and *A. lumbricoides* than targeting children only. Finally, let aside the question of the feasibility of achieving STH elimination, which is widely debated and currently unknown, deploying control community-wide would be the only way to eventually achieve it (Medley and Hollingsworth, 2015, Keenan et al., 2013, Anderson et al., 2015, Anderson et al., 2014, Truscott et al., 2014).

With specific regard to the setting of the present study, *S. stercoralis* infections were more prevalent in adults while post-treatment re-infection risk was similar across age groups argues for a community-wide treatment (Forrer et al., 2016). However the generalizability of those results is limited regarding transmission intensity and studies investigating *S. stercoralis* infection levels and reinfection rates across settings with various transmission intensities are needed. So are randomized control trials assessing the impact of targeting children and women *vs.* entire communities with mebendazole/albendazole combined with ivermectin on infection levels with STH and *S. stercoralis* (Clarke et al., 2017). Importantly, the community-level improved sanitation coverage, which impacts reinfection rates, should be assessed and controlled for (Nikolay et al., 2015, Forrer et al., 2016). Detailed local data would help developing adequate transmission dynamics models that could in turn be used to perform cost-effectiveness analysis of alternative strategies for an integrated programme deployed to control all four STH infections in the country.

The experience of integrated programmes such as STH and schistosomiasis combined treatment has shown that integration might exacerbate difficulties and in some instances might overstrain health systems (Kabatereine et al., 2010, Campbell et al., 2016). In the present analysis we made the assumption that the currently available health system resources would have sufficient resources to expand control to entire communities. However, sustaining community-wide control in the long run might be challenging due to system capacity and limited resources (Truscott et al., 2014, Kabatereine et al., 2010). The capacity -both in terms of finances and manpower, including village health volunteers - of the primary health care system to deliver an integrated community-wide control programme should be assessed locally, at the level of health operational districts.

As long as ivermectin will not be donated or extensively subsidized for *S. stercoralis* control, the cost per treated individual will remain far above the cost per treatment of any other helminth control programme. Most importantly, this high costs precludes the implementation of control in Cambodia, where the parasite is rampant (Khieu et al., 2014b, Khieu et al., 2013a, Khieu et al., 2014c). Drug donations will increase in the coming years along with the new momentum due to the 2012 London Declaration where NTDs control actors and multinational pharmaceutical companies pledged to provide the necessary resources to control or eradicate the major 10 NTDs (NTDs, 2012).

However, *S. stercoralis* is currently not recognized as a public health problem in most countries and no strategy for its control has been yet defined so it is not part of the London Declaration targets plan (NTDs, 2012). National level prevalence surveys as well as morbidity studies are needed to estimate the parasite regional and global burden. *S. stercoralis* is clearly a public health problem in Cambodia, where it causes significant morbidity and infects 30.5% of the population (Khieu et al., 2013b, Forrer et al., 2017). There is no question that *S. stercoralis* is needed in Cambodia but subsidization or donation of ivermectin is needed to allow starting control implementation.

6.5 Acknowledgements

We are grateful to all of the study participants. We sincerely thank the local authorities of Preah Vihear. We sincerely thank Ms Molyden Vann for her precious help in the field. We are grateful to the laboratory technicians and staff from the Helminth Control Programme of the National Centre for Parasitology, Entomology and Malaria Control in Phnom Penh and from the Preah Vihear Provincial Health Department for their excellent laboratory and field work.

Chapter 7

Strongyloides stercoralis: spatial distribution of a highly prevalent and ubiquitous soil-transmitted helminth in Cambodia

Armelle Forrer[1,2], Virak Khieu[3], Penelope Vounatsou[1,2], Paiboon Sithithaworn[4], Sirowan Ruantip[5], Rekol Huy[3], Sinuon Muth[3], Peter Odermatt[1,2*]

1 Swiss Tropical and Public Health Institute, Basel, Switzerland; **2** University of Basel, Basel, Switzerland; **3** National Centre for Parasitology, Entomology and Malaria Control, Ministry of Health, Phnom Penh, Cambodia; **4** Department of Parasitology and Cholangiocarcinoma Research Institute, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand **5** Graduated School of Biomedical Science, Khon Kaen University, Khon Kaen, Thailand;

This paper has been submitted to PLoS Neglected Tropical Diseases, Sept 2018.

Abstract

Background

Strongyloides stercoralis is a neglected soil-transmitted helminth that occurs worldwide and can cause long-lasting and potentially fatal infections due to its ability to replicate within its host. *S. stercoralis* causes gastrointestinal and dermatological morbidity. The objective of this study was to assess the *S. stercoralis* infection risk, and using geostatistical models, to predict its geographical distribution in Cambodia.

Methodology / Principal Findings

A nation-wide community-based parasitological survey was conducted among the population aged 6 years and above. *S. stercoralis* was diagnosed using a serological diagnostic test detecting antigens in urine. Data on demography, hygiene and knowledge about helminth infection were collected. *S. stercoralis* prevalence among 7,246 participants with complete data record was 30.5% and ranged across provinces between 10.9% and 48.2%. The parasite was ubiquitous in Cambodia, with prevalence rates below 20% only in five south-eastern provinces. Infection risk increased with age both in men and women although girls aged less than 13 years and women aged 50 years and above had lower odds of infection than their male counterparts. Open defecation was associated with higher odds of infection while declaring having some knowledge about health problems caused by worms was protective. Infection risk was positively associated with night maximum temperature, minimum rainfall, and distance to water, and negatively associated with land occupied by rice fields.

Conclusions / Significance

S. stercoralis infection is ubiquitous and rampant in Cambodia. The parasite needs to be addressed by control programmes delivering ivermectin. However the high cost of this drug in Cambodia currently precludes control implementation. Donations, subsidization or the production of affordable generic production are needed so *S. stercoralis*, which infests almost a third of the Cambodian population, can be addressed by an adequate control programme.

Authors Summary

The threadworm, *Strongyloides stercoralis*, is a most neglected worm infection transmitted through infective larvae on the soil. Threadworms occur worldwide and particularly in tropical climates. It may cause long-lasting and potentially fatal infections due to its ability to replicate within its host. This study aimed to assess the risk of threadworm infection in at the national level in Cambodia.

We conducted a nation-wide community-based parasitological survey among the population aged 6 years and above. The threadworm was diagnosed using a serological diagnostic test detecting antigens in urine. Data on demography, hygiene and knowledge about helminth infection were collected. The threadworm infection risk was calculated by using geostatistical models to predict its geographical distribution in Cambodia. About one third (30.5%) of the enrolled study participants (n=7,246) were infected with threadworms. The lowest and hightest infection rates a province level was 10.9% and 48.2%, respectively. Prevalence rates below 20% were found only in five south-eastern provinces. The risk of an infection with threadworms increased with age in men and women. Open defecation was associated with higher risk of infection while declaring having some knowledge about health problems caused by worms was protective. Furthermore, the threadworm infection risk was positively associated with environmental factors such as night maximum temperature, minimum rainfall, and distance to water, and negatively associated with land occupied by rice fields.

Threadworm infection is highly prevalent in Cambodia and adequate control measures are warranted, including access to treatment, in order to address the burden of this NTD in Cambodia.

7.1 Introduction

Strongyloides stercoralis is a highly neglected intestinal nematode which larvae living in fecally polluted soil infect humans transcutaneously, like hookworms. *S. stercoralis* occurs worldwide but thrives in warm regions with poor sanitation conditions (Schär et al., 2013). *S. stercoralis* has been under-detected and overlooked for decades because its larvae are not detected by standard field diagnostic techniques (Schär et al., 2013, Olsen et al., 2009, Montes et al., 2010, Bisoffi et al., 2013, Krolewiecki et al., 2013). Up to recently, the only available estimates originated from a review conducted in the late 80s estimating that there would be 30-100 million cases worldwide (Genta, 1989). More recent estimates include prevalence rates between 10% and 40% in subtropical and tropical countries, while, based on the ratio of hookworm *vs*. *S. stercoralis* cases in studies using diagnosis approaches adequate for the latter, *S. stercoralis* prevalence could be half of hookworm’s, i.e. 200-370 cases worldwide (Schär et al., 2013, de Silva et al., 2003, Pullan et al., 2014).

In Cambodia, *S. stercoralis* was found to be highly prevalent in two community-based large-scale surveys documenting prevalence rates of 25% and 45% in the southern province of Takeo and the northern province of Preah Vihear, respectively (Khieu et al., 2014b, Khieu et al., 2014c). *S. stercoralis* infection is more prevalent among adults due to its unique ability among STH to replicate within the host, which leads to infections that can last for decades in absence of treatment (Grove, 1996). Importantly, in case of immunosuppression, this auto-infection cycle accelerates and results in hyperinfection, a condition that is 100% fatal if untreated (Keiser and Nutman, 2004, Nutman, 2016, Marcos et al., 2008). Additionally, chronic infection with *S. stercoralis* may cause abdominal pain, nausea, vomiting, diarrhea, as well as urticaria and larva currens (Becker et al., 2011, Khieu et al., 2013b, Forrer et al., 2017). The latter is a serpiginous intermittent moving eruption due to the parasite migration under the skin. Its location on the buttocks, thighs and trunk, together with the high speed of migration, i.e. 5 to 10 centimeters an hour, makes it a highly specific symptom of strongyloidiasis (Nutman, 2016, Grove, 1996). Finally, and although this aspect of infection needs to be confirmed, *S. stercoralis* infection might be associated with growth retardation in children (Forrer et al., 2017). Due to this combination of significant morbidity and high prevalence, *S. stercoralis* has been recognized as a public health problem in Cambodia. However, the national prevalence and the location of high risk zones are unknown.

A highly sensitive diagnostic approach consists in combining the Baermann and Koga agar plate culture techniques but this method is costly, time and labor consuming and requires laboratory staff specifically trained to identify *S. stercoralis* larvae by microscopy. Serological diagnosis is more sensitive than most coprological approaches but its use may be limited in endemic settings due to cross-reaction with other helminths species (Requena-Méndez et al., 2013, Siddiqui and Berk, 2001). Another issue is that serology may overestimate prevalence in endemic areas as it detects parasite-specific antibodies or antigens that can still be present long after contact with the parasite or cure, and cannot distinguish current from past infections (Requena-Méndez et al., 2013). While this last aspect would be an issue for cure assessment, it would not affect prevalence estimates in a population naïve to treatment against the investigated parasite. A serological test using an antigen from *S. ratti* to detect antibodies in urine was recently developed in Thailand (Eamudomkarn et al., 2015, Eamudomkarn et al., 2018). This technique has several strengths. While collecting urine samples is much easier than fecal samples, this test has a high sensitivity for *S. stercoralis* detection and does not cross-react with other soil-transmitted helminth (STH) species (Eamudomkarn et al., 2015, Eamudomkarn et al., 2018).

Geostatistical models have been increasingly used in the past decade to delineate risk zones for helminthic infections at small and large scale and help targeting control efforts in areas of highest need (Raso et al., 2005, Chammartin et al., 2014a, Chammartin et al., 2013b, Karagiannis-Voules et al., 2015b, Lai et al., 2013, Soares Magalhães et al., 2015, Brooker et al., 2004b, Clements et al., 2006b). Based on the association between environmental variables and infection levels at survey locations, such models can be used to predict infection levels through entire geographical zones.

The aim of this work was to estimate *S. stercoralis* prevalence in Cambodia and to predict *S. stercoralis* infection risk throughout the country to help guiding control efforts. A national parasitological survey was conducted in 2016 in all provinces of Cambodia to assess the infection with *S. stercoralis* based on a serological diagnosis using antigens of *S. ratti* (Eamudomkarn et al., 2015). Subsequently, geostatistical modeling was used to predict infection risk throughout the country.

7.2 Methods

7.2.1 Ethics statement

The study was approved by the National Ethics Committee for Health Research, Ministry of Health, Cambodia (NECHR, reference number 188, dated 02.05.2016). Prior to enrolment, all participants were explained the study goals and procedures. All participants aged 16 years and above provided written informed consent and parents or legal guardians provided consent for participants aged 6–15 years. All *S. stercoralis* cases were treated with a single oral dose of ivermectin (200µg/kg BW) and all other diagnosed parasitic infections were treated according to the national guidelines (CNM, 2004).

7.2.2 Study setting

Cambodia counted 15.6 million inhabitants in 2015, 79.3% of whom lived in rural areas. (WorldBank, 2016). The country has been undergoing fast economic development in the past decades, and with a Human Development Index rank of 143/188 in 2016 Cambodia belonged to the group of lower middle-income country in the World Bank classification (WorldBank, 2016, Unicef, 2016). Although poverty strongly decreased in the past years and the proportion if the population living in extreme poverty was down to 2.2% in 2016, about one person in 5 (21.6%) still lived with less than 3.1 US$/day in 2016 (WorldBank, 2016). Adult literacy and primary school net enrolment were 74% and 95%, respectively, in the years 2010-2014 and 32% of children aged below 59 months were stunted in 2015 (Unicef, 2016). Regarding water and sanitation in 2015, 42% and 69% of the rural population had access to improved sanitation facilities and improved water, respectively, while those figures were 88% and 100% for the urban population, respectively (Unicef, 2016).

7.2.3 Study population and design

A cross-sectional community-based survey was conducted among the general population in all 25 provinces of Cambodia between May and August 2016. In each province, 10 villages were randomly selected. Overall, eighteen villages originally selected were replaced because there remoteness compromised the quality of collected samples for parasitological data. In each village, households were selected using a systemic proportional sampling and all household members present on the survey day were enrolled until a maximum of 35 participants per village was reached. All household members aged 6 years and above were eligible. All *S. stercoralis* cases were treated with a single oral dose of ivermectin (200µg/kg BW) and all other diagnosed parasitic infections were treated according to the national guidelines (CNM, 2004).

7.2.4 Assessment of Strongyloides stercoralis infection

Participants were asked to provide a urine sample on which *S. stercoralis* was diagnosed using an enzyme-linked immunosorbent assay (ELISA) based on *S. ratti* antigens

(Eamudomkarn et al., 2015). After collection, urine specimens were preserved in NaN3 with the final concentration of 0.1% and kept at 4 °C at all until required for analysis. Samples were sent to the central laboratory of the National Centre for Parasitology, Entomology and Malaria Control (CNM) in Phnom Penh and from then sent to Khon Kaen University, Thailand, to proceed to the ELISA test. This method has shown to have no cross-reactivity with other STH, and has a high sensitivity, of 92.7%. *S. ratti* antigens may cross-react with filarial parasites, which are merely absent now from Cambodia, as well as with the liver fluke *Opisthorchis viverrini*, although very weakly (Eamudomkarn et al., 2018, Khieu et al., 2018).

7.2.5 Individual risk factor data

An individual questionnaire including demographics (age, sex, education attainment, main occupation), the number of household members, as well as access to sanitation (latrine availability at home, usual defecation place) and knowledge on worm infections (transmission route of and health problems caused by helminths) was administered to all study participants.

7.2.6 Environmental data

Environmental parameters were extracted from freely available remote sensing (RS) sources for the period September 2015-August 2016, which corresponds to 1 year back from the last month of the study. Day and night land surface temperature (LST), international geosphere biosphere programme (IGBP) type 1 land use/land cover (LULC) as well as normalized difference vegetation index (NDVI) and enhanced vegetation index (EVI) were extracted at 1 x 1 km resolution from Moderate Resolution Imaging Spectroradiometer (MODIS) Land Processes Distributed Active Archive Center (LP DAAC), U.S. Geological Survey (USGS) Earth Resources Observation and Science (EROS) Center (http://lpdaac.usgs.gov). Rainfall data was obtained from WorldClim (www.worldclim.org). Digital elevation data were retrieved from the NASA Shuttle Radar Topographic Mission

(SRTM) and CGIAR-CSI database, whereas distance to large water bodies was obtained from Health Mapper.

7.2.7 Data management

Laboratory and questionnaire data were double-entered and validated in EpiData version 3.1 (EpiData Association; Odense, Denmark). Environmental data processing, geo-referencing and maps were done in ArcGIS version 10.2.1 (ESRI; Redlands, CA, United States). LULC 18 classes were merged into four categories according to similarity and respective frequencies. Year and seasonal means, maxima and minima of monthly means of EVI, LST and RFE were calculated and standardized. Environmental data were linked to parasitological and questionnaire data according to geo-referenced location. Data management and non-Bayesian data analysis were done in STATA version 13.0 (StataCorp LP; College Station, United States of America). Bayesian geostatistical models were fitted using WinBUGS version 1.4.3 (Imperial College & Medical Research Council; London, UK). Age was grouped in five classes, as follows: (i) 6-12 years, (ii) 13-18 years, (iii) 19-30 years, (iv) 31-50 years, and (v) >50 years. Predictions at un-surveyed locations were performed in Fortran 95 (Compaq Visual Fortran Professional version 6.6.0, Compaq Computer Corporation; Houston, United States of America).

7.2.8 Statistical Analysis

Chi-square (χ^2) test was used to compare proportions. The association between infection risk and covariates was assessed, using mixed non spatial bivariate logistic regressions accounting for village clustering, i.e. with a non-spatial village-level random effect. Covariates exhibiting an association at a significance level of at least 15%, as determined by the likelihood ratio test (LRT), were included in the multivariate logistic regression models. In case of correlated variables, the variable resulting in the model with the smallest Akaike's information criterion (AIC) was selected. For the risk factor analysis, variables exhibiting high Wald p-values were removed one by one and kept outside the model if their removal resulted in a lower AIC. Summary measures of continuous environmental variables, i.e. LST day and night, rainfall and distance to water were standardized before inclusion in the multiple regression models. To explore the relationship between *S. stercoralis* infection risk and age, smoothed age-prevalence curves were produced with the "mkspline" command in STATA that regresses each outcome against a new age variable containing a restricted cubic spline of age.

For geostatistical models, a stationary isotropic process was assumed, with village-specific random effects following a normal distribution with mean zero and a variance-covariance matrix being an exponential function of the distance between pairs of locations. Vague prior distributions were chosen for all parameters. Further information on model specification is available in Appendix 7.6.1. Markov chain Monte Carlo (MCMC) simulation was used to estimate model parameters (Gelfand and Smith, 1990). Geostatistical models were run using the WinBUGS "spatial.unipred" function (Lunn et al., 2000). Convergence was assessed by examining the ergodic averages of selected parameters. For all models, a burn-in of 5,000 was followed by 30,000 iterations, after which convergence was reached. Results were withdrawn for the last 10,000 iterations of each chain, with a thinning of 10. Model fit was appraised with the Deviance Information Criterion (DIC). A lower DIC indicates a better model (Spiegelhalter et al., 2002).

Three types of Bayesian mixed logistic models were run. First, models without covariates using alternately a geostatistical or an exchangeable random effect were run to quantify the extent of village-level spatial correlation and unexplained variance of *S. stercoralis* prevalence. Second, a risk factor analysis model was used to assess individual-level demographic, sanitation, and knowledge risk factors, as well as environmental covariates associated with infection risk. Third, a model including only environmental covariates was aimed at predicting infection risk at non-surveyed locations.

7.2.9 Prediction of S. stercoralis at non-surveyed locations

For model validation, 199 (80%) randomly selected villages were used for fitting and the 50 (20%) remaining were used as test locations. A pair of models containing the same covariates but including alternately a non-spatial (exchangeable) or spatial (geostatistical) random effect was run. Model predictive ability was assessed by comparing the Mean Squared Error (MSE), which is obtained by squaring the average of absolute differences between predicted and observed prevalence rates at test locations.

Using the model with the best predictive ability, *S. stercoralis* infection risk was predicted at 68,410 pixels of 2x2 km resolution using Bayesian Kriging (Diggle et al., 1998).

7.3 Results

7.3.1 Study population

Among the 8,661 participants enrolled in the study, 1407 did not provide any urine and 338 were discarded because they did not provide stool sample –which was requested for other assessments not presented in this work-, 8 participants did not have any questionnaire data. Overall 7,246 participants living in 2,585 households and 249 villages were included in the analysis. The mean number of participants per village was 30.2 with an interquartile range of 6, and a minimum of 5. Except the villages Ou Tracheak Chet in Preah Sihanouk Province (5 participants) and Kampong Chrey in Preah Vihear province (9 participants), all villages had more than 10 participants and 93.6% of villages had 20 participants or more. Table 7.1 shows the characteristics of participants with complete parasitological and questionnaire data.

Table 7.1: Characteristics of the 7,246 participants included in the analysis

Variable	**Category**	**N (%)**
Sex	Male	3,081 (42.5)
	Female	4,165 (57.5)
Age (years)	6-12	1,747 (24.1)
	13-18	954 (13.2)
	19-30	1,142 (15.8)
	31-50	1,850 (25.5)
	>50	1,553 (21.4)
Usual defecation place	Toilet	4,961 (68.5)
	Forest	1,768 (24.4)
	River, rice field, other	517 (7.1)
Education attainment	Primary school	4,183 (57.7)
	No school	1,279 (17.7)
	Secondary	1,283 (17.7)
	High school and over	501 (6.9)
Main occupation	Farmer	3,879 (53.5)
	At school	2,488 (34.4)
	At home	343 (4.7)
	Other	536 (7.4)
Any knowledge about worms	No	3,103 (42.8)
	Yes	4,143 (57.2)
Knowledge about source of infection with worms	No	3,995 (55.1)
	Yes	3,251 (44.9)
Knowledge about health problems caused by worms	No	4,507 (62.2)
	Yes	2,739 (37.8)
Walking barefoot is a cause of worm infection	No	5,889 (81.3)
	Yes	1,357 (18.7)
Lack of hygiene is a cause of infection	No	5,100 (70.4)
	Yes	2,146 (29.6)
Open defecation is a cause of worm infection	No	5,993 (82.7)
	Yes	1,253 (17.3)
Not washing hands is a cause of worm infection	No	5,796 (80.0)
	Yes	1,450 (20.0)
Toilet at home	No	2,306 (31.8)
	Yes	4,940 (68.2)

Data were obtained from a cross-sectional survey conducted in 2016 among in 249 villages of the 25 provinces of Cambodia, among individuals aged 6 years and older.

Females (57.5%) were overrepresented in the sample compared to their proportion in the Cambodian population (51.5%) as assessed by the 2013 inter-census population survey (National Institute of Statistics, 2013b). The age distribution of the sample was very similar to that of the total Cambodian population: children and adolescents aged up to 14 years represented 29.95% and 29.4%, adolescents and adults aged 15 to 64 years adults represented 65.6% and 64.2%, and elderly adults aged 65 and above represented 5.8% and 5.0% of the sample and the Cambodian population, respectively.

The proportion of males and females were similar in participants excluded or included in the analyzed sample, while children and young adults aged between 6 and 30 years were less represented (53.0%) in the sample than among excluded participants 64.3%). Similarly, farmers were overrepresented (53.6% of the sample *vs.* 41.1% of excluded participants) and scholars underrepresented (34.3% of the sample *vs.* 51.6% of excluded participants) in the final sample. There was no difference between participants excluded from, or included in, the analyzed sample in terms of usual defecation place.

7.3.2 Strongyloides stercoralis prevalence

Overall, *S. stercoralis* prevalence was 30.7% (95% confidence interval (CI): 29.7 – 31.8), ranging at province level from 10.9% (95%CI: 7.4 – 14-4) in Prey Veng province to 48.2% (95%CI: 42.2 – 54.1) in Koh Kong province. Figure 7.1 shows the provinces of Cambodia and Figure 7.2 displays province level prevalence rates. Prevalence was highly variable at village level. The smallest prevalence rate, of 2.9% (95% CI: 0.1 – 14.9), was found in a village of Kandal province, where only 1 of 35 participants was infected. The highest rates were 88.9% (95%CI: 51.8 – 99.7) and 80% (95%CI: 63.1 – 91.6), observed in one village in Preah Vihear province and another village in Koh Kong province, respectively. However there were only 9 participants in the Preah Vihear province village across provinces. The map presented in Figure 7.3 displays observed *S. stercoralis* prevalence in each surveyed village.

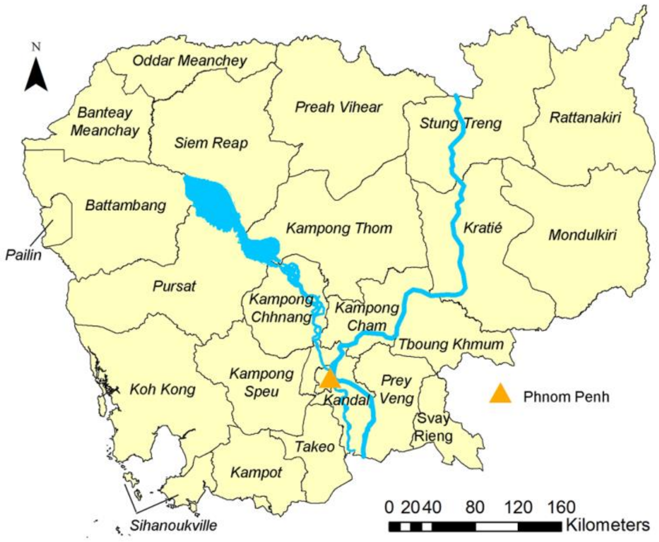


Figure 7.1: Map of Cambodian provinces.

This map was created with ArcGIS version 10.0 (ESRI; Redlands, CA, USA) specifically for this study by Forrer et al.

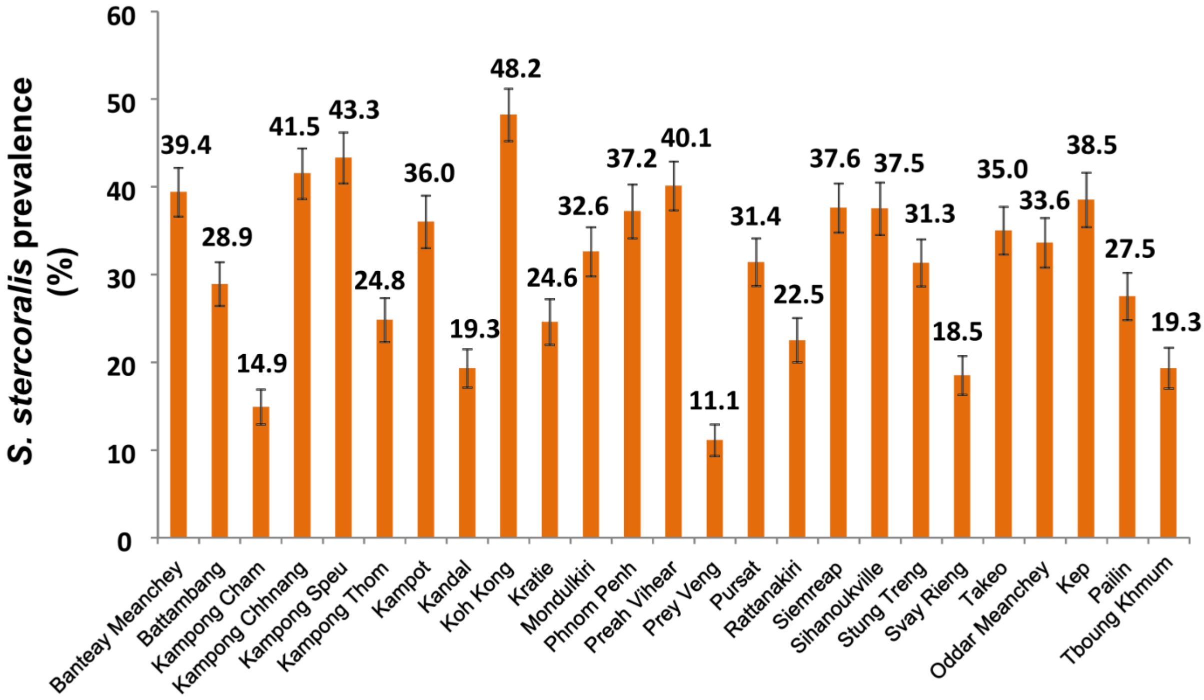


Figure 7.2: Province-level *S. stercoralis* prevalence in 25 provinces of Cambodia

Data were obtained from a cross-sectional survey conducted in 2016 in 249 villages of Cambodia, among 7,246 participants aged 6 years and above.

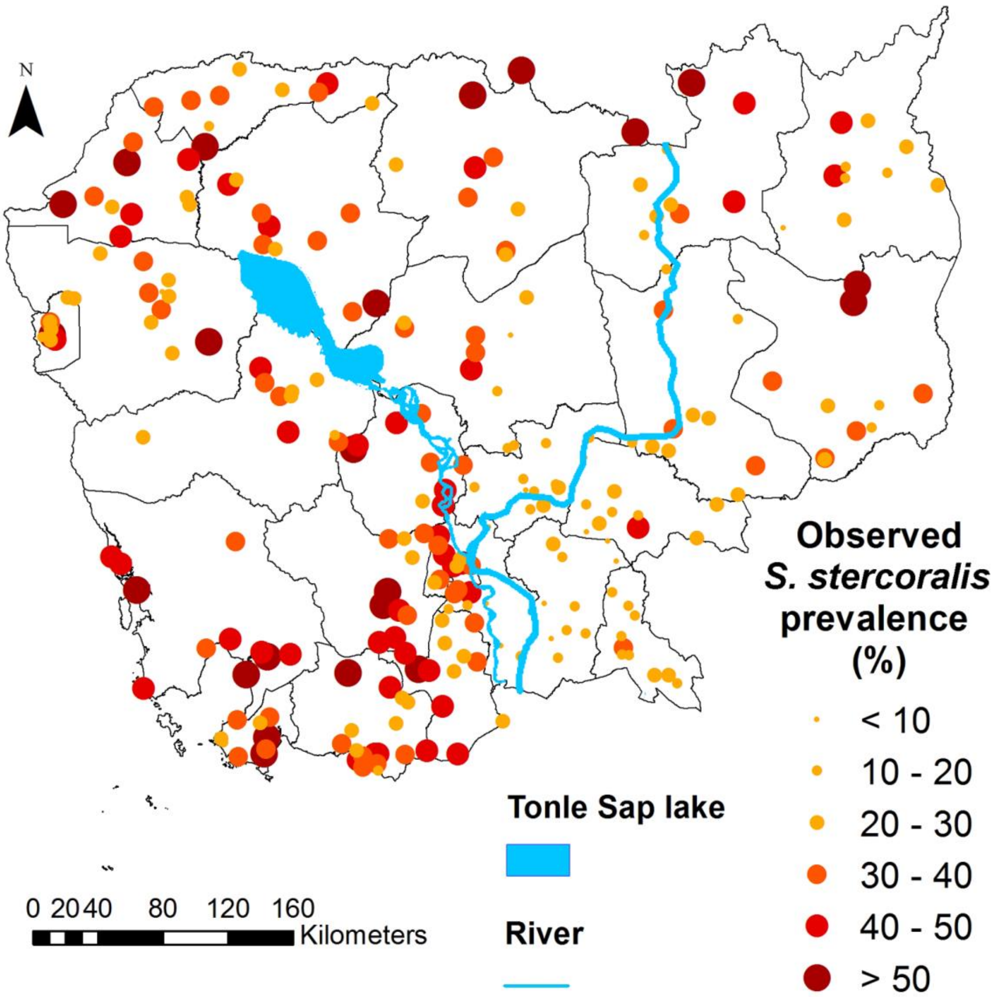


Figure 7.3: Map of Cambodia showing observed *S. stercoralis* prevalence in the 249 study villages, Cambodia.
Data were obtained from a cross-sectional survey conducted in 2016 in 249 villages of Cambodia, among 7,246 participants aged 6 years and above. This map was created with ArcGIS version 10.0 (ESRI; Redlands, CA, USA) and display the results obtained specifically from this study by Forrer et al.

7.3.3 Spatial correlation

The model parameters of three geostatistical models, i.e. (i) model without covariates, (ii) the predictive model including only environmental variables, and (iii) the risk factor analysis model including environmental, demographic and behavioural covariates are presented in Table 7.2. In absence of explanatory variables, *S. stercoralis* risk clustered at a distance of 85 km (range). Most of *S. stercoralis* tendency was due to environmental covariates as indicated by the range of 3.2 km after introducing environmental variables (predictive model).

Table 7.2: Parameters of three geostatistical models

	No covariates (a)		**Predictive model** (b)		**Risk factor analysis model** (c)	
	median	95% BCI	median	95% BCI	median	95% BCI
Range (km)	85.3	1.10 - 185.8	3.20	1.10 - 99.4	2.80	1.10 - 49.7
ρ	3.8	1.78 - 240.0	105.60	3.35 - 282.6	116.10	6.55 - 283.60
σ^2	0.36	0.21 - 0.59	0.27	0.19 - 0.40	0.29	0.21 - 0.41
DIC					8180.98	-

σ^2 is the location-specific unexplained variance.

ρ is the decay parameter. The range (range=$3/\rho$) is the distance at which the spatial correlation becomes less than 5%.

(a) Geostatistical model without covariates

(b) Predictive model: with environmental covariates only

(c) Risk factor analysis model: with environmental and demographic and behavioural covariates

7.3.4 Result of the model validation and predictive model

The predictive ability of the geostatistical model (MSE=182.9, DIC=6894.3) including environmental covariates (predictive model) was slightly higher than that of its non-spatial counterpart (MSE = 187.7, DIC = 6894.4). Therefore the geostatistical model was used to predict *S. stercoralis* risk at non-surveyed locations. The geographical distributions of the covariates used in the geostatistical predictive model, together with elevation, are displayed in Appendix 7.6.2. Odds ratios of those covariates are presented in Table 7.3.

Table 7.3: Results of the geostatistical predictive model

Variable	**Category**	**OR**	**95% BCI**
LST night dry season maximum (°C)	-	1.21	1.05 - 1.33
Rainfall year minimum (mm/month)	-	1.35	1.10 - 1.49
Distance to water (km)	-	1.10	0.97 - 1.22
Land use, land cover	Crops & natural vegetation mosaic, grass	1	-
	Cropland	0.82	0.67 - 1.03
	Forest and savanna	1.17	0.89 - 1.52
	Water and wetlands	1.29	0.88 - 1.91
Range (km)	-	3.20	1.10 - 99.4
ρ	-	105.6	3.35 - 282.6
σ^2	-	0.27	0.19 - 0.40

LST: Land surface temperature; BCI, Bayesian credible interval; OR: odds ratio; OR in bold are significant at 5% level

σ^2 is the location-specific unexplained variance.

ρ is the decay parameter. The range (range=3/ρ) is the distance at which the spatial correlation becomes less than 5%.

7.3.5 Risk factors for S. stercoralis infection

The results of non-spatial bivariate mixed regressions are presented in Appendix 7.6.3. The results of the multivariate Bayesian geostatistical risk factor analysis are presented in Table 7.4.

Table 7.4: Results of the risk factor analysis

		S. stercoralis negative N = 5,019	*S. stercoralis* positive N = 2,227		
Variable	**Category**	**n (%)**	**n (%)**	**OR**	**95% BCI**
Sex [(a)]	Male	2,138 (69.4)	943 (30.6)	1.00	-
	Female	2,881 (69.2)	1,284 (30.8)	**0.87**	0.67 - 1.12
Effect of age among men (years) [(b)]	6 - 12	746 (82.8)	155 (17.2)	1.00	-
	13 - 18	335 (73.6)	120 (26.4)	**1.9**	1.44 - 2.50
	19 - 30	301 (72.0)	117 (28.0)	**2.14**	1.61 - 2.85
	31 - 50	452 (64.1)	253 (35.9)	**3.15**	2.49 - 4.07
	≥ 50	304 (50.5)	298 (49.5)	**6.11**	4.82 - 7.85
Interaction: effect of age among women (years)					
	6-12	717 (84.8)	129 (15.2)	1.00	-
	13 - 18	381 (76.4)	118 (23.6)	**1.89**	1.41 - 2.51
	19 - 30	505 (79.8)	219 (30.2)	**2.67**	2.06 - 3.46
	31 - 50	715 (62.5)	430 (37.5)	**4.01**	3.17 - 5.10
	≥ 50	563 (59.2)	388 (40.8)	**4.79**	3.77 - 6.07
Interaction: females compared to males, in each age group					
	6-12	-	-	1.00	-
	13 - 18	-	-	0.86	0.64 - 1.18
	19 - 30	-	-	1.08	0.82 - 1.43
	31 - 50	-	-	1.1	0.89 - 1.36
	≥ 50	-	-	**0.68**	0.55 - 0.85
Usual defecation place	Toilet	3,503 (70.6)	1,458 (29.4)	1.00	-
	Forest	1,180 (66.7)	588 (33.3)	**1.24**	1.06 - 1.45
	River, rice field, other	336 (65.0)	181 (35.0)	**1.41**	1.12 - 1.80
Knowledge of signs of worm infection	No	3,117 (69.2)	1,390 (30.8)	1.00	-
	Yes			**0.86**	0.75 - 0.98
Land use, land cover	Crops & natural vegetation mosaic, grass	2,676 (69.0)	1,204 (31.0)	1.00	-
	Cropland	1,281 (73.8)	456 (26.2)	**0.81**	0.64 - 0.997
	Forest and savanna	802 (65.0)	431 (35.0)	1.21	0.92 - 1.57
	Water and wetlands	260 (65.7)	136 (34.3)	1.27	0.86 - 1.91
		Median (IQR)	**Median (IQR)**		
LST night dry season maximum (°C)	-	26.1 (1.4)	27.0 (1.6)	**1.22**	1.09 - 1.35
Rainfall year minimum (mm/month)	-	0.81 (0.70)	0.89 (0.91)	**1.38**	1.23 - 1.53
Distance to water (km)	-	14.9 (26.2)	16.0 (31.1)	**1.12**	1.01 - 1.25

Table 7.4 (*cont.*)

Model parameters				**Median**	**95% BCI**
Range (km)	-	-	-	2.80	1.10 - 49.7
ρ	-	-	-	116.1	6.55 - 283.60
σ^2	-	-	-	0.2914	0.21 - 0.41

LST: Land surface temperature; BCI, Bayesian credible interval; OR: odds ratio; OR in bold are significant at 5% level

σ^2 is the location-specific unexplained variance.

ρ is the decay parameter. The range (range=3/ρ) is the distance at which the spatial correlation becomes less than 5%.

(a) Main effect of sex. Due to the interaction the OR corresponds to the effect of sex among the baseline age group (6-12 years).

(b) Main effect of age. Due to the interaction, the OR corresponds to the effect of age among males.

Results were obtained with the multivariate geostatistical model and data from a cross-sectional survey conducted in 2016 among 7,246 participants living in 249 villages across the 25 provinces of Cambodia.

Sex was an effect modifier of age. Infection risk increased with age for both genders but women aged 50 years and above had a lower risk of being infected than males. The relationship between *S. stercoralis* infection risk and age is presented in Figure 7.4. Participants usually practicing open defecation (31.5% of participants defecating either in forests, or rice field and water) had higher odds of being infected, while individuals who had some knowledge about health problems resulting from worm infection has lower odds of harboring *S. stercoralis*. As for environmental factors, *S. stercoralis* infection risk was positively associated with increasing night land surface temperature (LST night) dry season maximum, increasing minimum year rainfall and increasing distance to water. Finally, the odds of *S. stercoralis* infection were lower among participants living in villages located in croplands, i.e. rice fields.

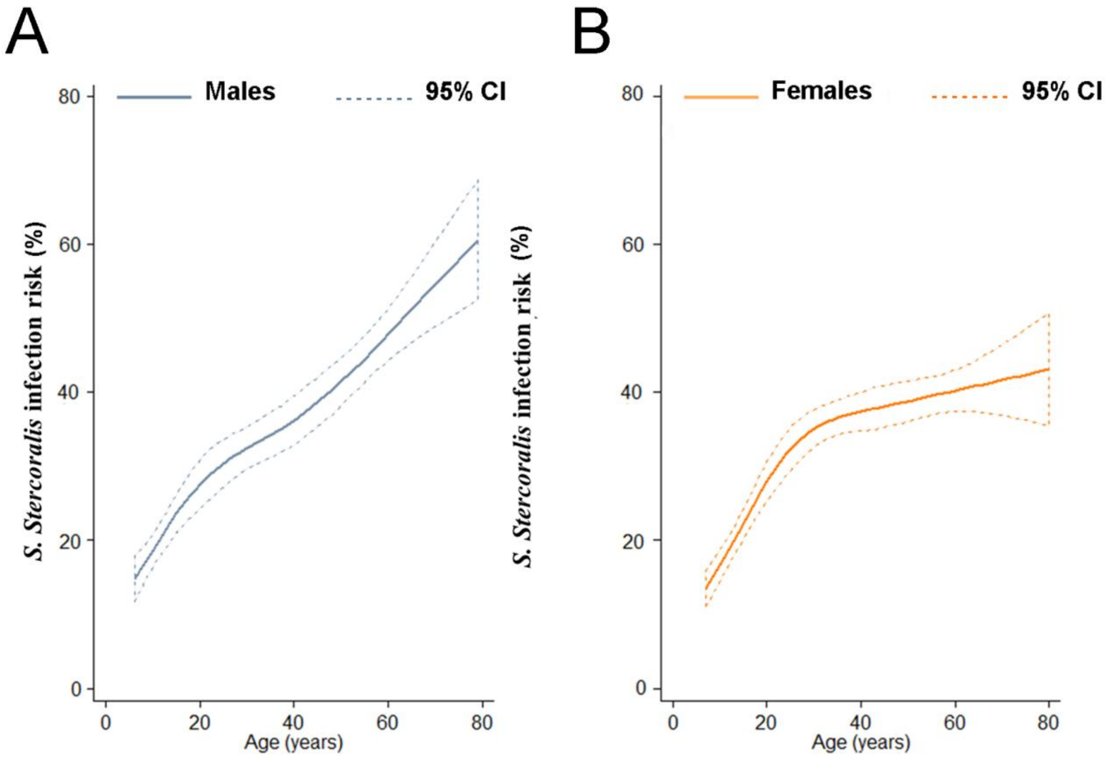


Figure 7.4: Smoothed age-prevalence of *S. stercoralis*, Cambodia
Data were obtained from a cross-sectional survey conducted in 2016 in 249 villages of Cambodia, among 7,246 participants aged 6 years and above. Restricted cubic splines were used. Data are stratified for males (A) and females (B). Uncertainty is expressed as 95% confidence interval (CI).

7.3.6 Spatial prediction of S. stercoralis infection risk

Figure 7.5 and 7.6 display the predicted median *S. stercoralis* prevalence in Cambodia and the lower and upper estimates, respectively. Prevalence was consistently higher than 10% except in a small area of Prey Veng province. *S. stercoralis* predicted risk was below 20% only in five provinces, i.e. Kampong Cham, Tboung Khmum, Prey Veng, Kandal and Svay Rieng. Predicted prevalence was particularly high in the north of Preah Vihear and Stung Treng provinces near the Lao border, as well as in the South, in areas of Kampong Speu, Koh Kong, Preah Sihanouk, and Kampot provinces.

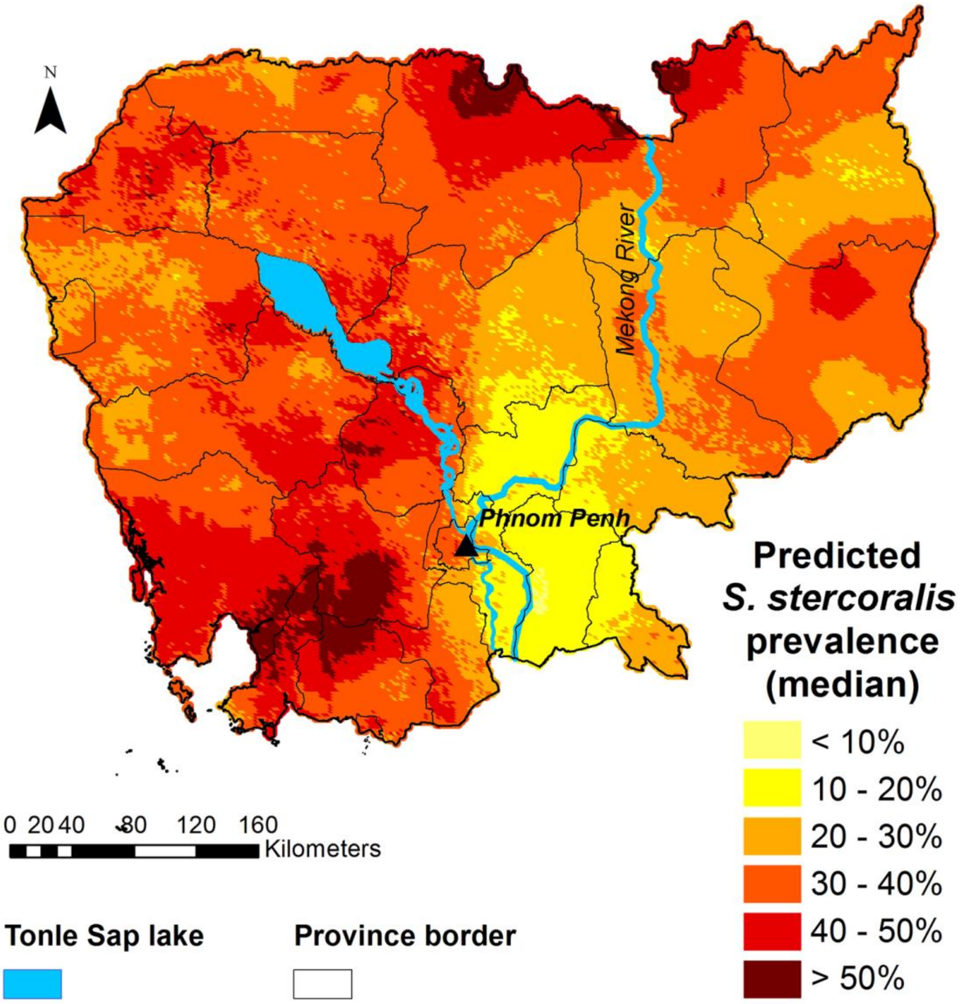


Figure 7.5: Map of the predicted prevalence (median) of *S. stercoralis* in Cambodia

Predictions were obtained with the geostatistical model shown in Table 7.3 based on survey data collected in 2016 in 249 villages of Cambodia among 7,246 participants aged 6 years and above. This map was created with ArcGIS version 10.0 (ESRI; Redlands, CA, USA) and display the results obtained specifically from this study by Forrer et al.

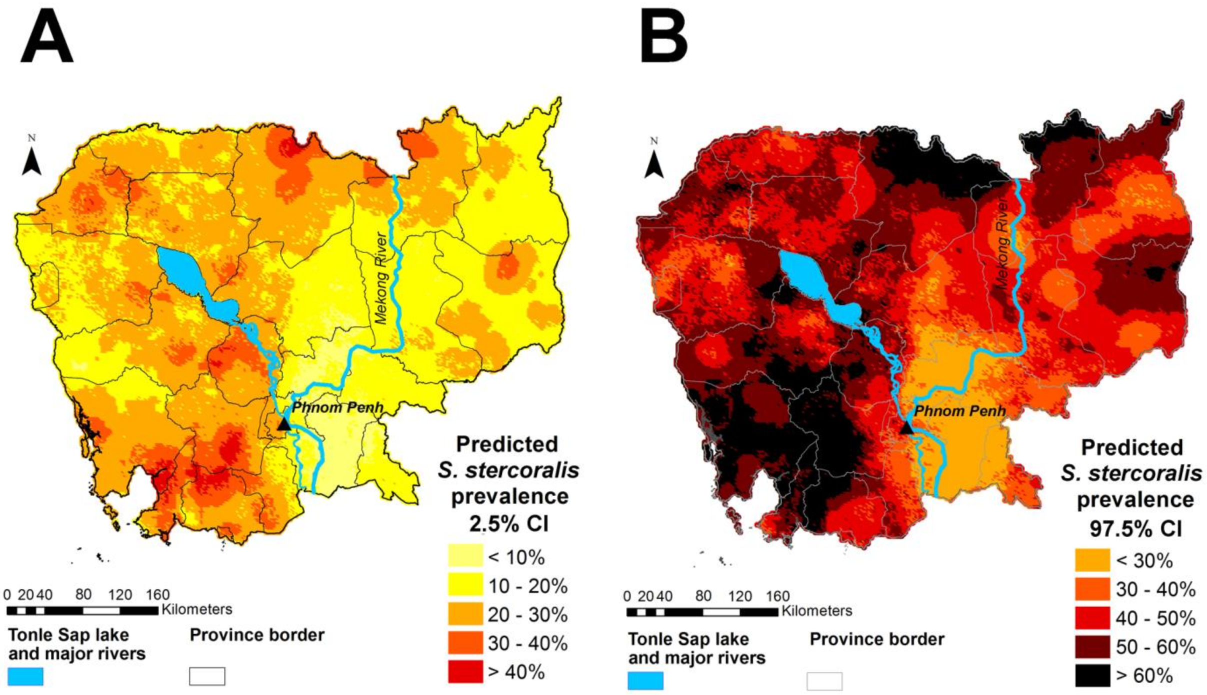


Figure 7.6: Lower (A) and upper (B) estimates of predicted *S. stercoralis* prevalence in Cambodia. The lower and upper estimates are the 2.5% CI and the 97.5% CI, respectively. This map was created with ArcGIS version 10.0 (ESRI; Redlands, CA, USA) and display the results obtained specifically from this study by Forrer et al.

7.4 Discussion

We present here, to our knowledge, the first national prevalence estimate and nation-wide infection risk map of *S. stercoralis*. The infection is ubiquitous in Cambodia. Based on a sample encompassing all the 25 country provinces and including over 7,200 participants, *S. stercoralis* occurs in Cambodia at prevalence rates systematically over 10%, with a national prevalence of 30%.

Infection risk was the lowest in the Southeast of the country, i.e. in the provinces of Prey Veng, Kandal, Kampong Cham, as well as the West and South parts of Tboung Khmum and Kampong Thom provinces, respectively. The highest province-level prevalence rates, above 40%, were found in Preah Vihear in the North, Kampong Chhnang in the Centre and in the South, Koh Kong and Kampong Speu.

The size of *S. stercoralis* infection clusters was relatively small, 85 km, similarly to that observed for hookworm infection risk in the country (Karagiannis-Voules et al., 2015b). Almost all spatial correlation of *S. stercoralis* infection was explained by its association with environmental factors (as indicated by the dramatic drop of the range, down to 3.2 km, after introducing environmental covariates in the model). This result is not surprising as in absence of available treatment the parasite biological requirements would mostly condition its distribution. The distribution of hookworm prevalence among school-aged children in Cambodia was similar to that of *S. stercoralis*, likely due to the resembling transmission routes of those two nematodes. Yet, the area with lower hookworm prevalence was larger, probably because of ongoing STH deworming programmes (Karagiannis-Voules et al., 2015b).

The odds of being infected increased with increasing maximum night temperature and increasing minimum rainfall. In presence of sufficient humidity hookworm has a good tolerance for high temperatures with its larvae having the ability to migrate in the soil, and *S. stercoralis* larvae might have the same ability (Brooker et al., 2004a). The positive association between temperature and risk is more surprising, although this might relate to a particularity of *S. stercoralis* life cycle. The number of females and infective larvae developing in the external environment depends on temperature, with numbers of infective larvae being maximum when temperatures are of 30°C and above (Grove, 1996). Hence, night maximum temperatures which range between 24°C and 32°C in Cambodia might have an impact on the amount of infective larvae present in the environment.

Regarding the environmental predictors of *S. stercoralis* infection, distance to water and the land cover category of cropland were not significantly associated with infection risk in the predictive model but became significant in the risk factor analysis after adjusting for demographic and behavioural factors. We found a positive association between *S. stercoralis* infection risk and distance to water. The development and survival of *S. stercoralis* larvae is affected by immersion, so seasonal flooding might affect their survival in areas close to water bodies (Anamnart et al., 2013, Cimino and Krolewiecki, 2014). Similarly, this relationship between larvae survival and water might explain the lower infection in areas occupied by croplands, which mostly correspond to rice fields that are regularly flooded. Yet it is also possible that distance to water captured other unmeasured features, including factors relating to socio-economic features and human activity (Soares Magalhães et al., 2015). In Cambodia, people have a clear preference for pour-flush latrines and would rather not have a toilet than pit latrines, but pour flush latrines function only with water (Pedi D, 2014). Limited availability of water due to living away from permanent water bodies might result in decreased access to, or use of, sanitation facilities.

Studies that investigated risk factors for *S. stercoralis* infection mostly report a higher risk in men, whereas the relationship between age and *S. stercoralis* prevalence seems to vary across settings (Steinmann et al., 2007b, Knopp et al., 2010b, Khieu et al., 2014b, Khieu et al., 2014c). In this national survey among more than 7,200 individuals aged six years and above, we found that prevalence increased with age both in men and women, although men aged 50 years and above had higher odds of *S. stercoralis* infection than women of the same age. Boys aged between 6 and 12 years were also more likely to harbor *S. stercoralis* than their female counterparts. Previously to this national survey, prevalence was found to increase with age and reach a plateau in adulthood in North Cambodia, while no cases were found in individuals aged less than 15 years in Yunnan, China (Steinmann et al., 2007a, Khieu et al., 2014b, Forrer et al., 2016). Yet, no association between age and *S. stercoralis* infection was found in Lao PDR, South Cambodia, or Zanzibar (Knopp et al., 2010b, Khieu et al., 2014c, Vonghachack et al., 2014). Age-specific infection risk is of particular importance to target control programmes and should be further documented.

An interesting finding was that individuals who declared having some knowledge about health problems caused by worm infections had lower odds of being infected with *S. stercoralis*, but having some knowledge about sources of infection was not associated with infection risk. While knowledge does not necessarily translate into behaviour change, this result suggests that being aware of personal disease risk, –which is an important driver in health promotion and increases compliance to helminth control programmes– might be a better trigger of hygienic behaviour than knowing exposure sources (Krentel et al., 2013, Palmeirim et al., 2018).

The protective effect of improved sanitation against STH infection is widely acknowledged (Freeman et al., 2013b, Strunz et al., 2014, Freeman et al., 2014, Ziegelbauer et al., 2012, Echazú et al., 2015). We found that, compared to open defecation, defecating in latrines was protective against *S. stercoralis* infection. This result is in line with other studies conducted in Cambodia or Ecuador, and with a recent meta-analysis that included 9 studies investigating the impact of sanitation on *S. stercoralis* infection risk, and estimated a pooled OR of 0.50 (95%CI: 0.36-0.70) (Khieu et al., 2013a, Khieu et al., 2014b, Moore et al., 2015, Echazú et al., 2015, Freeman et al., 2017). Of note, village-level sanitation coverage was also found to reduce re-infection risk one year after treatment in North Cambodia (Forrer et al., 2016).

The present work has several limitations. First, women were overrepresented in the sample compared to the general Cambodian population and the lower prevalence among young girls and women aged 50 years and above, compared to males, might have resulted in a slight underestimation of prevalence. Most importantly, our sample was representative of the 2013 Cambodian general population in terms of age, which is strongly associated with infection risk (National Institute of Statistics, 2013b).

Second, it was the first time that the serological diagnostic method of detecting IgG anti-bodies employed in this study was used for a large-scale survey. This method has proven a high sensitivity for *S. stercoralis* detection, and it does not suffer from cross-reactivity with other STH (Eamudomkarn et al., 2015, Eamudomkarn et al., 2018). However, validation of the method in different settings should be carried out. Finally, our risk factor analysis did not adjust for socio-economic status which was found associated with infection risk in North Cambodia, but results from the few studies that accounted for it are heterogeneous (Conlan et al., 2012, Khieu et al., 2014b, Khieu et al., 2014c, Forrer et al., 2016, Forrer et al., 2018). Given the strong association between poverty and other STH infections, it is likely that *S. stercoralis* risk distribution is also associated with socioeconomic status and future studies should account for it. Of note, the socioeconomic status was not a confounder of the relationship between age or sex and *S. stercoralis* infection risk in North Cambodia and would probably not have substantially affected the estimates for sex and age in the present study (Forrer et al., 2016, Forrer et al., 2018).

Our study represents a clear risk map of *S. stercoralis* of a highly endemic setting. Based on these data the population at risk can be quantified and planning of concrete control approach become realistic. Further developing this operational approach in other settings and with further validated diagnostic approaches will result in data bases for global planning. The mainstay of the WHO strategy to control soil-transmitted helminths is preventive chemotherapy, i.e. the regular treatment with mebendazole or albendazole to prevent high intensity infections and the associated morbidity to entire populations or at-risk groups (WHO and Crompton, 2006, WHO, 2010). However those drugs are not efficacious at a single oral dose against *S. stercoralis* for which the drug of choice is ivermectin (Gann et al., 1994, Igual-Adell et al., 2004, Henriquez-Camacho et al., 2016). A single oral dose (200µg/kg Body Weight) of ivermectin has recently been found to achieve a high cure rate and result in re-infection rates below 15%, one year after treatment in a highly endemic setting of Cambodia (Gann et al., 1994, Igual-Adell et al., 2004, Forrer et al., 2016). As our results demonstrate, *S. stercoralis* is highly endemic in all provinces in Cambodia and the inclusion of ivermectin in the control programme would be required (WHO, 2009, Nutman, 2016, Henriquez-Camacho et al., 2016). Yet, this drug is not subsidized in in regions where onchocerciasis is absent, let alone to treat *S. stercoralis*, and its high cost in Cambodia, 10 USD per tablet –while up to 5 tablets may be needed to treat an individual, depending on their weight–, precludes the deployment of control measures in the country.

In absence of data on age specific morbidity, the fact that individuals of any age appear to have the same risk for reinfection one year after treatment suggests community-wide control (Forrer et al., 2016). Yet, it appeared in a study investigating *S. stercoralis* related morbidity in Cambodia that children and adolescents with higher parasite loads had higher odds of being stunted, while *S. stercoralis* infection was found to be associated with anemia but not stunting in Argentina (Echazú et al., 2017, Forrer et al., 2017). The relationship between *S. stercoralis* parasite loads, morbidity and transmission intensity needs to be assessed, as well as age-related infection levels, using appropriately designed longitudinal studies. Cost-effectiveness studies of various control options are needed while mathematical models could help better appraising the parasite transmission dynamics and guide control efforts, as the complex life cycle of *S. stercoralis* life might yield transmission dynamics that are different from the other STH.

Cambodia benefits from a well-established STH control network and was among the first countries to reach the 75% national coverage target (Sinuon et al., 2005, Montresor et al., 2008). STH control was recently scaled up to reach children in secondary and high schools, including in private schools, and women of child bearing age in factories (National Center for Parasitology, 2014). Additionally, schistosomiasis has been successfully controlled with no severe cases recorded recently and lymphatic filariasis has been eliminated as a public health problem and is now under surveillance for elimination (Sinuon et al., 2007, Montresor et al., 2008, WHO, 2016a).

However, in this country that has demonstrated its capacity to efficiently address helminthic infections, the control of *S. stercoralis* is currently hindered by the high cost of ivermectin, which cannot be entirely supported by the Ministry of Health. Either subsidizations, donations, or the production of affordable generics are necessary to start tackling this dangerous parasite that infects almost a third of the Cambodian population.

7.5 Acknowledgements

We are grateful to all of the study participants. Our sincere thanks go to the laboratory technicians and staff at the Helminth Control Programme of the National Centre for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia, and the staff of the laboratory staff of the Parasitology department of the Khon Kaen University, Khon Kaen, Thailand. We thank the Provincial Health Departments of all provinces for their support and field work, and the local authorities for their support.

7.6 Appendix

7.6.1 Bayesian Model Formulation

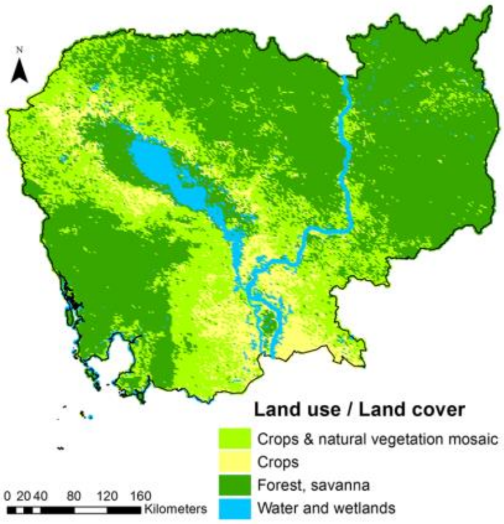
For the individual level risk factor analysis we assumed the infection status Y_{ij} of an individual *i* at a village *j* followed a Bernouilli distribution: $Y_{ij} \sim Be(p_{ij})$ where p_{ij} is the probability of being infected by *S. stercoralis* for an individual *i* in a village *j*.

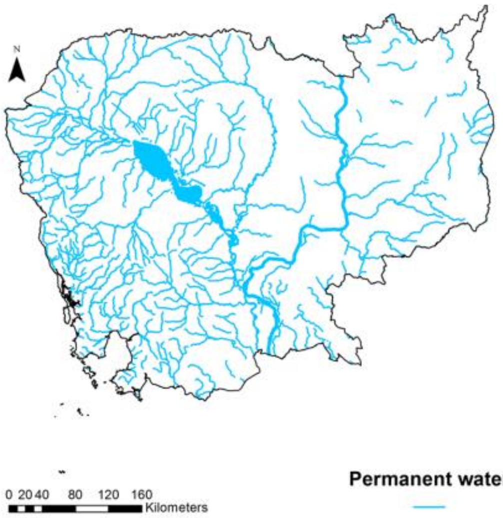
For the predictive model using environmental variables at village level only we assumed that at each village *j*, the number of infected Y_j followed a Binomial distribution: $Y_j \sim Bin(N_j, p_j)$ where N_j is the number of screened individuals and p_j is the probability of infection.

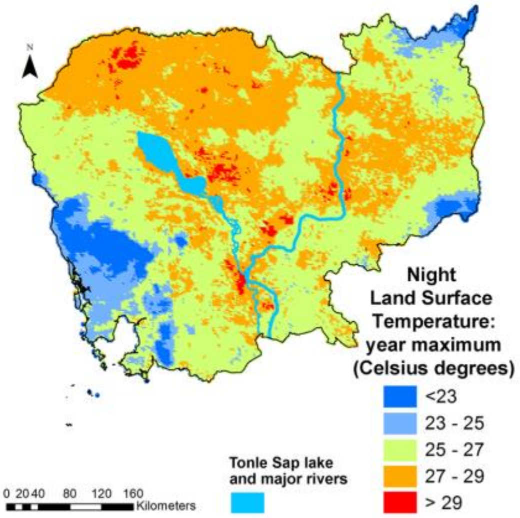
Spatial random effects ϕ_j, accounted for unobserved spatial processes at every village *j*. Covariates β_k and random effects ϕ_j were modelled on a log *it* scale: $\log it\left(p_{ij}\right) = \alpha + \sum_{k=1}^{n} \beta_k X_{kij} + \phi_j$, with *n* being the number of covariates. The random effect $\underset{-}{\phi} = (\phi_1, \phi_2, ..., \phi_j)^T$ is assumed to follow a Normal distribution $\underset{-}{\phi} \sim N(0, \Sigma)$, where Σ is the covariance matrix. A stationary isotropic process was assumed in the present work, with the covariance matrix $\Sigma_{ij} = \sigma^2 corr_{ij}(d_{ij}, \rho)$ and an exponential correlation function $corr_{ij}(d_{ij}, \rho) = \exp(-d_{ij}, \rho)$ where d_{ij} is the shortest distance between two locations s_i and s_j, and ρ is a measure of how spatial correlation decreases with the distance. The distance at which the spatial correlation between villages gets under 5% is equal to $3/\rho$ and is called the range.

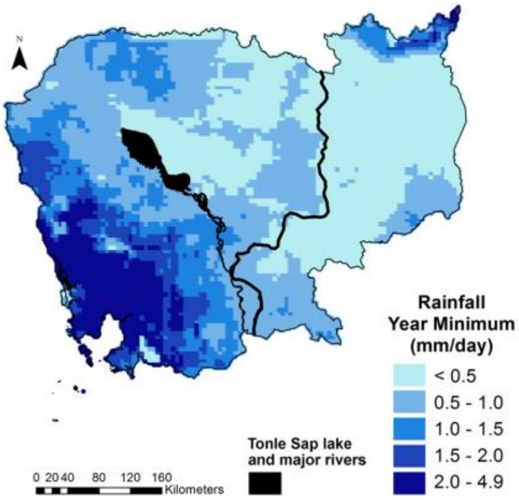
According to Bayesian modelling specification, we chose prior distributions for all parameters to be estimated. We chose a Normal distribution with a mean of zero and a variance of 100 for the regression coefficients. An inverse gamma vague prior with mean equal to 1 and variance equal to 100 was adopted for σ^2, and a uniform prior for ρ with parameters calculated as a function of the minimum and maximum distance between sampled villages:

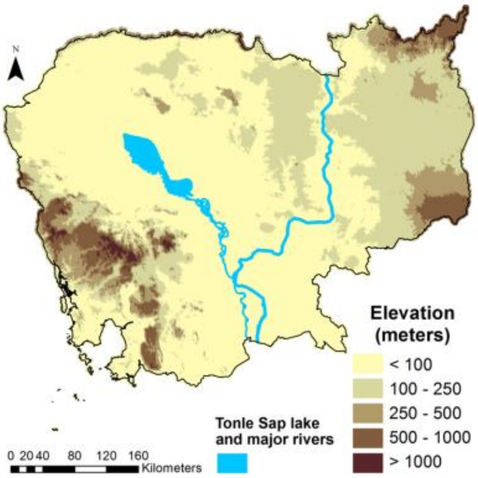
7.6.2 Maps of environmental predictors











7.6.3 Results of the bivariate non-spatial regressions for individual-level risk factors

Variable	Category	OR	95% CI	LRT p-value
Sex	Male	1.00	-	0.8695
	Female	1.01	0.9 -1.12	
Age group (years)	6 - 12	1.00	-	< 0.0001
	13 - 18	1.8	1.47-2.21	
	19 - 30	2.29	1.89-2.77	
	31 - 50	3.38	2.86-3.99	
	≥ 50	4.8	4.03-5.72	
Usual defecation place	Toilet	1.00	-	0.0287
	Forest	1.15	1.00-1.33	
	River, rice field, other	1.29	1.03-1.62	
Education attainment	Primary	1.00	-	< 0.0001
	No school	1.81	1.57-2.09	
	Secondary	1.17	1.01-1.35	
	High school and higher	0.97	0.78-1.21	
Main occupation	Farmer	1.00	-	< 0.0001
	At school	0.38	0.34-0.43	
	At home	0.76	0.58-0.99	
	Other	0.8	0.65-0.99	
Number of household members	-	0.99	0.96-1.01	0.2517
Do you know anything about worms?	No	1.00	-	
	Yes	1.29	1.14-1.46	< 0.0001
Do you know how to get infected with worms?	No	1.00	-	
	Yes	1.13	1.00-1.27	0.0491
Do you know what health problems worm infection can cause?	No	1.00	-	
	Yes	1.11	0.98-1.25	0.1043
Source of infection: walking barefoot (a)	No	1.00	-	
	Yes	0.97	0.83-1.13	0.6909
Source of infection: lack of hygiene (a)	No	1.00	-	
	Yes	0.99	0.87-1.13	0.8795
Source of infection: open defecation (a)	No	1.00	-	
	Yes	1.06	0.91-1.24	0.4313
	No	1.00	-	
Source of infection: no handwashing (a)	Yes	1.04	0.90-1.20	0.6236

(a) spontaneous specific answers to the question "Do you know how to get infected with worms"?
Data were obtained from a cross-sectional survey onducted in 2016 among 7,246 participants living in 249 villages across the 25 provinces of Cambodia.

Chapter 8

Strongyloides stercoralis and hookworm co-infection: spatial distribution and risk factors in Preah Vihear province, Cambodia.

Armelle Forrer[1,2], Virak Khieu[3], Fabian Schär[1,2], Penelope Vounatsou[1,2], Frédérique Chammartin[1,2], Hanspeter Marti[1,2], Sinuon Muth[3], Peter Odermatt[1,2*]

1 Swiss Tropical and Public Health Institute, Basel, Switzerland; **2** University of Basel, Basel, Switzerland; **3** National Centre for Parasitology, Entomology and Malaria Control, Ministry of Health, Phnom Penh, Cambodia

This paper has been published in Parasites & Vectors 2018, 11(1)

Abstract

Background

Strongyloides stercoralis and hookworm are two soil-transmitted helminths (STH) that are highly prevalent in Cambodia. *S. stercoralis* causes long-lasting infections and significant morbidity but is largely neglected, while hookworm causes the highest burden among STH. The two parasites have the same infection route, i.e. skin penetration. The extent of co-distribution, which could result in potential high co-morbidities, is unknown in highly endemic settings like Cambodia. The aim of this study was to predict the spatial distribution of *S. stercoralis*-hookworm co-infection risk and to investigate determinants of co-infection in Preah Vihear province, north Cambodia.

Methods

A cross-sectional survey was conducted in 2010 in 60 villages of Preah Vihear province. Diagnosis was performed on two stool samples, using combined Baermann technique and Koga agar culture plate for *S. stercoralis* and Kato-Katz technique for hookworm. Bayesian multinomial geostatistical models were used to assess demographic, socioeconomic, and behavioural determinants of *S. stercoralis*-hookworm co-infection and to predict co-infection risk at non-surveyed locations.

Results

Of the 2,576 participants included in the study, 48.6% and 49.0% were infected with *S. stercoralis* and hookworm, respectively; 43.8% of the cases were co-infections. Females, preschool-aged children, adults aged 19–49 years, and participants who reported regularly defecating in toilets, systematically boiling drinking water and having been treated with anthelmintic drugs had lower odds of co-infection. While *S. stercoralis* infection risk did not appear to be spatially structured, hookworm mono-infection and co-infection exhibited spatial correlation at about 20 km. Co-infection risk was positively associated with longer walking distances to a health centre and exhibited a small clustering tendency. The association was only partly explained by climatic variables, suggesting a role for underlying factors, such as living conditions and remoteness.

Conclusions

Both parasites were ubiquitous in the province, with co-infections accounting for almost half of all cases. The high prevalence of *S. stercoralis* calls for control measures. Despite several years of school-based deworming programmes, hookworm infection levels remain high. Mebendazole efficacy, as well as coverage of and compliance to STH control programmes should be investigated.

Author summary

S. stercoralis and hookworm are two intestinal parasitic worms. Hookworm is a well-known parasite that causes a high disease burden, but *S. stercoralis* has been overlooked for decades, despite being an important cause of morbidity, because it is not detected by the usual diagnostic techniques. Both parasites are very common in Cambodia. Parasitic infections are addressed through control programmes that regularly distribute deworming drugs, but the drugs are not active against *S. stercoralis*. This study aimed to assess the geographical distribution of co-infection with both parasites in Preah Vihear province, Cambodia, and to investigate the determinants of co-infection. More than two thirds of the participants were infected with one or two types of parasites. Both *S. stercoralis* and hookworm infected almost half of the population (48.6% and 49.0 %, respectively). Females, young children and adults aged 19–49 years had lower odds of infection. Hygienic behaviours, such as defecating in toilets and boiling drinking water, were protective factors. Both parasites and co-infection cases were found across the whole province. Further studies should investigate why school-aged children, despite regular deworming, were found with high hookworm infection levels. The high prevalence and ubiquity of *S. stercoralis* underlines the need to initiate its control.

8.1 Introduction

Strongyloides stercoralis and hookworms are parasitic intestinal nematodes that belong to the group of soil-transmitted helminths (STH). For both parasites, infection occurs when larvae living in faecally-polluted soil penetrate intact skin. STH mostly affect the poorest, locking them into poverty through a cycle of gastro-intestinal symptoms, malnutrition and long-term impact on fitness and productivity (Stephenson et al., 2000b, Hotez et al., 2008, Bethony et al., 2006). With overlapping geographical distributions, STH are mostly prevalent in rural areas with poor sanitation conditions and a warm and humid climate that favour larvae survival in the environment (Hotez et al., 2008, Brooker et al., 2004a, Schär et al., 2013).

Hookworm infection is of major public health importance in low and middle-income countries, with 439 million cases reported worldwide in 2010 (Pullan et al., 2014). Hookworm causes the highest burden among STH, with detrimental effects on children's physical and cognitive development and agricultural workers' productivity. It also gives rise to hookworm disease, which occurs in cases of high worm load and causes iron-deficiency anaemia that affects infant and maternal mortality and leads to low birth weights (Hotez et al., 2008, Hotez et al., 2009, Bethony et al., 2006, Brooker et al., 2008).

As for *S. stercoralis*, it is one of the most neglected of the neglected tropical diseases (NTDs), mostly because its larvae, present in human stool, are not detected by the diagnostic coprological techniques used in endemic countries to screen for helminth eggs (Olsen et al., 2009, Bisoffi et al., 2013, Krolewiecki et al., 2013, Schär et al., 2013). However, the parasite has recently gained some attention from the scientific and global health community and recent studies in Cambodia found high prevalence rates of up to 45% (Khieu et al., 2013a, Khieu et al., 2014b, Khieu et al., 2014c). Not only is this parasite very common, but it also causes significant dermatological and gastro-intestinal morbidity and is associated with chronic malnutrition in children (Khieu et al., 2013b, Forrer et al., 2017). Moreover, its ability to replicate within its host leads to long-lasting infections and potentially fatal dissemination of the parasite (Vadlamudi et al., 2006, Fardet et al., 2007, Marcos et al., 2008).

The backbone of the WHO strategy to control STH is preventive chemotherapy (PC), i.e. regular treatment to prevent high-intensity infections and associated morbidity administered to entire populations or at-risk groups. In the case of hookworm, children and women of childbearing age are particular targets for treatment (WHO and Crompton, 2006, WHO, 2010). However, continued exposure to contaminated environments due to unhygienic behaviour leads quickly to re-infection (Yap et al., 2013, Jia et al., 2012, Forrer et al., 2016) and re-treatment is required.

To date, *S. stercoralis* is not included in the WHO recommended preventive chemotherapy control strategy used against STH, in which anti-helminthic treatment is the main pillar. The drug of choice against *S. stercoralis*, ivermectin, is safe, well tolerated and highly efficacious (Gann et al., 1994, Igual-Adell et al., 2004, Henriquez-Camacho et al., 2016). It was recently found that a single oral dose (200µg/kg body weight) of ivermectin achieved a high cure rate and resulted in re-infection rates below 15%, one year after treatment in a highly endemic setting in Cambodia (Igual-Adell et al., 2004, Gann et al., 1994, Forrer et al., 2016).

Both *S. stercoralis* and hookworm are highly prevalent in Preah Vihear province (Sinuon et al., 2005, Montresor et al., 2008). Considering the need to integrate *S. stercoralis* control into existing STH programmes, it is important to know the extent to which the distributions of the two parasites overlap geographically and across age groups. Geostatistical models using survey parasitological data combined with remote sensing (RS) environmental data provide unique tools for estimating parasite distribution over small or large areas, thereby providing a rapid and cost-effective means of identifying the areas of greatest need and guiding control efforts (Brooker et al., 2009, Jex et al., 2011, Utzinger et al., 2012).

The aim of the present work was to assess the geographical distribution and explore the underlying factors of *S. stercoralis*-hookworm co-infection risk in the rural province of Preah Vihear, North Cambodia.

8.2 Methods

8.2.1 Study setting and design

The study was conducted among the general population of Preah Vihear province, located in Northern Cambodia and bordering Thailand. With a population of 171,139 in 2008 and a total area of 16,132 km^2, the province stretches from 13°06’ to 14°44’ N latitude and 104°37’ to 106°91’ longitude (National Institute of Statistics, 2013a). The poverty incidence, defined as the proportion of individuals living in households with an average per capita expenditure below the poverty line of 6,347 riels (1.55 USD), was 33.5% in 2009 (Haslett et al., 2013). It has a monsoon type climate, with a rainy season occurring from May to October. *S. stercoralis* and hookworms, as well as protozoa, are highly endemic in this region (Khieu et al., 2014b).

Sixty of the 184 villages in Preah Vihear province were selected for a large-scale, cross-sectional study carried out from February to June 2010. Six of seven districts were included; the district of Chaeb was excluded because the distance between its villages and the study labs was too large to ensure sufficiently fast transfer of samples to preserve their integrity. In each village, 15 households were randomly selected from the list of all households and all household members aged one and over were eligible for inclusion in the study.

8.2.2 Parasitological data and case definition

Two stool samples were collected on consecutive days from each participant. *S. stercoralis* was diagnosed using both Koga agar plate (KAP) culture and the Baermann technique performed on each sample (Baermann, 1917, Koga et al., 1991). A detailed description of this laboratory procedure is given elsewhere (Khieu et al., 2014b). Hookworm was diagnosed using Kato-Katz thick smears, KAP culture and the Baermann technique on each sample. *S. stercoralis* larvae and hookworm eggs were identified through microscope examination and based on morphology. For quality control, technicians were specifically trained to differentiate *S. stercoralis* and hookworm larvae. In addition, they were rigorously supervised by a qualified microscopist from the Swiss Tropical and Public Health Institute (Swiss TPH), Basel, Switzerland. Any unclear diagnosis was immediately discussed with both the qualified microscopist and the study supervisor.

In this analysis, infection status was defined as follows. For *S. stercoralis*, a participant was considered positive if at least one larva was found in any of the four samples (KAP and Baermann technique on two samples) or negative if no larva was detected in the four samples. Participants with only negative results but with fewer than four analysed samples were not included in the analysis. For hookworm, a participant was considered positive if at least one larva was found in any of the six samples (Kato-Katz, KAP, and Baermann technique on two samples), and negative if both Kato-Katz slides were negative. Negative participants with one missing Kato-Katz examination were not included in the analysis. All reported results in this paper use the definitions described above.

8.2.3 Demographic, socioeconomic, knowledge and hygiene practices data

Data on demographic factors (age, sex, main occupation, level of education), hygiene practices (hand washing, shoe wearing, regular place of defecation), and worm-related knowledge (sources of infection, health problems caused by worms) were collected with an individual questionnaire. Heads of households were administered an additional questionnaire to collect information on household size, water and sanitation conditions, house material and household asset ownership. Age was categorized into four classes, as follows: (i) <6 years, (ii) 6–18 years, (iii) 19–49 years and (iv) ≥ 50 years. About 17% (432/2576) of the participants reported their occupation as “other”, most of whom declared being at home (361/432), while the remaining occupations varied from teachers, nurses and military, to village chiefs and construction workers. Original categories of variables with frequencies under 5% were grouped with similar categories.

An asset-based socioeconomic index was built using house construction material, ownership of household assets and multiple correspondence analysis (MCA), a data reduction technique that was developed for categorical data (Asselin and Tuan Anh, 2008, Booysen et al., 2008). Households were classified into one of three wealth classes, from the least poor to the poorest.

8.2.4 Environmental data

Environmental parameters were extracted from freely available remote sensing (RS) sources. Day and night land surface temperature (LST), land use/land cover (LULC), and enhanced vegetation index (EVI) were extracted at 1 x 1 km resolution from Moderate Resolution Imaging Spectroradiometer (MODIS) Land Processes Distributed Active Archive Center (LP DAAC), U.S. Geological Survey (USGS) Earth Resources Observation and Science (EROS) Center (http://lpdaac.usgs.gov). EVI was used instead of the normalized difference vegetation index (NDVI), as it is more sensitive to differences in heavily vegetated areas and, thus, more appropriate for Southeast Asia (see http://earthobservatory.nasa.gov/Features/MeasuringVegetation/measuring_vegetation_4.php) . Rainfall estimates (RFE) at 0.1 degree (about 10 x 11 km) resolution were obtained from the National Oceanic and Atmospheric Administration’s (NOAA) Climate Prediction Center (CPC) Famine Early Warning System (FEWS) Rainfall Estimates South Asia, version 2.0 (http://www.cpc.ncep.noaa.gov/products/fews/SASIA/rfe.shtml). Digital elevation data at a resolution of 90 x 90 m were retrieved from the NASA Shuttle Radar Topographic Mission's (SRTM) Consortium for Spatial Information of the Consultative Group for International Agricultural Research (CGIAR-CSI) database. Soil type data, including bulk density, soil organic carbon content and pH, at a spatial resolution of 9 x 9 km, were extracted from the International Soil Reference and Information Center's (ISRIC) World Inventory Soil Emission Potentials (WISE), version 1.0 (http://www.isric.org). District information (i.e. district name) was downloaded from the Global Administrative Areas website (http://www.gadm.org). The 18 land cover type 1 classes (IGBP) were merged into five categories, according to similarity and respective frequencies. Yearly means, as well as minima and maxima of EVI, monthly LST and RFE were calculated for May 2009 to April 2010.

8.2.5 Statistical analysis

ArcGIS version 10.0 (ESRI; Redlands, CA, USA) was used for environmental data processing, geo-referencing and map drawing. Environmental data was linked to parasitological and questionnaire data according to location. Data management was performed in STATA version 13.0 (StataCorp LP; College Station, United States of America).

Bayesian mixed multinomial models, i.e. with a random effect accounting for village-level clustering, were used to jointly model the risks of mono-infection with *S. stercoralis*, mono-infection with hookworm, and *S. stercoralis*-hookworm co-infection. Such a model yields relative risk ratios for categorical outcomes (here, the two mono-infection and the co-infection risks) compared to a baseline outcome (in the present case, "no infection"). Two types of Bayesian mixed multinomial models were developed. First, a model using demographic, socioeconomic and behavioural data was developed to assess determinants of *S. stercoralis* and hookworm mono- and co-infection. Second, a model including only environmental covariates aimed to predict *S. stercoralis*-hookworm mono- and co-infection risks at non-surveyed locations in Preah Vihear province.

Variable selection for both types of models was done in STATA using simple multinomial models, based on 15% significance level as assessed by the likelihood ratio test (LRT). In case of correlation, the variable resulting in the model with the smallest Akaike's information criterion (AIC) was selected. Selected continuous covariates were standardized. We checked whether sex was an effect modifier of any other variable in the model.

A mixed logistic regression model for *S. stercoralis* infection was used to estimate the odds ratio for co-infection with hookworm; this was done in STATA, while adjusting for all the variables present in the determinant analysis. The unadjusted odds ratio was produced using a mixed bivariate logistic regression.

Bayesian multivariate mixed multinomial models were fitted using OpenBUGS version 3.2.3 (Imperial College & Medical Research Council; London, UK) (Lunn et al., 2000). Models without or with environmental covariates were run with either a spatial (geostatistical) random effect, using the OpenBUGS "spatial.unipred" function, or with an exchangeable random effect. To explore the clustering tendency of *S. stercoralis* and hookworm mono- and co-infection risks, a Bayesian spatial multinomial model was run in the absence of covariates. Spatial models assumed a stationary isotropic process, with village-specific random effects following a normal distribution with mean zero and a variance-covariance matrix being an exponential function of the distance between pairs of locations. Vague prior distributions were chosen for all other parameters. Markov Chain Monte Carlo (MCMC) simulation was used to estimate model parameters (Gelfand and Smith, 1990). Convergence was assessed by examining the ergodic averages of selected parameters. Further information about models specification is available in Appendix 8.8.1. For all models, a burn-in of 5,000 was followed by 30,000 iterations, after which convergence was reached. Results were withdrawn for the last 10,000 iterations of each chain, with a thinning of 10.

For model validation, 48 randomly selected villages were used for fitting and the 12 remaining villages were used as test locations. Model predictive ability was assessed with the Mean Squared Error (MSE), which was obtained for test locations by squaring the average of absolute differences between predicted and observed prevalence rates.

Based on environmental factors only and using Bayesian Kriging, predictions of *S. stercoralis* mono-infection, hookworm mono-infection and *S. stercoralis*-hookworm co-infection risks at non-surveyed locations were made at 16,532 pixels of a 1x1 km resolution (Diggle et al., 1998).

8.3 Results

8.3.1 Study population and size

Of the 3,560 participants with available questionnaire data, 221 did not provide any stool sample. The case definition used in the present study resulted in the further exclusion of 662 *S. stercoralis* negative participants with fewer than four available diagnostic tests (KAP and Baermann on two samples) and 101 hookworm negative participants with fewer than two available diagnostic examinations (Kato-Katz on 2 samples), resulting in a sample of 2,576 participants. While this sample was used for the predictive model, the final sample for the determinant analysis consisted in 2,502 participants (70.3%) with complete questionnaire data and covering in 769 households and 60 villages. The characteristics of those 2,502 participants are presented in Table 8.1.

The proportion of males and females, and those who had ever been treated for worms were similar in the groups of excluded and included participants. Children under the age of six were less represented in the analysed sample (7.7%) than in the group of excluded participants, due to missing diagnostic results (24.6%). This was also reflected in the variable “occupation”, for which participants spending their time “at home” were also less represented. This group of participants consisted mostly of children, as adults were evenly distributed in the two groups. Participants declaring regularly defecating in toilets or behind their house were less represented in the included group, as well as those declaring wearing shoes “sometimes or never”. All those variables were therefore adjusted for in the multivariate model, with the exception of occupation, which was collinear with age and thus removed from the model (see below).

Table 8.1: Characteristics of participants included in the analysis

Variable	**Category**	**n (%)**
Sex	Male	1,095 (43.8)
	Female	1,407 (56.2)
Age (years)	<6	193 (7.7)
	6-18	992 (39.7)
	19-49	1,015 (40.6)
	≥50	302 (12.1)
Occupation	Rice farmer	1,216 (48.6)
	School	817 (32.7)
	At home other	469 (18.7)
Level of education	No school	821 (32.8)
	Primary school	1,450 (58.0)
	Secondary school or higher	231 (9.2)
Socioeconomic status	Least poor	918 (36.7)
	Poor	815(32.6)
	Poorest	769 (30.7)
Ever treated for worms	Yes	1,754 (70.1)
	No or don't know	748 (29.9)
Reported regular defecation place	Forest	908 (36.3)
	Toilet	293 (11.7)
	Rice field or water	302 (12.1)
	Behind the house	999 (39.3)

Table 8.1 (*cont.*)

Variable	**Category**	**n (%)**
Wearing shoes, frequency	Often or always	2,139 (85.5)
	Sometimes or never	363 (14.5)
Washing hands before eating	Yes	2,304 (92.1)
	No	198 (7.9)
Washing hands after defecating	Yes	1,834 (73.3)
	No	668 (26.7)
Using soap or ashes when washing hands	Yes	768 (30.7)
	No	1,734 (69.3)
Boiling drinking water	Never	1,841 (72.3)
	At dry or wet season, but not both	191 (7.5)
	Yes, both seasons	514 (20.2)
Do you know anything about worms?	No	2,016 (80.6)
	Yes	486 (19.4)
Ever used health facility	Yes	1,935 (77.3)
	No	567 (22.7)
Distance to health facility (minutes)	Close (1 to 20 minutes)	701 (28.0)
	Less close (21 to 30 minutes)	729 (29.1)
	Least close (≥ 31 minutes)	502 (20.1)
	Not applicable	570 (22.8)
Toilet at home	No	2,216 (88.6)
	Yes	286 (11.4)
Own dog	No	873 (34.9)
	Yes	1,629 (65.1)
Own farm animals	No	201 (8.0)
	Yes	2,301 (92.0)
Main water source for general use	Open water body[a], rain	178 (7.1)
	Well	1,525 (61.0)
open water: pond canal river lake dam	Wellpump	799 (31.9)
District	Tbang Mean Chey	559 (22.3)
	Rovieng	626 (25.0)
	Chey Saen	354 (14.2)
	Choam Khsant	402 (16.1)
	Sangtom Thmei	248 (9.9)
	Kulean	313 (12.5)
Land use / Land cover	Savanna	769 (30.8)
	Forests	300 (12.0)
	Grassland	191 (7.6)
	Cropland and crop-natural vegetation mosaic	1,242 (49.6)
Soil organic carbon (g/kg)	5.00 – 9.99	1,190(47.6)
	10.00 – 19.99	1,312 (52.4)
	median (IQR)	
LST day year minimum (°K)	299.4 (2.1)	
LST night year mean (°K)	296.2 (0.7)	
Rainfall year maximum (mm/day)	16.7 (1.1)	

8.3.2 *S. stercoralis* and hookworm prevalence

About two in three (1,749/2,576) participants were infected with *S. stercoralis* (1,252 cases) or/and hookworms (1,263 cases). The overall prevalence of *S. stercoralis* was 48.6% (95% confidence interval (CI): 46.7 – 50.6), with 61.2% (95%CI: 58.4 – 63.9) of *S. stercoralis* cases being co-infected with hookworms. The overall prevalence of hookworm was 49.0% (95%CI: 47.1 – 51.0), with 60.6% (95%CI: 57.9 – 63.4) being co-infected with *S. stercoralis*. Of the 1,089 hookworm cases for which egg counts were available (i.e. 86.2% of hookworm cases with at least one positive Kato-Katz slide), most (1,044/1,089, 95.9%) were of light intensity according to the WHO classification (1–1,999 eggs/gram (EPG)), whereas the remaining infections were either of moderate (2.0%) or heavy (2.1%) intensity. Individuals infected with *S. stercoralis* had double the odds of being infected with hookworm (unadjusted odds ratio (OR): 2.49 (95%CI: 2.10 – 2.97), adjusted OR: 2.21 (95%CI 1.84 – 2.66).

8.3.3 Determinants of *S. stercoralis* and hookworm mono- and co-infection risks

The results of bivariate multinomial regressions are available in Appendix 8.8.2. Occupation and age were collinear, and occupation was removed from the model since it was not a confounder of any other variable and its removal yielded a lower AIC in the multivariate model (AIC with occupation: 6314.8, AIC without occupation: 6311.2). No interaction was found.

Table 8.2 presents the results of the multivariate Bayesian mixed multinomial regression, accounting for village heterogeneity and jointly assessing determinants of each parasite mono-infection or co-infection as compared to non-infected participants. Females had lower odds of any infection type. The risk of being infected with *S. stercoralis* peaked among participants over 50 years old, the odds of hookworm mono-infection was lower for preschool-aged (age <

six years) children, and co-infection risk was lower among preschool-aged children and adults between 19 and 49 years old. The poorest individuals had higher odds of either mono-infection or co-infection. Toilet use was a protective factor against hookworm mono-infection or co-infection, while co-infection risk was higher among participants living at larger distances from a health centre.

8.3.4 Clustering tendency of *S. stercoralis* and hookworm mono- and co-infection

S. stercoralis exhibited almost no spatial correlation as indicated by the low location-specific variance and range, even in the absence of parameters. Hookworm mono-infection and co-infection exhibited moderate clustering tendencies, both with cluster sizes of about 20 km. Parameters of the three following multinomial geostatistical models are presented in Table 8.3: (i) model without covariates, (ii) predictive model including only environmental factors, and (iii) determinant analysis model including district and demographic, socioeconomic and behavioural factors.

Table 8.2: Risk factors for hookworm and *S. stercoralis* mono- and co-infection

		S. stercoralis mono-infection		Hookworm mono-infection		Co-infection	
Variable	Category	RRR	95% BCI	RRR	95% BCI	RRR	95% BCI
Sex	Female	1.00		1.00		1.00	
	Male	**2.01**	1.56 - 2.54	**1.47**	1.15 - 1.88	**2.06**	1.65 - 2.62
Age (years)	6-18	1.00		1.00		1.00	
	< 6	0.69	0.40 - 1.22	**0.29**	0.15 - 0.52	**0.25**	0.15 - 0.40
	19-49	1.18	0.88 - 1.56	1.06	0.79 - 1.41	**0.74**	0.56 - 0.97
	≥ 50	**1.59**	1.06 - 2.37	0.87	0.57 - 1.33	0.86	0.57 - 1.27
Socioeconomic status	Least poor	1.00		1.00		1.00	
	Poor	1.1	0.82 - 1.49	0.79	0.58 - 1.08	1.13	0.84 - 1.51
	Poorest	**1.56**	1.13 - 2.17	**1.49**	1.07 - 2.05	**1.67**	1.22 - 2.33
Own dog	No	1.00		1.00		1.00	
	Yes	1.21	0.92 - 1.60	0.99	0.76 - 1.29	0.89	0.68 - 1.16
Reported regular defecation place	Forest	1.00		1.00		1.00	
	Toilet	0.65	0.41 - 1.01	**0.51**	0.31 - 0.83	**0.43**	0.25 - 0.71
	Rice field of water	0.84	0.54 - 1.31	0.72	0.44 - 1.16	0.92	0.62 - 1.37
	Behind the house	0.9	0.66 - 1.27	1.1	0.79 - 1.52	1.17	0.84 - 1.61
Boiling drinking water	Never	1.00		1.00		1.00	
	Yes during dry or wet season but not both	1.13	0.66 - 2.02	**1.9**	1.18 - 3.12	1.29	0.77 - 2.15
	Yes both dry and wet season	1.14	0.81 - 1.63	0.78	0.54 - 1.13	**0.67**	0.47 - 0.95
Wearing shoes, frequency	Often or always	1.00		1.00		1.00	
	Sometimes or never	**0.47**	0.29 - 0.76	0.71	0.46 - 1.09	0.88	0.61 - 1.30
Do you know anything about worms?	No	1.00		1.00		1.00	
	Yes	1.29	0.92 - 1.85	1.1	0.78 - 1.58	**1.48**	1.06 - 2.07

Table 8.2 (*cont.)*

		S. stercoralis mono-infection		Hookworm mono-infection		Co-infection	
Variable	Category	RRR	95% BCI	RRR	95% BCI	RRR	95% BCI
Distance to health facility (minutes)	Close (1 - 20)	1.00		1.00		1.00	
	Less close (21 - 30)	1.06	0.73 - 1.55	1.18	0.77 - 1.71	**1.53**	1.07 - 2.28
	Least close (≥ 31)	1.1	0.70 - 1.80	1.3	0.78 - 2.12	**1.7**	1.09 - 2.73
	Not applicable [(b)]	0.9	0.62 - 1.33	1.13	0.76 - 1.67	1.32	0.88 - 1.96
Ever treated for worms	No or don't know	1.00		1.00		1.00	
	Yes	0.79	0.59 - 1.06	**0.58**	0.43 - 0.77	**0.43**	0.32 - 0.57
District	Tbaeng Mean Chey	1.00		1.00		1.00	
	Rovieng	0.78	0.40 - 1.27	**0.29**	0.19 - 0.46	**0.24**	0.13 - 0.43
	Chey Saen	0.97	0.48 - 1.77	**0.34**	0.20 - 0.58	**0.51**	0.25 - 0.97
	Choam Khsant	1.96	0.93 - 3.76	0.59	0.32 - 1.04	**2.33**	1.18 - 4.31
	Sangkom Thmei	0.9	0.39 - 1.96	**2.09**	1.13 - 4.04	**2.35**	1.04 - 4.70
	Kuleaen	1.33	0.62 - 2.51	1.1	0.61 - 1.98	**2.49**	1.25 - 5.09

RRR: relative rate ratio (posterior median)

BCI: Bayesian credible interval

[a] The relative rate ratio for each multinomial outcome category compares the risk to that of non-infected participants (baseline outcome group).

[b] Participants who never used the health facility.

RRR in bold were significant at 5% level.

Data were obtained from a cross-sectional survey conducted in 2010 in 60 villages of Preah Vihear province, North Cambodia, among 2,502 participants aged 1 year and older.

Table 8.3: Model parameters of three geostatistical models

		Hookworm mono-infection		*S. stercoralis* mono-infection		Co-infection	
		median	95% BCI	median	95% BCI	median	95% BCI
Model without covariates	σ^2 [a]	0.59	0.32 - 1.20	0.23	0.10 - 0.46	1.00	0.57 - 1.91
	Range (km) [b]	23.48	3.98 - 65.2	0.44	0.21 - 12.32	19.95	5.73 - 57.76
Predictive model	σ^2 [a]	0.33	0.16 - 0.63	0.14	0.03 - 0.32	0.64	0.36 - 1.56
	Range (km) [b]	0.73	0.21 - 15.37	0.42	0.20 - 4.77	9.39	0.24 - 54.60
Determinant analysis model	σ^2 [a]	0.14	0.03 - 0.32	0.22	0.08 - 0.49	0.33	0.18 - 0.60
	Range (km) [b]	0.41	0.20 - 4.27	0.72	0.21 – 19.7	0.46	0.20 - 7.36

[a] σ^2 is the location-specific unexplained variance;

[b] the range is the distance at which the spatial correlation becomes less than 5%.

Predictive model: geostatistical multinomial model used to predict *S. stercoralis* and hookworm mono- and co-infection risk at un-surveyed locations.

Determinant analysis model: model including only socio-demographic, behavioural and district identification data.

8.3.5 Prediction of *S. stercoralis* and hookworm mono- and co-infection risks

Of the three pairs (exchangeable *vs.* geostatistical random effect) of models submitted to variable selection, model validation indicated that the geostatistical model, including LST day minimum, LST night mean, soil organic carbon, land use land cover, and RFE maximum, had the best predictive ability as assessed by the MSE. Results of the model validation are available in Appendix 8.8.3. Relative risk ratios (RRR) and parameters of the predictive model are presented in Table 8.4.

Table 8.4: Results of the geostatistical multinomial predictive model

		Hookworm mono-infection		*S. stercoralis* mono-infection		Co-infection	
		RRR [a]	95% BCI	RRR [a]	95% BCI	RRR [a]	95% BCI
LST day, year minimum		**0.73**	0.56 - 0.95	0.98	0.78 - 1.24	**0.60**	0.41 - 0.80
LST night, year mean		**0.79**	0.62 - 0.99	1.03	0.88 - 1.25	**0.66**	0.50 - 0.89
Rainfall, year maximum		1.27	0.99 - 1.60	**0.79**	0.66 - 0.95	0.91	0.67 - 1.62
Soil organic carbon (g/kg)	5.00 – 9.99			1.00			
	10.00 – 19.99	0.90	0.57 - 1.41	**0.57**	0.40 - 0.86	0.58	0.32 - 1.42
Land use/Land cover	Savanna			1-00			
	Forests	1.02	0.52 - 2.00	1.59	0.92 - 2.78	0.87	0.42 - 1.95
	Grassland	0.79	0.30 - 2.07	1.52	0.77 - 3.04	2.13	0.73 - 5.84
	Cropland and crop-natural vegetation mosaic	1.18	0.64 - 2.02	**1.71**	1.12 - 2.61	**2.20**	1.22 - 3.79

Figure 8.1 presents the maps of predicted *S. stercoralis* and hookworm mono- and co-infection risks. The two parasites, as well as co-infection cases, were ubiquitous in the province. The distributions of mono-infection with each parasite appeared complementary, with relatively higher risk of *S. stercoralis* and lower risk of hookworm in the North-West.

The highest rates of co-infection were found in the South-West, East and North of the area. Model uncertainty for each predicted outcome, as expressed by the ratio of the posterior median over its standard deviation (error coefficient), is presented in Figure 8.2. A smaller value indicates a larger degree of uncertainty.

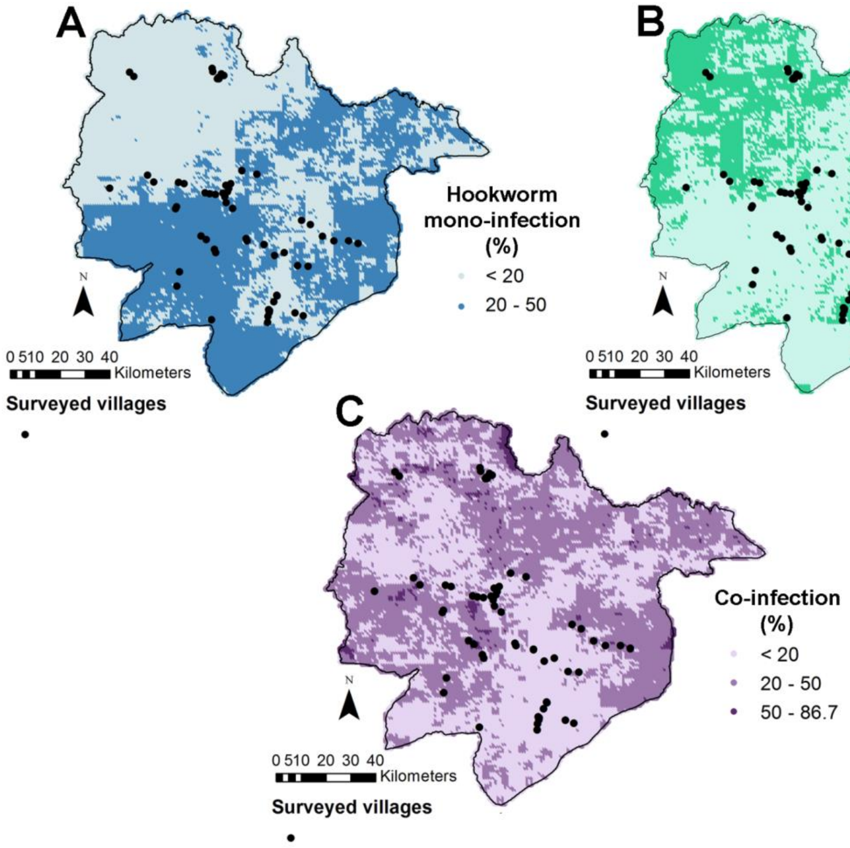


Figure 8.1: Maps of predicted hookworm mono-infection (A), *S. stercoralis* mono-infection (B) and co-infection (C) risk in Preah Vihear province, North Cambodia. Predictions correspond to the posterior median obtained with the geostatistical multinomial model described in Table 8.3.

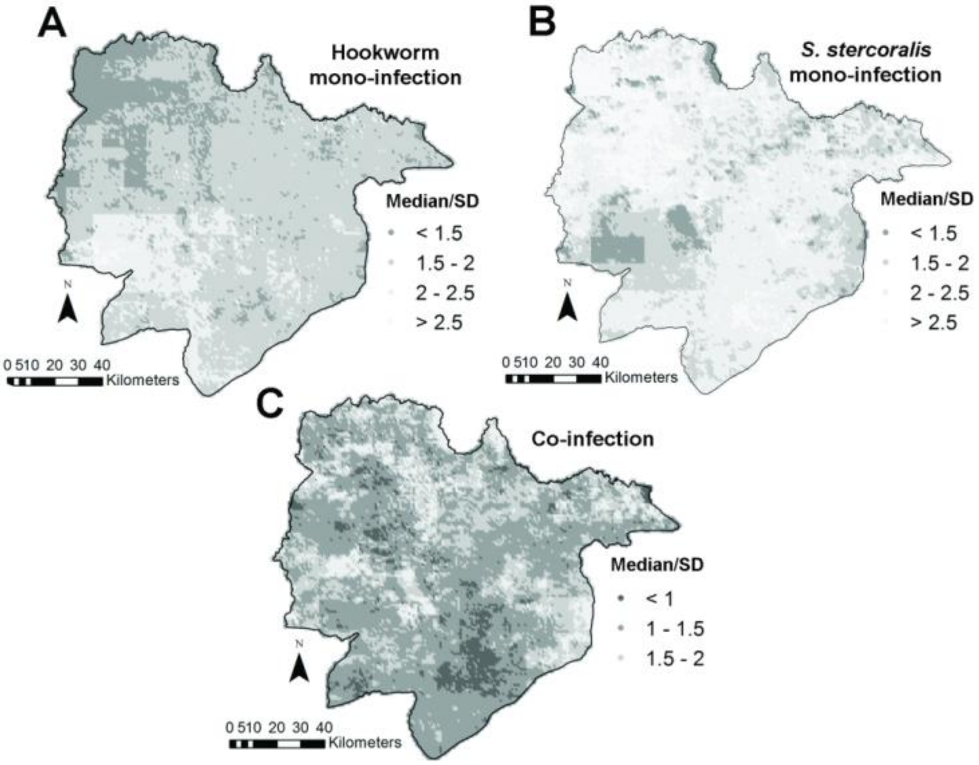


Figure 8.2 Error coefficients of the predicted hookworm mono-infection (A), *S. stercoralis* mono-infection (B) and co-infection (C) risk in Preah Vihear province, North Cambodia. The error coefficient is the ratio between predicted median and its standard deviation. Darker zones indicate higher uncertainty.

8.4 Discussion

This first *S. stercoralis*-hookworm co-risk map underlines the ubiquity of the two parasites and high prevalence rates in Preah Vihear province, with more than two in three participants infected with one of the parasites and almost half (44%) experiencing co-infections.

In Cambodia, *S. stercoralis* infection was recently found to be associated with significant morbidity, and calls for control have been acknowledged (Khieu et al., 2014b, Forrer et al., 2017). The hookworm situation, whereby half of the study participants experienced mostly light infections (2% both for moderate and heavy intensity classes), raises some questions (Forrer et al., 2018). Infection levels were particularly high in school-aged children (SAC), with a prevalence rate similar to that of the late nineties, and infection intensity levels remaining above the 1% WHO control target for each intensity class (Urbani and Albonico, 2003, Sinuon et al., 2005, Montresor et al., 2008, Montresor, 2011, WHO, 2013).

STH control programmes reportedly achieve high coverage rates in Cambodia, but the assessment methods remain unclear, particularly with respect to the impact of school enrolment (net ratio was 65.2% in 2005 in Preah Vihear) (Sinuon et al., 2005, Montresor et al., 2008, National Institute of Public Health, 2006). An important aspect of programme evaluation is to estimate both coverage, i.e. the proportion of eligible population who received tablets, and compliance, i.e. the proportion of eligible population who actually swallowed tablets; these components can be substantially different, with the latter being the best measure of programme implementation quality (Shuford et al., 2016).

Additionally, STH control programmes in Cambodia deliver mebendazole, which has a significantly weaker effect on hookworm infection than albendazole (Keiser and Utzinger, 2010, Steinmann et al., 2011, Levecke et al., 2014). Decreased mebendazole efficacy against hookworm has raised concerns in Southeast Asia (Soukhathammavong et al., 2012, Levecke et al., 2014, Flohr et al., 2007). Reasons for its low performance should be investigated and explore both potential resistance and efficacy on *Ancylostoma ceylanicum*, a hookworm of dogs and cats that is highly prevalent among humans in Southeast Asia, and in Preah Vihear province (Traub, 2013, Conlan et al., 2012, Inpankaew et al., 2014, Hotez et al., 2015, McCarty et al., 2014).

Another cause of persistent hookworm infection might be high reinfection rates, which can reach around 60% one year post-treatment for hookworm (Jia et al., 2012). Documenting hookworm reinfection rates in the country might help disentangle the reasons why hookworm infection levels remain high (Forrer et al., 2016).

Individuals living in the poorest households had higher odds of co-infection or mono-infection with any parasite, which reconfirms that STH infections, including *S. stercoralis*, are diseases of poverty (Bethony et al., 2006, Hotez et al., 2009, Hotez, 2003). The use of a robust method to construct the socioeconomic index might explain this result, since *S. stercoralis* was not associated with socioeconomic status in the few studies that accounted for it (Khieu et al., 2014c, Conlan et al., 2012). The higher odds of infection with either parasite alone or concurrently among males were in line with results from other studies conducted in Asia (Vannachone et al., 1998, Nontasut et al., 2005, Steinmann et al., 2007a, Conlan et al., 2012, Khieu et al., 2014c, Forrer et al., 2015). School-aged children and adolescents had the highest risk of being co-infected, suggesting that this age group is an important target for control of both parasites in this setting. Treatment decreased the odds of hookworm mono-infection, but did not affect the odds of being infected with *S. stercoralis,* against which it is not effective at a single oral dose (Keiser and Utzinger, 2010). Defecating in toilets, boiling drinking water and having been treated with anthelmintics were protective factors against co-infection. The role of sanitation in STH transmission is widely acknowledged, whereas the association between systematically boiling drinking water and co-infection is unexpected, given the infection route of these two parasites (Strunz et al., 2014, Freeman et al., 2014, Ziegelbauer et al., 2012, Echazú et al., 2015). This variable most likely acted as a proxy for overall hygienic health-promoting behaviour. Yet, an oral infection route has not been excluded for *S. stercoralis*; *Ancylostoma duodenale* can also infect orally, although it occurs rarely in this setting (Grove, 1996, Hawdon and Hotez, 1996, Inpankaew et al., 2014).

The spatial dependence of co-infection was mostly attributable to hookworm, as *S. stercoralis* exhibited almost no clustering tendency, even in the absence of covariates. This does not preclude, however, that *S. stercoralis* risk would cluster at a larger scale. Environmental factors partly explain the clustering of all outcomes (as indicated by the decrease in unexplained variance (σ^2) and the drop of the range after inclusion of environmental variables). Climatic parameters influencing the distribution of the two parasites have been discussed elsewhere, Two previously identified factors are increased temperature and rainfall, either preventing or favouring larvae survival through desiccation or wetness, respectively (Brooker et al., 2004a, Anamnart et al., 2013, Khieu et al., 2014b). Model parameters indicate that socio-demographic and behavioural variables played an important role in explaining the spatial variation of hookworm mono-infection and co-infection, but less so for *S. stercoralis* mono-infection. This result might reflect variability in mebendazole PC effectiveness across provincial regions, which cannot be excluded, despite adjusting for anthelmintic treatment. Indeed, we only adjusted for self-reported treatment, which is subject to recall bias, particularly after several deworming rounds (Chesnaye et al., 2011). We analysed data from 2010; since then, STH control with mebendazole has been scaled up to include additional age groups. Up-to-date studies accounting for programme coverage and compliance would help to assess the respective roles of environment, control and behaviour on infection levels with the two parasites.

Our study has some limitations. Although including only the negative participants with complete results ensured high specificity while keeping the maximum number of positive observations in the sample, it resulted in overestimated prevalence rates for both parasites, i.e. 48.6% vs. 44.7% with a complete case analysis for *S. stercoralis* and 49.0% vs. 39.6% for hookworm. Additionally, we did not address infection intensity of hookworm infection but most cases were of light intensity and geostatistical predictive models for infection intensity usually yield substantial uncertainty, particularly in low intensity settings (Clements et al., 2006a, Soares Magalhães et al., 2011a, Forrer et al., 2015). Children under six years were under-represented in the sample, due to insufficient stool amounts for performing the Baermann technique, a shortcoming that will keep occurring as long as combining Baermann and KAP provides the most sensitive diagnostic approach for prevalence surveys. This aspect underlines the need for new techniques to diagnose *S. stercoralis* in endemic settings (Buonfrate et al., 2015a, Albonico et al., 2016). No established prevalence thresholds exist for *S. stercoralis,* so we used those defined for other STH, although infection levels relating to the public health importance of this parasite might differ (Bisoffi et al., 2013, Forrer et al., 2018). The relationship between *S. stercoralis* infection intensity and morbidity, including potential co-morbidity arising from multiple infections needs to be investigated (Ezeamama et al., 2005, Steinmann et al., 2010).

There is a general agreement that *S. stercoralis,* now recognised as a major public health problem in Cambodia, needs to be integrated into existing STH control programmes (Khieu et al., 2013b, Khieu et al., 2014b, Khieu et al., 2014c, Khieu et al., 2014a, Forrer et al., 2016, Forrer et al., 2018). Under the present national policy, this integration would result in combining mebendazole with ivermectin, a combination found to be safe among schoolchildren in Zanzibar, but that has otherwise been rarely investigated (Reddy et al., 2007, Olsen, 2007, Knopp et al., 2010a, Keiser and Utzinger, 2010). The combination of ivermectin with albendazole has largely proven to be safe for long-term use to control filarial diseases and also appears to add-value in the treatment of STH, including hookworm . (Lammie et al., 2006, Reddy et al., 2007, Hotez, 2009, Knopp et al., 2010a, Keiser and Utzinger, 2010, WHO, 2010, WHO, 2013, Yong et al., 2014, Moore et al., 2015, Kuong et al., 2016, WHO, 2016b).

Drug combination is usually associated with a lower risk of emerging resistance, however the appearance of resistance to benzimidazoles might be accelerated by combining it with ivermectin, so any co-distribution should be closely monitored (Prichard et al., 2012). Cost-effectiveness studies of *S. stercoralis* control options, including the ancillary benefits of extending hookworm control coverage to adults and a potential switch to albendazole, are also needed.

The high impact that community-based, targeted ivermectin treatment achieved in Cambodia suggests that *S. stercoralis* control with PC is feasible. This assertion is supported by studies in several countries that documented a high ancillary impact of large-scale distribution of ivermectin against filarial diseases on *S. stercoralis* (Knopp et al., 2009a, Knopp et al., 2010a, Mohammed et al., 2012, Anselmi et al., 2015, Forrer et al., 2018). Still, longitudinal studies should be conducted to further confirm the impact of community-based PC with ivermectin in endemic settings of various transmission levels (Forrer et al., 2016).

A major issue limiting the implementation of *S. stercoralis* control in Cambodia is the high cost of ivermectin, which is not donated in areas free of onchocerciasis. Ivermectin subsidization, donation, or production in the form of affordable generics are more likely once *S. stercoralis* is recognised as a public health problem and listed in the WHO PC strategy. A sensitive rapid diagnostic test would be crucial for efficient data collection and prevalence estimation at large scale (Albonico et al., 2016).

In the meantime, the efficacy of both benzimidazoles against human and canine hookworm species occurring in the region need to be assessed. If a suboptimal efficacy of mebendazole was to be confirmed in the country, WHO should consider donating albendazole to Cambodia, whatever the control status of *S. stercoralis*. Indeed, the lost benefits of continuing to distribute an ineffective drug against one of the most prevalent STH in the country would be unfortunate, particularly in a country that benefits from a well-established and effective control network, was among the first countries to achieve the WHO STH control targets, and has eliminated lymphatic filariasis and trachoma as public health problems; in brief, a country that has a clear capacity to further tackle helminth infections (Sinuon et al., 2005, Flohr et al., 2007, Montresor et al., 2008, National Center for Parasitology, 2014, Hotez et al., 2015, WHO, 2016c, WHO, 2017b).

8.5 Conclusion

Both *S. stercoralis* and hookworm were highly prevalent in the province. Benzimidazoles delivered to control other STH are not effective against *S. stercoralis* infection and ivermectin should be integrated into STH control programmes to address strongyloidiasis, which is highly prevalent in Cambodia and is associated with significant morbidity. Infection levels of hookworm, despite several years of biannual school-based deworming with mebendazole, were also high, even among school-aged children. The potential heavy co-morbidity due to overlapping hookworm and *S. stercoralis* infections should be investigated. Additionally, the effectiveness of the ongoing STH control programme should be assessed, particularly with regard to the efficacy of mebendazole against hookworm infection, including the zoonotic *A. ceylanicum*, which commonly infects humans in Cambodia. Control programmes should also be assessed in terms of the coverage and compliance achieved. Finally, the high cost of ivermectin, at 10–40 USD per treatment, is a major obstacle to the implementing *S. stercoralis* PC control in the country. Subsidies or ivermectin donations are needed to start tackling *S. stercoralis*, which is a public health problem in Cambodia.

8.6 Ethics approval and consent to participate

Ethical approval was obtained from the National Ethics Committee for Health Research, Ministry of Health, Cambodia (NECHR, #004, dated 5 February 2010) and the ethics committee of the Cantons of Basel-Stadt and Baselland (EKBB; reference no. 16/10, February 1st, 2010). Written informed consent was obtained from all adult participants, and from the parents or legal guardian of participants aged between one and 18 years. S. stercoralis infections were treated with a single oral dose of ivermectin (200µg/kg BW), while hookworm infections were treated with a single oral dose of mebendazole (500 mg). Other helminths and protozoa were treated according to the guidelines of the National Helminth Control Program of Cambodia (CNM, 2004).

8.7 Acknowledgements

We are grateful to the participants and local authorities of Preah Vihear province. We deeply thank the laboratory technicians and staff from the Helminth Control Program of the National Centre for Parasitology, Entomology and Malaria Control in Phnom Penh and from the Preah Vihear Provincial Health Department for their excellent laboratory and field work under difficult field conditions. We thank Ms. Nadja Cereghetti for her help in the laboratory. We are very grateful to of the chiefs of Rovieng and Kulen Health Centres for their support. We thanks Mrs Amena Briet for her efficient English editing.

8.8 Appendix

8.8.1 Formulation of the multinomial model

Analysis of hookworm and *S. stercoralis* mono- and co-infection: multinomial model

Let Y_{jk} and n_j be the number of infected and number of screened, and let us define the probability of hookworm mono-infection (k = 1), *S. stercoralis* mono-infection (k = 2), co-infection with the two species (k = 3) and of no infection (k = 4) at each surveyed village j, j = 1,…. 60.

We assume that Y_{jk} follows a multinomial distribution, $Y_{jk} \sim MN(n_j, p_{jk})$ and model the log odds of infection in each multinomial category h, $h = 1 \ldots 3$, *vs.* the baseline category (no infection) as follows:

$\log(p_{jh}/p_{j4})$ = α +$\sum_{l=1}^{n}\beta_{lh}\,X_{jhl} + \phi_j$, where p_{jh}/p_{j4} is the risk ratio between infection status and no infection, X_{hlj} is the ith explanatory variable, β_{lh} are the vector coefficients for each explanatory variable and multinomial category h, and ϕ_j is the locational random effect for each village (see below).

Random effects

We consider $\phi_h = (\phi h_1, \dots, \phi_{h51})^T$, where h = 1 in the logistic model and h = 1 … 3 in the multinomial model (each multinomial category has a specific random effect) , to be either an exchangeable random effect (non-spatial models) or a geostatistical random effect (spatial models). In particular, we assume ϕ ~ N(0 , $\sigma^2 R_{ij}$) where σ^2 is the variance parameter and $R_{ij.}$is the correlation matrix between locations. Under an exchangeable prior distribution $R_{ij} = 0$ if i $\neq j$ and $R_{ij} = 1$ if $i = j$. Under the assumption of a spatial stationary isotropic process, $R_{ij} = \exp(d_{ij}, \rho)$, where d_{ij} is the Euclidean distance between two locations s_i and s_j, and ρ is a measure of how spatial correlation decreases with the distance. The distance at which the spatial correlation between villages gets under 5% is equal to $3/\rho$ and is called the range.

A vague inverse gamma prior with mean 1 and variance 100 was chosen for σ^2, and a uniform prior for ρ with parameters calculated as a function of the minimum and maximum distance between sampled villages was adopted, that is: .

We chose a vague Normal distribution with a mean of zero and a variance of 1000 for all the regression coefficients.

8.8.2 Results of the variable selection using bivariate multinomial regressions

Variable	**Category**	**_S.stercoralis_-hookworm co-infection**			**Hookworm mono-infection**			**_S. stercoralis_ mono-infection**		
		OR	**95% CI**	**LRT p-value**	**OR**	**95% CI**	**LRT p-value**	**OR**	**95% CI**	**LRT p-value**
Sex	Male	1.00								
	Female	0.58	0.47 - 0.70	< 0.0001	0.79	0.63 - 0.99	< 0.0001	0.58	0.46 - 0.72	< 0.0001
Age (years)	6-18	1.00								
	< 6	0.37	0.26 - 0.55	< 0.0001	0.33	0.2 - 0.55	< 0.0001	0.52	0.33 - 0.83	< 0.0001
	19-49	0.85	0.68 - 1.06		1.17	0.91 - 1.49		1.38	1.07 - 1.79	
	≥ 50	1.03	0.74 - 1.44		0.99	0.67 - 1.46		1.88	1.31 - 2.70	
Occupation	Rice farmer	1.00								
	At home, other	0.69	0.53 - 0.90	< 0.0001	0.46	0.33 - 0.63	< 0.0001	0.56	0.41 - 0.76	< 0.0001
	School	0.89	0.71 - 1.11		0.85	0.67 - 1.09		0.62	0.47 - 0.80	
Socioeconomic status	Least poor	1.00								
	Poor	1.18	0.93 - 1.49	< 0.0001	1.02	0.78 - 1.34	< 0.0001	1.07	0.82 - 1.39	< 0.0001
	Poorest	1.80	1.41 - 2.30		1.94	1.48 - 2.55		1.26	0.95 - 1.67	
Level of education	Primary school	1.00								
	No school	1.21	0.98 - 1.50	0.2188	0.92	0.72 - 1.18	0.2188	1.01	0.79 - 1.30	0.2188
	Secondary school or higher	0.94	0.66 - 1.34		1.04	0.70 - 1.52		1.2	0.82 - 1.75	
Reported regular defecation place	Forest	1.00								
	Toilet	0.24	0.17 - 0.36	< 0.0001	0.41	0.28 - 0.60	< 0.0001	0.61	0.43 - 0.85	< 0.0001
	Rice field of water	1.18	0.85 - 1.63		0.65	0.44 - 0.98		0.89	0.62 - 1.30	
	Behind the house	1.01	0.81 - 1.27		0.86	0.67 - 1.11		0.68	0.52 - 0.89	
Wearing shoes, frequency	Often or always	1.00								
	Sometimes or never	0.74	0.57 - 0.96	< 0.0001	0.55	0.40 - 0.76	< 0.0001	0.37	0.26 - 0.54	< 0.0001
Washing hands after defecating	Yes	1.00								
	No or don't know	1.13	0.91 - 1.41	0.5877	1.17	0.91 - 1.50	0.5877	1.07	0.83 - 1.38	0.5877
Washing hands before eating	Yes	1.00								
	No or don't know	1.18	0.83 - 1.69	0.7014	0.95	0.62 - 1.45	0.7014	1	0.66 - 1.52	0.7014
Using soap or ashes when washing hands	No	1.00								

Variable	**Category**	***S.stercoralis*-hookworm co-infection**			**Hookworm mono-infection**			***S. stercoralis* mono-infection**		
	Yes	0.82	0.66 - 1.02	0.118	0.78	0.61 - 1.00	0.118	0.97	0.77 - 1.24	0.118
Ever treated for worms	Yes	1.00								
	No or don't know	0.50	0.40 - 0.62	< 0.0001	0.58	0.45 - 0.74	< 0.0001	0.68	0.54 - 0.86	< 0.0001
Do you know anything about worms?	No	1.00								
	Yes	1.20	0.94 - 1.55	0.0875	1.11	0.83 - 1.48	0.0875	1.42	1.08 - 1.88	0.0875
Ever used the health facility	Yes	1.00								
	No	0.71	0.56 - 0.90	0.044	0.9	0.69 - 1.17	0.044	0.89	0.68 - 1.15	0.044
Distance to health facility (minutes)	Close (1 to 20 minutes)	1.00								
	Less close (21 to 30 minutes)	1.64	1.26 - 2.13	0.0004	1.36	1.01 - 1.83	0.0004	1.18	0.88 - 1.58	0.0004
	Least close (≥ 31 minutes)	1.69	1.26 - 2.25		1.45	1.05 - 2.01		0.97	0.69 - 1.36	
	Not applicable	1.00	0.76 - 1.33		1.13	0.82 - 1.54		0.94	0.69 - 1.27	
Toilet at home	No	1.00								
	Yes	0.24	0.17 - 0.35	< 0.0001	0.44	0.31 - 0.63	< 0.0001	0.73	0.53 - 0.996	< 0.0001
Main water source for general use	Open water body, rain									
	Well	0.50	0.34 - 0.75	0.0133	0.56	0.36 - 0.88	0.0133	0.6	0.38 - 0.95	0.0133
	Wellpump	0.50	0.33 - 0.76		0.66	0.41 - 1.06		0.69	0.43 - 1.12	
Source of drinking water, wet season	Well									
	Wellpump	0.98	0.75 - 1.28	0.1194	1.24	0.93 - 1.66	0.1194	1.06	0.79 - 1.42	0.1194
	Rain	1.12	0.88 - 1.42		1.24	0.94 - 1.62		0.99	0.75 - 1.30	
	Open water body	1.86	1.21 - 2.88		1.86	1.14 - 3.02		1.32	0.79 - 2.21	
Source of drinking water, dry season	Well									
	Wellpump	0.98	0.79 - 1.21	0.036	1.13	0.89 - 1.44	0.036	1.22	0.96 - 1.55	0.036
	Open water body, rain	1.78	1.18 - 2.68		1.55	0.97 - 2.48		1.26	0.76 - 2.08	
Boiling drinking water	Never									
	Yes during dry or wet season but not both	1.22	0.82 - 1.81	0.0001	1.79	1.18 - 2.69	0.0001	1.03	0.64 - 1.66	0.0001
	Yes both dry and wet season	0.64	0.50 - 0.83		0.79	0.60 - 1.05		1.09	0.83 - 1.42	

Variable	**Category**	***S.stercoralis*-hookworm co-infection**			**Hookworm mono-infection**			***S. stercoralis* mono-infection**		
Own dog	No									
	Yes	0.77	0.63 - 0.95	0.0064	0.9	0.71 - 1.13	0.0064	1.16	0.91 - 1.48	0.0064
Own farm animals	No									
	Yes	0.61	0.43 - 0.88	0.066	0.72	0.48 - 1.10	0.066	0.74	0.49 - 1.14	0.066
Number of family members		1.01	0.96 - 1.06	0.3535	0.96	0.91 - 1.02	0.3535	1.01	0.96 - 1.07	0.3535
Land use/Land cover	Savanna									
	Forests	1.09	0.77 - 1.55	< 0.0001	0.94	0.65 - 1.37	< 0.0001	1.46	0.98 - 2.18	< 0.0001
	Grassland	0.53	0.35 - 0.80		0.38	0.23 - 0.61		1.15	0.75 - 1.75	
	Cropland and crop-natural vegetation mosaic	1.15	0.92 - 1.45		0.67	0.52 - 0.86		1.52	1.16 - 2.00	
Soil organic carbon (g/kg)	5.00 – 9.99									
	10.00 – 19.99	0.37	0.30 - 0.45	< 0.0001	0.51	0.41 - 0.64	< 0.0001	0.69	0.55 - 0.86	< 0.0001
LST day, year minimum		0.80	0.74 - 0.86	< 0.0001	0.77	0.71 - 0.83	< 0.0001	1.02	0.94 - 1.11	< 0.0001
LST night, year mean		0.35	0.28 - 0.44	< 0.0001	0.47	0.36 - 0.60	< 0.0001	0.98	0.75 - 1.26	< 0.0001
Rainfall, year maximum		1.09	1.01 - 1.17	< 0.0001	1.3	1.19 - 1.41	< 0.0001	0.91	0.83 - 0.99	< 0.0001
District	Tbaeng Mean Chey									
	Rovieng	0.33	0.24 - 0.45	< 0.0001	0.3	0.22 - 0.41	< 0.0001	0.77	0.56 - 1.07	< 0.0001
	Chey Saen	0.75	0.53 - 1.07		0.41	0.28 - 0.60		1.14	0.79 - 1.65	
	Choam Khsant	1.98	1.42 - 2.75		0.51	0.34 - 0.77		1.62	1.11 - 2.36	
	Sangkom Thmei	2.79	1.83 - 4.25		2.05	1.33 - 3.16		0.99	0.56 - 1.74	
	Kuleaen	2.28	1.59 - 3.26		1.01	0.67 - 1.50		1.28	0.82 - 1.99	

OR: odds ratio; CI: confidence interval; LRT: likelihood ratio test.

[a] The relative rate ratio for each outcome category compares the risk to that of non-infected participants (baseline outcome group).

[b] open water: pond canal river lake dam.

Data were obtained from a cross-sectional survey conducted in 2010 in 60 villages of Preah Vihear province, North Cambodia, among 2,502 participants aged 2 years and above

8.8.3 Results of model validation for predictive models

Model	**Total MSE**
Model 1, non spatial	508.31
Model 1, spatial	468.85
Model 2, non spatial	722.94
Model 2, spatial	686.73
Model 3, non spatial	682.84
Model 3, spatial	700.96

Model 1: LST Day minimum, LST night mean, Soil organic carbon, land use land cover, RFE maximum

Model 2: LST Day minimum, LST night mean, Soil organic carbon, land use land cover, district

Model 3: LST Day minimum, LST night mean, Soil organic carbon, land use land cover, RFE maximum, district

non spatial: including an exchangeable random effect

spatial: including a geostatistical random effect

Chapter 9

Risk Profiling of Hookworm Infection and Intensity in Southern Lao People's Democratic Republic Using Bayesian Models

Armelle Forrer[1,2], Penelope Vounatsou[1,2], Somphou Sayasone[1,2,3], Youthanavanh Vonghachack[1,2,4], Dalouny Bouakhasith[4], Jürg Utzinger[1,2], Kongsap Akkhavong[3], Peter Odermatt[1,2*]

1 Department of Epidemiology and Public Health, Swiss Tropical and Public Health Institute, Basel, Switzerland, **2** University of Basel, Basel, Switzerland, **3** National Institute of Public Health, Ministry of Health, Vientiane, Lao People's Democratic Republic, **4** Faculty of Basic Sciences, University of Health Sciences, Vientiane, Lao People's Democratic Republic

This paper has been published in PLoS Neglected Tropical Diseases 2015, 9(3) e0003486.

Abstract

Background: Among the common soil-transmitted helminth infections, hookworm causes the highest burden. Previous research in the southern part of Lao People's Democratic Republic (Lao PDR) revealed high prevalence rates of hookworm infection. The purpose of this study was to predict the spatial distribution of hookworm infection and intensity, and to investigate risk factors in the Champasack province, southern Lao PDR.

Methodology: A cross-sectional parasitological and questionnaire survey was conducted in 51 villages. Data on demography, socioeconomic status, water, sanitation, and behaviour were combined with remotely sensed environmental data. Bayesian mixed effects logistic and negative binomial models were utilized to investigate risk factors and spatial distribution of hookworm infection and intensity, and to make predictions for non-surveyed locations.

Principal Findings: A total of 3,371 individuals were examined with duplicate Kato-Katz thick smears and revealed a hookworm prevalence of 48.8%. Most infections (91.7%) were of light intensity (1-1,999 eggs/g of stool). Lower hookworm infection levels were associated with higher socioeconomic status. The lowest infection levels were found in preschool-aged children. Overall, females were at lower risk of infection, but women aged 50 years and above harbored the heaviest hookworm infection intensities. Hookworm was widespread in Champasack province with little evidence for spatial clustering. Infection risk was somewhat lower in the lowlands, mostly along the western bank of the Mekong River, while infection intensity was homogeneous across the Champasack province.

Conclusions/Significance: Hookworm transmission seems to occur within, rather than between villages in Champasack province. We present spatial risk maps of hookworm infection and intensity, which suggest that control efforts should be intensified in the Champasack province, particularly in mountainous areas.

9.1 Introduction

Hookworm disease is caused by chronic infection with *Ancylostoma duodenale* or *Necator americanus* and is of considerable public health importance in low- and middle- income countries in the tropics and subtropics (Hotez et al., 2004). In 2010, an estimated 439 million people were infected with hookworm, causing a global burden of 3.2 million disability-adjusted life years (Murray et al., 2012, Pullan et al., 2014). Depending on hookworm infection intensity, the morbidity may range from mild and transient symptoms to severe disease, negatively impacting on child growth and cognitive development, and worker productivity in older age (Bethony et al., 2006). Chronic hookworm infection cause intestinal blood loss and may result in poor iron status and iron deficiency anemia, particularly in children, women of reproductive age, and individuals with high worm loads (Bethony et al., 2006, Brooker et al., 2008, Smith and Brooker, 2010).

Preventive chemotherapy – that is the periodic administration of albendazole or mebendazole to school-aged children and other high-risk groups – is the backbone of the global control against hookworm and other soil-transmitted helminth infections (WHO and Crompton, 2006, WHO, 2010). The Ministry of Health (MoH) of Lao People's Democratic Republic (Lao PDR) has adopted the national scheme for school deworming. Since 2005, two annual rounds of deworming are conducted among school-aged children (Phommasack et al., 2008). In addition, anthelmintic treatment has been provided to preschool-aged children through the Expanded Program on Immunization (EPI), and alongside vitamin A distribution campaigns (Kounnavong et al., 2011, WHO, 2012b). However, in face of continued exposure to contaminated environment and unhygienic behaviour, re-infection with soil-transmitted helminths is rapid (Jia et al., 2012, Yap et al., 2013). Hence, the identification and geographic delineation of areas that are at high risk of hookworm transmission is crucial for spatial targeting and fostering control activities.

The purpose of this study was to identify risk factors for hookworm infection and intensity in Champasack province, southern Lao PDR, and to predict infection risk and intensity in non-surveyed locations. These findings are important to enhance local control efforts and to inform the national helminthiasis control programme.

9.2 Methods

9.2.1 Ethics Statement

The study was approved by the institutional research commission of the Swiss Tropical and Public Health Institute (Swiss TPH; Basel, Switzerland). Ethical approval was obtained from the MoH of Lao PDR (reference no. 027/NECHR) and the ethics committee of Basel (EKBB; reference no. 255/06). Permission for field work was obtained from the MoH, the Provincial Health Office (PHO), and the District Health Office (DHO). Because literacy is low in Champasack province, individual oral consent was obtained from all adult household members. Additionally, written informed consent was obtained from heads of households. This consent procedure was approved by the aforementioned ethics committees. Participants infected with hookworm were treated with a single 400 mg oral dose of albendazole. Other parasitic infections were treated according to national guidelines (MOH, 2004).

9.2.2 Study Area

The study was carried out in Champasack, the largest province of southern Lao PDR, with an area of 15,415 km^2 and a population of 603,370 in 2005 (National Statistics Centre of the Lao, 2005). The province stretches from 13°55' to 15°29' N latitude and from 105°11' to 106°46' E longitude and is crossed by the Mekong River from North to South. The climate is of monsoon tropical type and the rainy season occurs between May and October. Soil-transmitted helminths, *Opisthorchis viverrini*, *Schistosoma mekongi*, and minute intestinal flukes (MIF) are endemic in this province (Rim et al., 2003, Chai et al., 2005, Chai et al., 2007, Muth et al., 2010, Sayasone et al., 2011, Forrer et al., 2012, Chai et al., 2013).

9.2.3 Parasitological, Demographic, Socioeconomic, and Behavioural Data

Epidemiological data were obtained from a cross-sectional, community-based survey carried out between January and May 2007 in all nine rural districts of Champasack province. The tenth district, which is primarily urban, was excluded. Sample selection was achieved using a two-stage sampling method: first a random selection of villages and, second, random selection of 10-15 households per village. All individuals aged 6 months and above were eligible. Overall, 4,380 participants in 51 villages were selected. A single stool sample was collected from each participant. Samples were screened for eggs of soil-transmitted helminths, *O. viverrini*, and S. *mekongi*. Duplicate 41.7 mg Kato-Katz thick smears were prepared from each stool sample the same day of collection, and the slides were examined under a microscope within 30-45 min by experienced laboratory technicians (Katz et al., 1972). Helminth eggs were counted and recorded for each species separately. A random sample of 10% of the Kato-Katz thick smears were re-examined by a senior technician for quality control.

Data on demography (age, sex, ethnic group, main occupation, and educational attainment) and hygiene (hand washing, wearing shoes) were obtained from each participant by means of a pre-tested questionnaire. Heads of household were interviewed, and information collected about household characteristics, water and sanitation, and asset ownership. The geographic coordinates of each household were recorded using a hand-held global positioning system (GPS) device (Garmin Ltd.; Olathe, United States of America).

9.2.4 Environmental Data

Enhanced vegetation index (EVI), day and night land surface temperature (LST), and land use/land cover (LULC) consisting of 18 land cover type 1 classes (IGBP) at a spatial resolution of 1 x 1 km were downloaded from Moderate Resolution Imaging Spectroradiometer (MODIS). Instead of normalized difference vegetation index (NDVI), EVI was used, since it is more sensitive to differences in heavily vegetated areas, and is more appropriate in areas such as Southeast Asia (see; http://earthobservatory.nasa.gov/Features/MeasuringVegetation/measuring_vegetation_4.php). Rainfall estimates (RFE) at 0.1 degree (about 10 x 11 km) resolution were obtained from the National Oceanic and Atmospheric Administration's (NOAA) Climate Prediction Center (CPC) Famine Early Warning System (FEWS) Rainfall Estimates South Asia, version 2.0 (see; http://www.cpc.ncep.noaa.gov/products/fews/SASIA/rfe.shtml). Digital elevation data at a resolution of 90 x 90 m were retrieved from the NASA Shuttle Radar Topographic Mission's (SRTM) Consortium for Spatial Information of the Consultative Group for International Agricultural Research (CGIAR-CSI) database. Soil type data at a spatial resolution of 9 x 9 km, including soil pH, bulk density, and organic carbon content, were extracted from the International Soil Reference and Information Center's (ISRIC) World Inventory Soil Emission Potentials (WISE), version 1.0 (see; http://www.isric.org). LULC classes were merged into four categories according to similarity and respective frequencies. Yearly means, minima, and maxima of EVI, LST, and RFE were calculated for a 1-year period (May 2006 to April 2007).

9.2.5 Statistical Analysis

All survey data were double-entered using EpiData version 3.1 (Epidata Association; Odense, Denmark) and validated. Environmental data processing, geo-referencing, and map drawing were made in ArcMap version 10 (ESRI; Redlands, United States of America). Environmental data were linked to parasitological and questionnaire data, by unique location. Data management and analysis of proportions and means were done in STATA version 12 (StataCorp LP; College Station, United States of America).

Two outcomes were considered in this study. First, the hookworm infection status of a participant, which was considered positive, if at least one hookworm egg was found in any of the two Kato-Katz thick smears. Second, the intensity of infection, which was calculated as follows. For each participant, the two hookworm egg counts from the duplicate Kato-Katz thick smears were summed up and multiplied by a factor 12 to obtain a standard measure of eggs per 1 g of stool (EPG). Intensity classes were created based on cut-offs put forward by the World Health Organization (WHO): light (1-1,999 EPG), moderate (2,000-3,999 EPG), and heavy (≥4,000 EPG) (WHO, 2002). Age was categorized into five groups: (i) <5 years; (ii) 5-17 years, (iii) 18-34 years, (iv) 35-49 years, and (v) ≥50 years. Original categories of variables with frequencies under 5% were merged with similar categories. A socioeconomic index was built using house construction material and asset ownership, using multiple correspondence analysis (MCA) (Asselin and Tuan Anh, 2008, Booysen et al., 2008). Households were classified in five wealth quintiles, the first quintile corresponding to the most poor and the fifth to the least poor.

The geometric mean EPG was calculated including both positive and zero counts and using the natural logarithm of the EPG augmented by 1 (ln(EPG+1)). Interactions were checked for hookworm infection prevalence and intensity, in absence of random effects in STATA, using logistic and negative binomial (NB) regression, respectively, and the likelihood ratio test (LRT). To explore the relationship between each outcome and age, smoothed age-prevalence and age-intensity curves were produced with the “mkspline” command in STATA, that regresses each outcome against a new age variable containing a restricted cubic spline of age. The same approach was used to describe the relationship between hookworm prevalence and infection intensity at village-level. Mean intensity of infection was regressed against a new prevalence variable containing restricted cubic splines of village-level prevalence, using a NB regression model.

9.2.6 Model Selection for Hookworm Infection Risk

Mixed effects logistic regressions were used to model hookworm infection risk. First, to identify the best set of environmental variables to model infection risk, variable selection was performed with a Bayesian approach, the stochastic search variable selection (SSVS), using alternately non-spatial (exchangeable) or geostatistical random effects (George and McCulloch, 1993). An explicit description of this method is available elsewhere (Giardina et al., 2012). Summary measures for LST, EVI, RFE, altitude, soil pH, bulk density (aimed as a proxy for soil type, a bulk density of 1.6 kg/dm^3 corresponding to sandy soils), carbon organic content for the upper 20 cm soil layer, and land cover were fed into the aforementioned models.

Second, model validation was used to identify the model with the best predictive ability. Models including environmental covariates selected as described above were run using 10 of the 51 surveyed villages (19.6%) as test locations, while model fitting was performed on the remaining 41 villages (80.4%). To assess the predictive ability of each model, predictions for the 10 test locations were compared to observed prevalence using the mean squared error (MSE), which is the squared difference between the predicted and the observed values. The deviance information criterion (DIC) was also used to compare model fit.

9.2.7 Model Selection for Hookworm Infection Intensity

Intensity of infection was approximated by the number of excreted eggs (Hotez et al., 2008). Because worm burden tends to be highly aggregated across individuals, intensity data contains many zero egg counts and are over-dispersed compared to Poisson. Count distributions accounting for data over-dispersion include negative binomial (NB), zero-inflated Poisson (ZIP), or zero-inflated negative binomial (ZINB). The NB distribution incorporates extra-Poisson variation through a dispersion parameter, r. Zero-inflated models account for over-dispersion by assuming two sources of zeros, (i) structural (non-random) zeros with probability π (mixing proportion), and (ii) zeros arising from the count distribution, i.e., Poisson (ZIP) or NB (ZINB), with probability $1 - \pi$ (Vounatsou et al., 2009). Further information about model specification is available in Appendix 9.6.1.

Similarly to prevalence, variable selection was first conducted to identify the best set of environmental covariates, for spatial and non-spatial NB, ZIP, and ZINB models. Then, to identify the model with the best predictive ability, geostatistical and non-spatial NB, ZIP, and ZINB models were fitted, using the environmental sets of covariates selected for each model. Similarly as for infection risk, 41 locations were used for model fitting and the remaining 10 villages were used as test locations. Village-level means of predicted intensities at test locations obtained from all distributions were compared to the observed intensity, using the MSE. The DIC measure was also used to compare model fit.

9.2.8 Bayesian Models of Hookworm Prevalence and Intensity of Infection

Three series of models were run for each outcome, using the model formulation with the best predictive ability identified by model validation. First, models without covariates using alternately a geostatistical and an exchangeable random effect were run to quantify the extent of village-level spatial correlation and unexplained variance of hookworm prevalence or intensity of infection. Second, a risk factor analysis was performed for each outcome, using demographic, socioeconomic, behavioural, and environmental determinants. Third, predictions of infection risk and intensity at non-surveyed locations were made with models using environmental covariates only.

9.2.9 Risk Factor Analysis of Hookworm Infection Risk and Intensity

For each outcome, the model with the best predictive ability was used to identify the most important demographic, socioeconomic, and behavioural factors, using the SSVS procedure as described previously. Environmental variables obtained during the model selection were fed into the variable selection model together with the following questionnaire-derived variables: sex, age categories, and wealth quintiles. These variables were considered as confounders and fixed in the regression model. The same set of demographic, socioeconomic, water and sanitation, and behavioural variables was subjected to selection for each outcome and included ethnicity, education attainment, main occupation, toilet availability, raising farm animals, vegetable farming, house floor material, unsafe drinking water source, distance to drinking water source in the dry season, disposal of infant feces, wearing shoes, and drinking bottled or boiled water.

9.2.10 Prediction of Hookworm Infection Risk and Intensity

Predictions at non-surveyed locations were made using models with the best predictive ability for infection risk and intensity, at a 1 x 1 km spatial resolution resulting in a 15,156-pixel grid.

9.2.11 Parameter Estimation

Geostatistical models were fitted in WinBUGS version 1.4.3, using the "*spatial.unipred"* function (Imperial College & Medical Research Council; London, United Kingdom) (Spiegelhalter et al., 2002). A stationary isotropic process was assumed with village-specific random effects, following a multivariate normal distribution with mean zero, and a variance-covariance based on an exponential correlation function of the distance between pairs of locations. Non-informative prior distributions were chosen for all parameters. Sensitivity analysis was conducted to verify that similar results were obtained with alternate vague priors. Further information is provided in Appendix 9.6.1. Markov chain Monte Carlo (MCMC) simulation was used to estimate model parameters (Gelfand and Smith, 1990). For all models, two chains were run with a burn-in of 5,000. Depending on the model, variable selection was run on 100,000 to 600,000 iterations. Results were recorded for the last 1,000 iterations of each chain. For all other models, 100,000 iterations were run and results were withdrawn for the last 10,000 iterations of each chain, with a thinning of 10. Convergence was assessed through visualization of history and density plots of selected parameters. Before drawing samples, it was verified for each parameter that the Monte Carlo (MC) error, which measures the uncertainty due to simulation error, was below 5% of the standard deviation (Lunn et al., 2000). The DIC was withdrawn after 5,000 additional iterations.

9.3 Results

9.3.1 Study Population

Overall, 4,380 people were invited, among whom 280 were absent during registration, 709 missed the parasitological or the questionnaire survey, and 20 had incomplete questionnaire data. Hence, the final sample consisted of 3,371 individuals from 815 households in 51 villages.

Table 9.1 shows the characteristics of the 3,371 participants who had complete parasitological and questionnaire data. There were slightly more females (n=1,776, 52.7%) than males. The mean age was 26.9 years and the inter-quartile range was 32.4 years. The age structure was as follows: <5 years (9.6%), 5-17 years (36.6%), 18-34 years (19.6%), 35-49 years (17.2%), and ≥50 years (16.9%). The illiteracy rate was 28.6% with a significant sex difference (32.6% among females and 24.2% among males; χ^2=29.02, p<0.001). The most frequent occupation was rice farmer (39.7%). Over three quarters of the participants (76.3%) did not have access to sanitation of any type, and 27.4% (14/51) of villages had no toilets facilities at all. No village had toilets for all households. About one third of the study population (31.1%) did not have access to safe drinking water. About one out of five participants declared not wearing shoes outside their house.

Table 9.1: Characteristics of 3,371 study participants from Champasack province, southern Lao PDR in 2007.

Variable	Category	n (%)
Sex	Male	1,595 (47.3)
	Female	1,776 (52.7)
Age (years)	<5	325 (9.6)
	5-17	1,235 (36.6)
	18-34	661 (19.6)
	35-49	579 (17.2)
	≥50	571 (16.9)
Ethnic group	Lao Loum	2,755 (81.7)
	Other	616 (18.3)
Educational attainment	Illiterate	965 (28.6)
	Primary school	1,767 (52.4)
	Secondary school and higher	639 (19.0)
Main occupation	Rice farmer	1,339 (39.7)
	School pupil	897 (26.6)
	Tertiary sector and other	403 (12.0)
	No occupation	732 (21.7)
Socioeconomic status	Most poor	582 (17.3)
	Very poor	657 (19.5)
	Poor	739 (21.9)
	Less poor	715 (21.2)
	Least poor	678 (20.1)
Access to toilets	No	2,572 (76.3)
	Yes	799 (23.7)
Source of drinking water, dry season	Safe (village pump, protect well, pipe)	2,322 (68.9)
	Unsafe (river, pond, canal, rain)	1,049 (31.1)
Walking distance to drinking water source (min)	≤4	1,640 (48.7)
	5-9	887 (26.3)
	≥10	844 (25.0)
Consumption of bottled of boiled drinking water	No	1,518 (45.0)
	Yes	1,853 (55.0)
Does your house have a place to grow vegetables?	No	876 (26.0)
	Yes	2,495 (74.0)
Do you raise farm animals?	No	278 (8.3)
	Yes	3,093 (91.7)
House floor material	Wood	2,980 (88.4)
	Clay or bamboo	162 (4.8)
	Concrete	229 (6.8)
Disposal of infant feces	Not applicable	609 (18.1)
	Safe disposal	1,667 (49.5)
	Unsafe disposal	1,095 (32.5)
Wear shoes outside house	No	655 (19.4)
	Yes	2,716 (80.6)

9.3.2 Hookworm Infection Prevalence and Intensity

The overall prevalence of hookworm infection was 48.8% (95% confidence interval (CI): 47.1-50.5%). Infected individuals were found in all villages and the prevalence ranged from 7.3% (95% CI: 2.4%-16.4%) to 85.0% (95% CI: 70.2%-94.3%) at the unit of the village. Most infections (91.7%) were of light intensity according to WHO classification (1-1,999 EPG), whereas the remaining infections were either moderate or heavy (4.1% for both classes). The geometric mean intensity of infection was 12.9 EPG (95% CI: 11.6-14.3). Village-level geometric means ranged between 0.44 EPG (95% CI: 0.04-0.98) and 115.3 EPG (95% CI: 53.3-248.1). The most heavily infected individual, a 13-year-old boy, had a fecal egg count of 38,748 EPG. Smoothed age-prevalence curves, stratified by sex, are shown in Figure 9.1A (males) and 9.1B (females). Smoothed age-infection intensity curves, for infected participants only, are displayed in Figure 9.1C for males and in Figure 9.1D for females. Figure 9.2 displays a smoothed curve of infection intensity as a function of village-level prevalence, which were found to be positively correlated.

9.3.3 Spatial Correlation of Hookworm Infection Risk and Intensity

Models run in absence of covariates indicated very little spatial correlation of hookworm infection risk and intensity. The parameters of those models are presented in Table 9.2. The small residual (unexplained) within-village variance (σ^2) in the spatial model indicates a weak clustering tendency both for infection risk (σ^2=0.56) and intensity (σ^2=0.66). Accordingly, the spatial correlation of infection risk and intensity became less than 5% after 2.26 km and 2.04 km, respectively, indicating small spatial correlation.

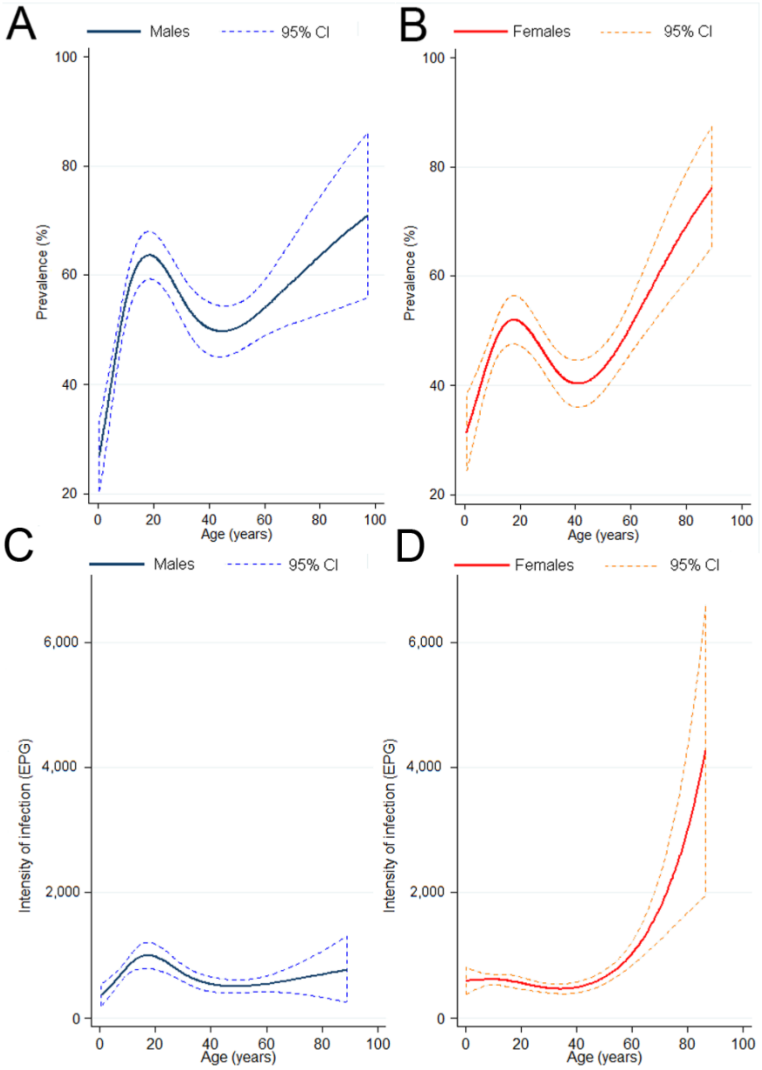


Figure 9.1: Smoothed age-prevalence and intensity curves of hookworm infection, Champasack province, southern Lao PDR.

Restricted cubic splines were used. For hookworm prevalence data are stratified for males (A) and females (B). For intensity of infection, only participants with an infection were included and data are presented separately for males (C) and females (D). Uncertainty is expressed as 95% confidence interval (CI).

Data were obtained from a cross-sectional survey carried out among 3,371 participants in 51 villages of Champasack province in 2007.

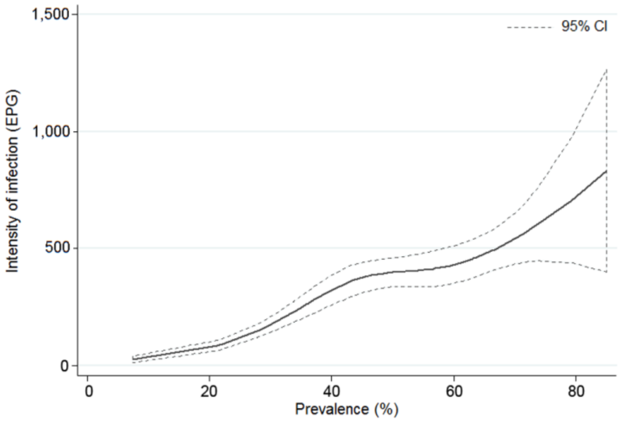


Figure 9.2: Smoothed age-intensity curve for hookworm infection intensity according to village-level prevalence, Champasack province, southern Lao PDR. Data were obtained from a cross-sectional survey carried out among 3,371 participants in 51 villages of Champasack province in 2007. The smoothed age-intensity curve is based on a restricted cubic spline of hookworm infection intensity at the unit of the village.

9.3.4 Results of Model Validation

In the sensitivity analysis, all tested alternative vague priors produced similar estimates for all parameters. For infection risk, comparing the geostatistical and non-spatial logistic models, both the MSE and DIC indicated that the geostatistical (MSE=0.0337; DIC=4,337.2) and the non-spatial models (MSE=0.0345; DIC=4,336.6) had similar predictive abilities and fitted equally well. Therefore the non-spatial model was used to analyse and predict hookworm infection risk.

Among the three distributions (NB, ZIP, and ZINB) used to model hookworm infection intensity, the NB models were found to have the best predictive ability. Results of the model validation (i.e., MSE of each model), are available in Appendix 9.6.2. The MSE indicated a better predictive ability for the NB geostatistical model (MSE=36,070) than for its non-spatial counterpart (MSE=36,826). However, model fit was similar as indicated by the DIC of the geostatistical model (DIC=29,446.5) and the non-spatial model (DIC=29,446.3). Although the spatial model had a 2% better predictive ability, based on the low spatial correlation of both hookworm infection risk and intensity, the similar fit, and consistency with infection risk results, the most parsimonious model, i.e. the NB model with an exchangeable random effect, was used for the analysis of infection intensity.

9.3.5 Risk Factors for Hookworm Infection Risk and Intensity

The results of the mixed effects multivariate logistic and NB models, including variables selected by the SSVS procedure, for each model, are presented in Table 9.3. Female sex and a higher socioeconomic status (i.e., belonging to the less or least poor quintiles) were associated both with lower hookworm infection risk and intensity. With regard to age, preschool-aged children had the lowest risk. No significant interaction between age and sex was found for hookworm prevalence, but sex was an effect modifier of age for infection intensity, as indicated by a significant LRT (χ^2=27.24, p<0.001). Interaction terms were accordingly included in the Bayesian mixed effects NB model. No additional important interaction was found. Preschool-aged boys harboured the lightest hookworm intensities, whilst all other age groups had similar hookworm intensities. Comparing females across age groups, women ≥50 years harboured the heaviest hookworm intensities.

Table 9.2: Parameters for the non-spatial and geostatistical logistic and NB models without covariates.

Model parameters	Prevalence				Intensity of infection			
	Non-spatial		Spatial		Non-spatial		Spatial	
	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI
σ^2 [a]	0.56	0.36-0.94	0.56	0.35-0.92	0.66	0.38-1.09	0.66	0.38-1.12
ρ [b]	n.a.	n.a.	143.5	14.5-286.4	n.a.	n.a.	158.8	27.7-287.5
Range (km) [c]	n.a.	n.a.	2.26	1.13-22.6	n.a.	n.a.	2.04	1.12-12.64
r [d]	n.a.	n.a.	n.a.	n.a.	0.09	0.09-0.10	0.09	0.09-0.10
DIC[e]	4,337.2	n.a.	4,336.9	n.a.	29,447.5	n.a.	29,447.1	n.a.

Parasitological data were obtained from a cross-sectional survey carried out among 3,371 participants in 51 villages of Champasack province in 2007.

CI, credible interval;

Prevalence models: Bayesian geostatistical logistic model (spatial model) and Bayesian model with an exchangeable random effect (non-spatial model);

Intensity models: Bayesian geostatistical NB model (spatial model) and Bayesian NB model with an exchangeable random effect (non-spatial model);

[a] σ^2 is the location-specific unexplained variance;

[b] ρ is the decay parameter;

[c] The range (range=3/ρ) is the distance at which the spatial correlation becomes less than 5%;

[d] r is the dispersion parameter from the negative binomial distribution that quantifies the amount of extra-Poisson variation;

[e] Deviance information criterion.

Table 9.3: Determinants of prevalence and intensity of hookworm infection.

Covariate		Prevalence		Intensity of infection	
		OR	95% CI	IRR	95% CI
Sex[a]	Male	1.00		1.00	
	Female	0.75	0.65-0.86	0.56	0.39-0.82
Age (years)[b]	5-17	1.00		1.00	
	<5	0.31	0.23-0.41	0.17	0.10-0.32
	18-34	0.82	0.67-1.03	0.87	0.53-1.43
	35-49	0.77	0.62-0.96	0.64	0.40-1.05
	≥50	1.16	0.93-1.44	0.94	0.57-1.62
Interaction: effect of age among females	5-17	n.a.	n.a.	1.00	
	<5	n.a.	n.a.	0.62	0.34-1.26
	18-34	n.a.	n.a.	0.61	0.40-0.96
	35-49	n.a.	n.a.	0.85	0.51-1.40
	≥50	n.a.	n.a.	2.22	1.39-3.57
Interaction: females compared to males, among each age group	5-17	n.a.	n.a.	1.00	
	<5	n.a.	n.a.	2.02	0.93-4.44
	18-34	n.a.	n.a.	0.40	0.23-0.70
	35-49	n.a.	n.a.	0.74	0.41-1.29
	≥50	n.a.	n.a.	1.32	0.72-2.38
Ethnic group	Other	n.a.	n.a.	1.00	
	Lao Loum	n.a.	n.a.	1.58	0.98-2.72
Socioeconomic status	Most poor	1.00		1.00	
	Very poor	0.78	0.59-1.04	0.70	0.44-1.10
	Poor	0.82	0.63-1.08	0.71	0.46-1.18
	Less poor	0.64	0.49-0.85	0.48	0.29-0.77
	Least poor	0.50	0.36-0.69	0.44	0.25-0.75

Table 9.3 (*cont.)*

Covariate		**Prevalence**		**Intensity of infection**	
		OR	95% CI	IRR	95% CI
Disposal of baby stools	Not applicable	1.00		1.00	
	Safe disposal	1.07	0.85-1.30	0.84	0.59-1.21
	Unsafe disposal	1.36	1.06-1.72	1.35	0.90-2.01
Source of drinking water, dry season	Safe	n.a.	n.a.	1.00	
	Unsafe	n.a.	n.a.	1.34	0.89-2.00
Distance to drinking water, dry season	≤ 4 minutes	n.a.	n.a.	1.00	
	5 - 9 minutes	n.a.	n.a.	0.69	0.50-0.96
	≥ 10 minutes	n.a.	n.a.	0.98	0.67-1.44
House floor material	Wood	1.00		1.00	
	Clay/bamboo	0.85	0.57-1.27	0.78	0.41-1.58
	Concrete	1.30	0.93-1.88	0.75	0.42-1.30
Raise farm animals	No	n.a.	n.a.	1.00	
	Yes	n.a.	n.a.	1.49	0.94-2.30
LST day (monthly minimum)		1.23	0.99-1.54	1.32	1.06-1.65
Soil bulk density	[1.2 -1.4[kg/dm^3	1.00		1.00	
	[1.4-1.6[kg/dm^3	0.50	0.30-0.81	n.a.	n.a.
Model parameters					
σ^2 (median)[c]		0.51	0.32-0.81	0.57	0.32-1.01
r (median)[d]		n.a.	n.a.	0.09	0.09-0.10
DIC[e]		4,243.3	n.a.	29,376.2	n.a.

Results obtained using multivariate non-spatial models and data from a cross-sectional parasitological and questionnaire survey, Champasack province, southern Lao PDR in 2007. Prevalence models: Bayesian non-spatial mixed effects logistic model;

Intensity models: Bayesian non-spatial mixed effects NB model;

CI, credible interval; OR, odds ratio (posterior median); IRR, incidence rate ratio (posterior median);

[a] For the intensity of infection, IRRs correspond to the effect of sex among the baseline age group (5-17 years);

[b] For the intensity of infection, IRRs correspond to the effect of age among males;

[c] σ^2 is the location-specific unexplained variance;

[d] r is the dispersion parameter from the negative binomial (NB) distribution that quantifies the amount of extra-Poisson variation;

[e] Deviance information criterion.

9.3.6 Prediction of Hookworm Infection Risk

Hookworm infection risk was predicted with the non-spatial logistic model, which included four covariates: LST day, land cover, soil bulk density, and organic carbon. Bayesian model fit indicated that no environmental predictor was important. ORs for this model are presented in Appendix 9.6.4. Figure 9.3A displays the median of the posterior predicted distribution of hookworm prevalence in Champasack province. Hookworm was found to be ubiquitous in this setting, with somewhat lower prevalence rates predicted in the lowlands, along the west side of the Mekong River in the center and southern parts of the province, and on both banks in the north-western part. Elevation is displayed in Figure 9.3B. The uncertainty of predictions was large, but the geographic distribution of prevalence was maintained in the lower (2.5%) and upper (97.5%) limits of the Bayesian credible intervals. Figure 9.4A and 9.4B display the lower and upper prediction boundaries for predicted prevalence, respectively. Maps of environmental characteristics of the province are available as supplementary information in Appendix 9.6.3.

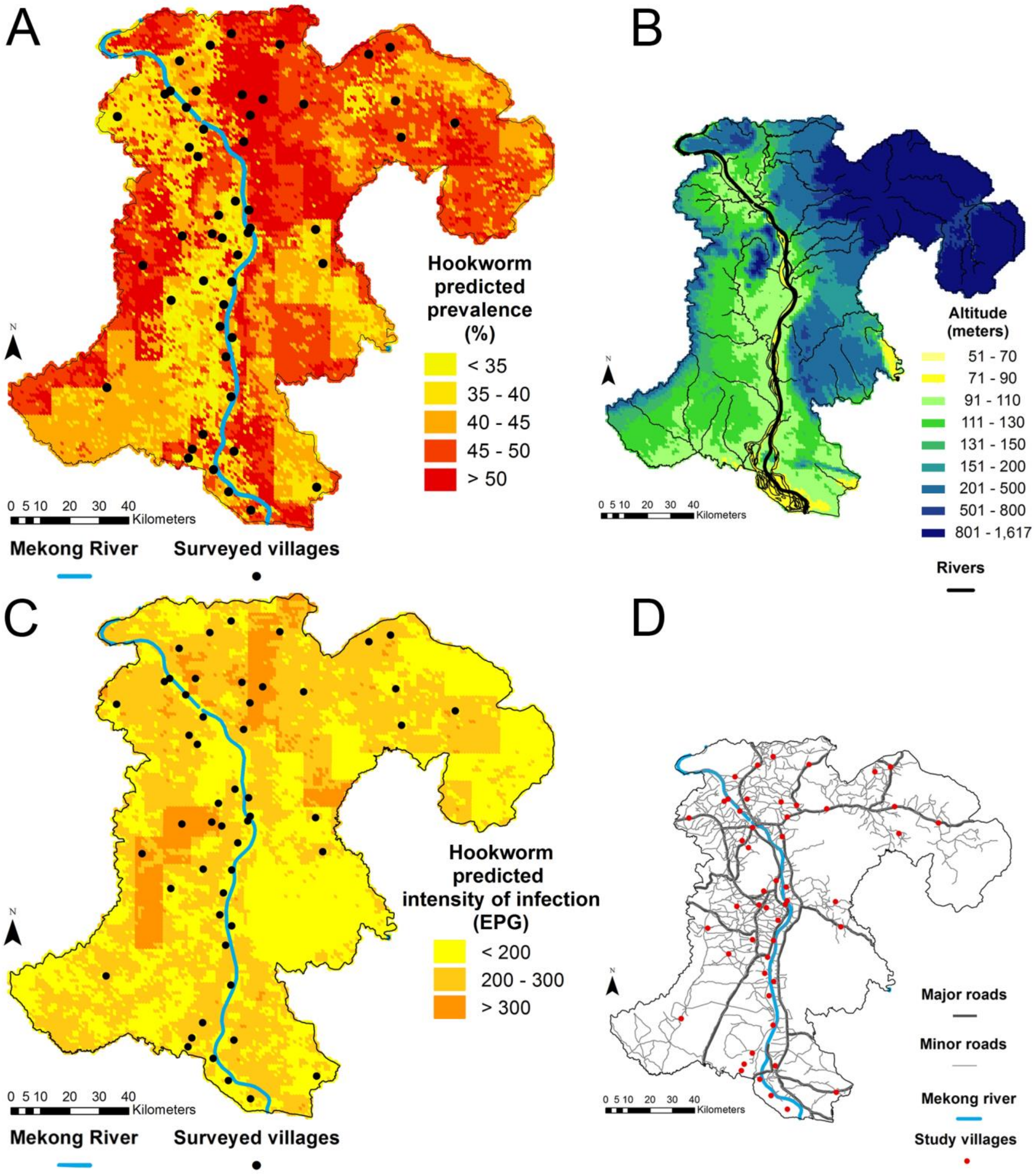


Figure 9.3: Maps of predicted hookworm prevalence (A), elevation (B), predicted hookworm infection intensity (C), and road network (D) in Champasack province, southern Lao PDR.

Predictions were based on the non-spatial mixed effects logistic (prevalence) and NB (infection intensity) models using environmental covariates only.

9.3.7 Prediction of Hookworm Infection Intensity

Hookworm infection intensity was predicted using the non-spatial NB model, with LST day and soil bulk density included as covariates. Higher minimum LST day was positively associated with hookworm infection intensity. IRRs for this model are available in Appendix 9.6.4. The median of the posterior predicted distribution of hookworm infection intensity is presented in Figure 9.3C. All estimates corresponded to light hookworm infection intensities (maximum: 599.3 EPG), with comparable levels all over the province. Slightly lighter infections were predicted closely around most survey villages located right on the Mekong River, and in areas with low population density and road network (i.e., in some zones of the south-west, in the center-east, on the borders of the north-east). Figure 9.3D presents the road network of Champasack province. The uncertainty of predicted infection intensity was substantial. Still, the pattern of predicted intensity persisted in the lower (2.5%) and upper (97.5%) limits of the Bayesian credible intervals, which are shown in Figure 9.4C and 9.4D, respectively.

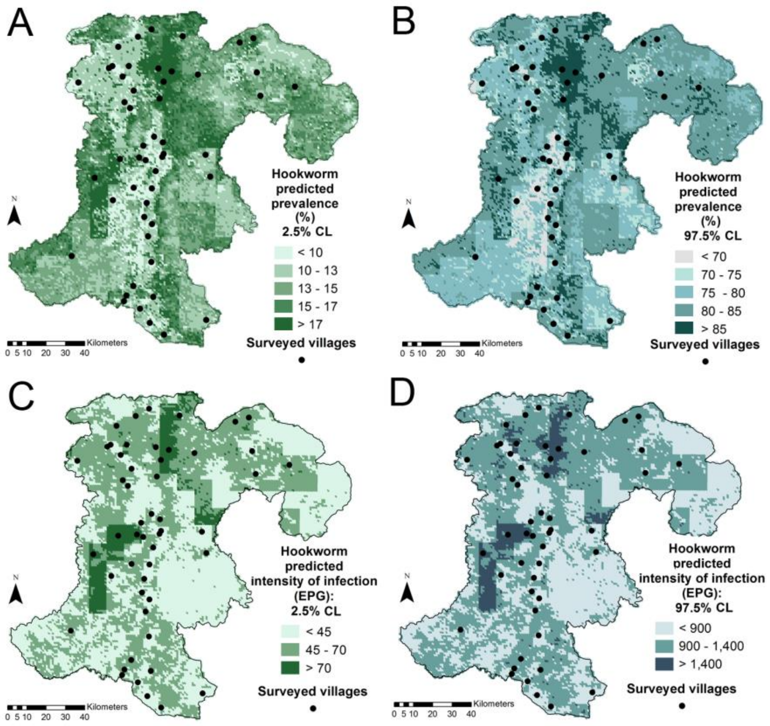


Figure 9.4: Uncertainty of hookworm predictions in Champasack province, southern Lao PDR.

Lower estimates (2.5% CL) of hookworm predicted risk (A) and infection intensity (C). Upper estimates (97.5% CL) of hookworm predicted risk (B) and infection intensity (D). CL, credible limit.

9.4 Discussion

Results from a cross-sectional parasitological and questionnaire survey reported here with more than 3,000 participating individuals from 51 villages confirm that hookworm infection is highly endemic in the Champasack province. The prevalence of hookworm was considerably higher than that of the other soil-transmitted helminths (i.e., *Ascaris lumbricoides* and *Trichuris trichiura*) (Rim et al., 2003, Sayasone et al., 2011, Soukhathammavong et al., 2012). Indeed, almost every second individual was infected with hookworm, while only few individuals were infected with *A. lumbricoides* and *T. trichiura*. Interestingly, hookworm infections were mainly of light intensity; 91.7% of the infections were below 2,000 EPG. We used duplicate Kato-Katz thick smears based on a single stool sample for hookworm diagnosis. Hence, the reported prevalence of infection underestimates the “true” situation, due to the low sensitivity of the Kato-Katz technique, particularly in areas where light infections predominate (Booth et al., 2003, Knopp et al., 2008a, McCarthy et al., 2012). This issue is underscored by a previous study: Kato-Katz thick smears examined from two stool samples, combined with a formalin-ethyl-acetate concentration technique on a third stool sample, revealed a hookworm prevalence of 76.8% in six villages in the same province (Sayasone et al., 2011). An additional concern might be the potential bias due to the 84% compliance in this study, but the missing data did not affect the age, sex, or socioeconomic composition of datasets, which were similar before and after excluding participants who did not have parasitological data.

Hookworm infections were encountered in all of the 51 villages surveyed and exhibited only little spatial clustering. This observation is in line with previous findings from Côte d’Ivoire and elsewhere in Africa (Raso et al., 2006a, Brooker and Clements, 2009).

The spatial correlation was negligible, and hence, predictions of hookworm infection risk and intensity were obtained using non-spatial models. Lower hookworm prevalence rates were predicted in the lowlands, mostly along the western side of the Mekong River. Light infection intensities were predicted all over the province, with an overall homogeneous distribution.

The current study has two main limitations that may have impacted the precision of our estimates. First, there is a lack of sampled locations in the south-west, the center-east, and the north-east mountains. While this is reflecting the lower population densities in these areas, it resulted in higher prediction uncertainty, which was particularly substantial for infection intensity. It might also explain the absence of association between hookworm prevalence and elevation, although lower risk was predicted in the lowlands, at altitudes below 150 m. We also found no association between elevation and *O. viverrini* infection risk in the same province, although risk zones for this parasite were clearly delineated by altitude (Forrer et al., 2012). Additionally, our models did not account for ongoing control efforts, which might have further increased uncertainty of estimates, particularly in this setting, where infection intensity is low, yielding a high sensitivity to treatment. Given the low precision of hookworm prevalence and infection intensity predictions, setting-specific estimates must be interpreted with caution. However, despite the large credible intervals of the predictions, the same patterns of predicted risk and intensity arose in the lower and upper prediction estimates, and appear to be reliable. Finally, the uncertainties we report here for both outcomes are in line with those reported by other mapping studies of hookworm, *S. mansoni*, and *S. haematobium* mono- or co-infections in different parts of Africa (Raso et al., 2006a, Clements et al., 2006a, Brooker and Clements, 2009, Soares Magalhães et al., 2011b). It is conceivable that our predictive risk model captured some unmeasured processes related to the province topography and associated human features, including helminthiasis control measures. The province zones with lower predicted prevalence (i.e., the lowlands), and particularly the areas bordering the Mekong River, have a higher population density, a more advanced infrastructure with a denser road network, offer better living conditions, access to health care, and benefit from a higher coverage of helminthiasis control interventions than mountains and remote areas (i.e., province South-West) (Sayasone et al., 2011). Importantly though, the first round of the national deworming programme targeting school- and preschool-aged children was delivered in the Champasack province in October 2006 (i.e., 3-9 months before the present survey) (Phommasack et al., 2008).

However, infection levels of children attending primary schools (i.e., who received a single oral dose of mebendazole (500 mg) through the deworming programme), were similar to infection levels of children attending secondary schools, and who were not dewormed. This suggests either high re-infection rates, or a limited impact of the initial deworming round in 2006. Monitoring of the control programme after two rounds of deworming showed disappointing results for hookworm infections, probably due to the low efficacy of mebendazole against this helminth species (Phommasack et al., 2008, Montresor et al., 2008, Keiser and Utzinger, 2008). A recent study conducted in Champasack province found that a single oral dose of mebendazole (500 mg) resulted in a low cure rate (CR) of only 17.6% and a moderate egg reduction rate (ERR) of 76.3% against hookworm infection (Soukhathammavong et al., 2012). It must be noted, however, that our data did not allow adjusting for individual treatment. The available treatment data were self-reported, and hence are prone to reporting bias. Indeed, only 13.2% of participants and 12.0% of children attending primary school reported to ever have received deworming drugs, although the latter benefited from the national school-based deworming programme which reported a 95% national coverage in 2007 (Phommasack et al., 2008, Montresor et al., 2008).

Although no causality can be inferred given the cross-sectional design of the present survey, an interesting result was that women aged ≥50 years had heavier hookworm infections than younger females. Elder women were also found to have high hookworm infection intensities in Vietnam, Hainan province of the People's Republic of China, and Uganda (Bethony et al., 2002, Brooker et al., 2004a, Pullan et al., 2010b). Although the use of night soil in agriculture could explain this result in Vietnam, this practice was absent in Hainan province, similarly to our study setting (Bethony et al., 2002, Brooker et al., 2004a). Interestingly, 68.1% of participants aged 50 years and above who reported no occupation outside the household were women, while 30.6% of women aged ≥50 years reported having no occupation, a proportion raising to 55.8% and 88.3% for women aged ≥60 and ≥70 years, respectively. By spending more time at home, they might be more exposed to contaminated environment at and around their place of residence. This assumption is consistent with the results of two household-based studies conducted in Uganda and Brazil, which concluded that exposure to hookworm was mostly concentrated in the peri-domiciliary environment (Brooker et al., 2006, Pullan et al., 2010b). However, the relative roles of exposure, immune response, and host genetic susceptibility in the epidemiology of hookworm infections are still poorly understood (Brooker et al., 2004a, Pullan et al., 2008).

Poor hygiene, lack of sanitation, and clean water are well known to influence hookworm transmission (Raso et al., 2006a, Pullan et al., 2010b, Pullan et al., 2010a, Freeman et al., 2013b). However, we did not find any association between hookworm infection levels and most of water and sanitation indicators, including self-reported availability of toilets, which contradicts findings from two recent systematic reviews and meta-analyses (Ziegelbauer et al., 2012, Strunz et al., 2014). No village had full sanitation coverage, and hence, soil contamination with hookworm and other helminth species occurred in all survey locations.

This lack of association might also relate to the exposure to specific helminth species such as *Ancylostoma ceylanicum*, a hookworm of dogs and cats widely distributed and highly prevalent among carnivore pets in Asia (Traub et al., 2008, Traub, 2013). This hookworm species can produce patent infections among humans and recent studies suggest that its frequency and role in human infections in Southeast Asia might be largely underestimated (Ngui et al., 2012, Conlan et al., 2012, Traub, 2013). Indeed, a recent study conducted in a rural Cambodian village found that 52% of participants infected with hookworm were actually harboring *A. ceylanicum* (Inpankaew et al., 2014). Although very little, the 2 km range we found both for prevalence and intensity of hookworm infection was larger than the smallest distance between two surveyed villages. This suggests that transmission occurs within and around villages rather than between them, and could relate to the distribution of defecation sites inside villages and around living places, but maybe also to zones where human cohabit with pets and semi-domesticated cats and dogs. *A. ceylanicum* infections in humans should be further assessed in Southeast Asia. In a first step, infection levels should be investigated at larger scales and in various settings. Subsequently, treatment efficacy and the potential impact of this zoonotic parasite on deworming programmes effectiveness and sanitation-related control measures should be determined.

In view of the high prevalence of hookworm infection but the relatively low overall infection intensity, our results suggest a low morbidity associated with hookworm mono-infections in Champasack province. Yet, hookworm is co-endemic with other soil-transmitted helminths and trematodes (e.g., *O. viverrini* and *S. mekongi*) in this setting (Rim et al., 2003, Chai et al., 2005, Chai et al., 2007, Muth et al., 2010, Sayasone et al., 2011, Forrer et al., 2012). High prevalence rates for these intestinal helminth species, coupled with a high proportion of poly-parasitized individuals reported in 2006 suggest potential co-morbidities that should not be overlooked (Sayasone et al., 2011). Multiparasitism is the rule rather than the exception in Lao PDR and elsewhere, and hence, co-morbidity must be studied in connection with co-infections (Steinmann et al., 2010). Our results suggest that hookworm control efforts should be intensified in the Champasack province, particularly in mountainous areas. In the meantime, school-based deworming targeting soil-transmitted helminthiasis, opisthorchiasis, and schistosomiasis mekongi, which resumed in 2008 and 2006, respectively, has been implemented, although irregularly (Sayasone et al., 2011, Forrer et al., 2012). An up-to-date assessment would be needed to evaluate the impact of these programmes after several years of implementation and the evolution of parasitic infections in the region, including in the most remote areas, in light of achieved coverage, target age groups, re-infection rates, and efficacy of single-oral dose of currently delivered drugs in deworming programmes.

9.6 Acknowledgments

We are grateful to all study participants. Our sincere thanks go to staff at the Centre for Malariology, Parasitology and Entomology, Vientiane, and the Station for Malariology, Parasitology and Entomology of the Champasack province, Pakse, for their support and field work assistance. We thank the Champasack provincial health office and the district health offices for their support. We thank Prof. Pascal Boivin for providing insight into soil data, and Dr. Steffen Vogt and Prof. Rüdiger Glaser for downloading and management of remote sensing data.

9.7 Appendix

9.7.1 Formulation of logistic, NB, ZIP and ZINB models

1. Analysis of hookworm infection risk: logistic model

Let Y_{ij} be the infection status of individual *i* in village *j*, taking the values 0 or 1 for a negative or positive status, respectively. For an individual *i* in village *j*, we assume that

Y_{ij} ~ Bernouilli(p_{ij}) where p_{ij} is the probability of infection, and model p_{ij} as follows:

$\log it\left(p_{ij}\right)=\alpha+\sum_{k=1}^{n}\beta_k X_{kij}+\phi_j$, where X_{kij} is the k$^{\text{th}}$, n=1,….n, covariate for individual *i* in village *j*, and ϕ_j is the locational random effect for village *j* (see section 3.1).

2. Analysis of hookworm infection intensity: NB, ZIP and ZINB models

2.1 Negative binomial (NB) model

Let Y_{ij} be the eggs count of individual *i* in village *j*. We assume that $Y_{ij} \sim NB(p_{ij}\,, r)$ and model the mean eggs count λ_{ij}, as follows:

$p_{ij} = \frac{r}{(r+ \lambda_{ij})}$, log($\lambda_{ij}$) = α +$\sum_{k=1}^{n} \beta_k \, X_{kij} + \phi_j$, where X_{kij} is the n^th^ explanatory variable, and ϕ_j is the locational random effect for village (see section 3.1). Under this parameterization, the NB is a Poisson-Gamma mixture, where *r* is a real number that has the meaning of a dispersion parameter (Ntzoufras, 2009).

2.2 Zero-inflated Poisson (ZIP) and zero-inflated negative binomial (ZINB) models

Zero-inflated models assume that a proportion π (mixing proportion) of zeros are structural (non-random) and that the remaining zeros arise from the count distribution (Poisson (i.e. ZIP) or Negative binomial (i.e. ZINB)), with probability 1 – π. We assume that the mixing proportion π is constant across individuals and introduce covariates only on the mean parameter of the count.

Let Y_{ij} be the eggs count of individual *i* in village *j*. We assume $Y_{ij} \sim Poisson(mu_{ij})$, $mu_{ij} = \lambda_{ij} * (1 - u_{ij})$ (ZIP distribution) and $Y_{ij} \sim NB(p_{ij}\,, r)$, $p_{ij} = \frac{r}{(r+ \lambda_{ij})*(1- u_{ij})}$ (ZINB distribution). We model log(λ_{ij}) = α +$\sum_{k=1}^{n} \beta_k \, X_{kij} + \phi_j$, $u_{ij} \sim Be(\pi)$, where π is the mixing proportion (probability of structural zeros of the inflation model) and u_{ij} is a binary indicator that specifies whether the zeros are structural or arise from the count distribution.

3. Specification of prior distributions

3.1 Random effects

For all models (logistic, NB, ZIP and ZINB), we consider $\boldsymbol{\phi} = (\phi_1, \dots, \phi_{51})^T$ to be either an exchangeable random effect (non-spatial models) or a geostatistical random effect (spatial models). In particular, we assume $\phi \sim N(0\,, \sigma^2 R_{ij})$ where σ^2 is the variance parameter and R_{ij}.is the correlation matrix between locations. Under an exchangeable prior distribution $R_{ij} = 0$ if i $\neq j$ and $R_{ij} = 1$ if $i = j$. Under the assumption of a spatial stationary isotropic process, $R_{ij} = \exp(d_{ij}, \rho)$, where d_{ij} is the Euclidean distance between two locations s_i and s_j, and ρ is a measure of how spatial correlation decreases with the distance. The distance at which the spatial correlation between villages gets under 5% is equal to $3/\rho$ and is called the range.

A vague inverse gamma prior with mean 1 and variance 100 was chosen for σ^2, and a uniform prior for ρ with parameters calculated as a function of the minimum and maximum distance between sampled villages was adopted, that is:

.

3.2 Other priors

We chose a vague Normal distribution with a mean of zero and a variance of 1000 for all the regression coefficients. The prior for the dispersion parameter *r* of the NB distribution was chosen to be a gamma with mean 1, variance 100 and a restricted domain within the range (0.001, 100). The mixing probability π of the ZIP and ZINB distributions was assumed to follow a flat beta distribution Beta(1,1).

4. Sensitivity analysis

For π, the mixing proportion of ZIP and ZINB models, two additional beta distributions were tested: Beta(0.5,0.5) and Beta(0.125,0.125), with mean 0.5 and variances 0.125 and 0.2, respectively. For the village-level inverse variance tau parameter (logistic, NB, ZIP and ZINB models) and the over-dispersion r parameter of the NB and ZINB models, gamma distributions with mean 1 and variances 10, 100 and 1000 were tested.

9.7.2 Results of the model validation for hookworm prevalence and intensity risk profiling.

Prevalence	MSE[a]
non-spatial	0.0345
spatial	0.0337
Intensity of infection	MSE[a]
NB non-spatial	36,826.47
NB spatial	36,069.89
ZINB non-spatial	38,258.58
ZINB spatial	40,069.97
ZIP non-spatial	46,303.53
ZIP spatial	38,645.35

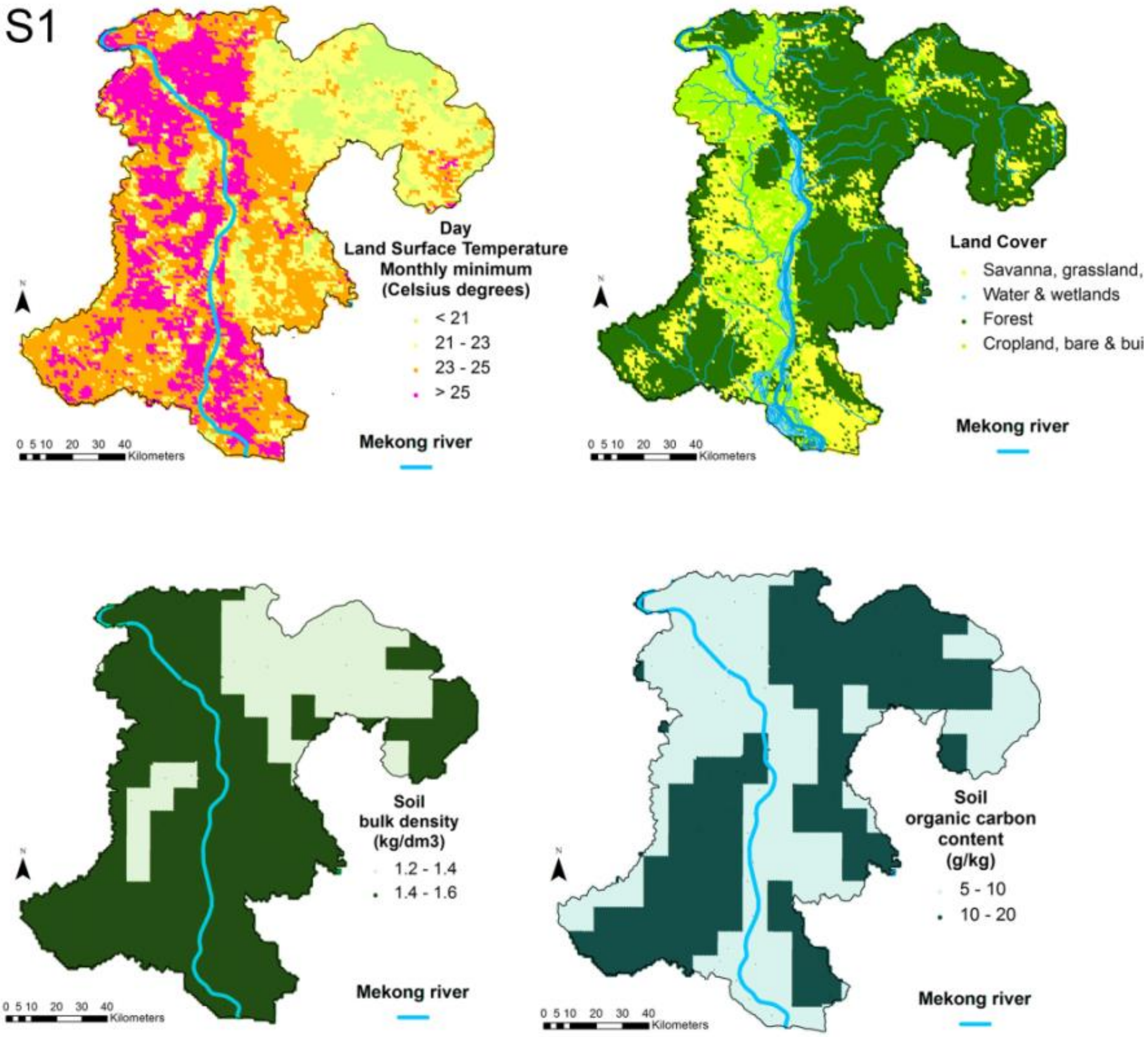
Parasitological data were obtained from a cross-sectional parasitological and questionnaire survey, Champasack province, southern Lao PDR in 2007. Shown are spatial and non-spatial hookworm prevalence models, and spatial and non-spatial NB, ZINB, and ZIP models for hookworm infection intensity, including environmental covariates only (predictive models).

[a] Mean Squared error;

A lower MSE indicates a better predictive ability;

Included environmental covariates were specifically selected with the SSVS selection variable method, for each variant of the models (i.e. non-spatial *vs.* spatial), and for each distribution.

9.7.3 Distribution of environmental factors in Champasack province, southern Lao PDR.



9.7.4 Odds ratios (ORs) and incidence rate ratios (IRRs) of environmental covariates in the predictive models.

		Prevalence		**Intensity of infection**	
Covariate		OR	95% CI	IRR	95% CI
LST day (monthly minimum)		1.19	0.97 - 1.47	1.31	1.07 - 1.67
Soil bulk density	1.20 - 1.39 kg/dm^3	1.00		1.00	
	1.40 - 1.59 kg/dm^3	0.63	0.31 - 1.35	0.60	0.35 - 1.09
Soil organic carbon content	5.00 – 9.99 g/kg	1.00		n.a.	n.a.
	10.00 – 19.99 g/kg	0.72	0.42 - 1.31	n.a.	n.a.
Land Cover	Savannah, grassland, shrubland	1.00		n.a.	n.a.
	Water and wetlands	0.87	0.47 - 1.61	n.a.	n.a.
	Forest	1.05	0.48 - 2.40	n.a.	n.a.
	Cropland, bare and built soil	0.59	0.34 - 1.06	n.a.	n.a.
Model parameters					
σ^2 (median) [a]		0.50	0.31 - 0.83	0.52	0.27 - 0.92
r (median) [b]		n.a.	n.a.	0.09	0.09 - 0.10
DIC [c]		4,336.59	n.a.	29,446.30	n.a.

OR, Odds Ratio (posterior median); CI, Credible Interval; IRR, Incidence Rate Ratio (posterior median);

[a] σ^2 is the location-specific unexplained variance;

[b] r is the dispersion parameter from the Negative Binomial distribution that quantifies the amount of extra-Poisson variation;

[c] Deviation Information Criterion.

Parasitological data were obtained from a cross-sectional parasitological and questionnaire survey, Champasack province, southern Lao PDR in 2007. Results obtained with the non-spatial logistic model for hookworm infection prevalence and non-spatial NB model for hookworm infection intensity.

Chapter 10

Discussion

10.1 Major findings on *Strongyloides stercoralis* infection and control in Cambodia

The following section summarizes the main findings according to working objectives relative to *S. stercoralis*, i.e. contributing to the rationale and evidence base to guide *S. stercoralis* control efforts in Cambodia.

S. stercoralis infection is rampant in Cambodia. Based on the estimated prevalence rate of 30.7% (95%CI: 29.7 – 31.8) of the population infected, there would be approximately 4,783,000 *S. stercoralis* cases in Cambodia. Prevalence ranges from 10.9% in Preah Veng province to 48.2% in Koh Kong province. *S. stercoralis* is ubiquitous in the country, with prevalence below 20% only in the provinces of Kampong Cham, Prey Veng, Kandal, and the west of Svay Rieng (Forrer et al., Submitted Oct. 10, 2018). Of note, those provinces were also found to have a lower risk of hookworm infection (Karagiannis-Voules et al., 2015b).

Significant gastrointestinal and dermatological morbidity was associated with chronic infection, with abdominal pain and urticaria, two bothersome symptoms, being common (Forrer et al., 2017).

The control of *S. stercoralis* is feasible with chemotherapy, with ivermectin achieving a high cure rate, resolving symptoms associated with chronic infections, and resulting in low re-infection rates one year post-treatment, while the impact of chemotherapy was enhanced by community sanitation coverage (Forrer et al., 2016).

Control implemented through MDA targeting either children and women or entire communities was more cost effective than targeted (i.e. following diagnosis) treatment in areas of prevalence above 20% but would not be affordable under the current high price of ivermectin in the country (Forrer et al., in preparation). Affordable generics, subsidies or donations of ivermectin are needed to start implementing control in Cambodia.

10.1.1 Community-effectiveness of ivermectin treatment

a. Impact of ivermectin treatment on *Strongyloides stercoralis* infection

Results obtained from the study based on a *S. stercoralis* cohort of about 1,200 individuals indicate that the control of *S. stercoralis* is feasible with preventive chemotherapy and is highly beneficial to endemic communities. A single dose of ivermectin (i) achieved a high cure rate of 96.6%, (ii) resolved gastrointestinal and dermatological symptoms associated with chronic infection, and (iii) resulted in low re-infection rates one year post treatment, i.e. below 15%, whereas those rates range between 57% and 94% for the other STH (Jia et al., 2012, Forrer et al., 2016). Those low re-infection rates suggest that treatment delivery interval could be longer than one year, at least in areas of similar transmission intensity.

b. Age risk groups and effect of sanitation reinfection rates

A major question was to identify age-specific infection risk to help identifying target groups for control. *S. stercoralis* prevalence tends to increase with age, probably due to infection maintenance through time in absence of treatment. Interestingly, the relationship with age was found to differ between men and women in the national sample of over 7,200 participants, with risk sharply increasing with age in men, but increasing sharply only until around 30 years in women and more slowly thereafter (Forrer et al., Submitted Oct. 10, 2018). This relationship is shown in Figure 7.4. However, acquiring a new *S. stercoralis* infection (incident risk) did not depend on age. The data exhibited a non-significant trend of decreasing incident infection risk with increasing age. Further studies conducted in settings with various prevalence rates and transmission intensity would be needed to further confirm or invalidate that individuals of any age have similar risk to acquire an incident *S. stercoralis* infection.

An important finding was that increasing community-level sanitation coverage enhanced the impact of chemotherapy, with increasing community-level resulting in lower incident infection rates (Forrer et al., 2016). Those findings are in line with sanitation measures being previously found to be more beneficial in combination with treatment and with another study assessing the impact of treatment on STH infection which also found that increased sanitation coverage was protective against hookworm infection (Esrey et al., 1991, Nikolay et al., 2015).

c. Morbidity associated with chronic *Strongyloides stercoralis* infection

Chronic infection was found to cause urticaria, abdominal pain, nausea, vomiting, and to a lesser extent diarrhea and cough (Forrer et al., 2017). STH morbidity is known to arise with high infection intensity and a surprising result was the lack of association between *S. stercoralis* parasite load and clinical signs, as participants harbouring low parasite loads experienced the same symptoms as those harbouring moderate or high parasite loads.

Additionally, we found that children and adolescents aged between 6 and 19 years harbouring high *S. stercoralis* parasite loads were at higher risk of being stunted (Forrer et al., 2017). The assessment was cross-sectional and by no means could infer causality. There would be many sources of confounding that could explain this association, including helminth and/or protozoa infection in infancy and younger age, in particular before STH control started. However, this association is not surprising given the gastrointestinal symptoms caused by infection, as diarrhea, abdominal pain and vomiting can all result in lower food intake and malnutrition (Stephenson et al., 2000b). The association between STH and growth retardation due to malnutrition, which may originate from the same mechanisms, is widely acknowledged (Stephenson et al., 2000b, Koski and Scott, 2001, Hotez, 2003, Bethony et al., 2006).

The impact of *S. stercoralis* on growth should be explored although such assessments have been challenging for other STH (Campbell et al., 2016). Most studies having investigated STH impact on childhood growth are observational and yielded highly variable results, probably due to the high disparities in infection levels across settings, but also to confounding with micro- (i.e. minerals, vitamins) and macronutrient (proteins, carbohydrates, fatty acids) deficiencies, as well as with other parasitic infections that may also be associated with malnutrition such as *Giardia lamblia* or *Entemoeba histolytica* (Lustigman et al., 2012b, Campbell et al., 2016). The confirmation that *S. stercoralis* infection causes growth retardation in children would greatly add to the parasite burden as stunting has long term impact on adult fitness and productivity (Brooker et al., 2004a, Bethony et al., 2006, Hotez et al., 2008).

Given the large overlapping of *S. stercoralis* and other STH infections in the country, particularly hookworm, disentangling the role of various parasites on childhood growth might be particularly challenging. The co-morbidity of *S. stercoralis* and hookworm, which have the same transmission mode, should be investigated, as they commonly occur as co-infections (Forrer et al., 2018). An additional challenge would be to assess the health impact of the zoonotic hookworm, *A. ceylanicum*, which is highly prevalent among humans in Cambodia (Inpankaew et al., 2014). The use of combined diagnostic methods that are highly sensitive to detect *S. stercoralis*, STH and protozoan, for example combining the Baermann and KAP methods, Kato-Katz and FECT on two samples, may help identifying clinical signs associated with single or specific co-infections. Such assessments could in addition use tailored infection status definitions as done in our work, i.e. include only negatives with all diagnosis results available, to maximize diagnosis specificity.

An additional important point to eventually estimate *S. stercoralis* burden is the assessment of a relationship between parasite load and morbidity, which has not yet been appraised (Grove, 1996, Krolewiecki et al., 2013). *S. stercoralis* intensity is challenging to estimate due to the auto-infection ability of the parasite and potentially variable infection intensity at individual level. and Yet, studies on *S. stercoralis* infection intensity are paramount to appraise transmission dynamics and establish morbidity related infection intensity thresholds for the establishment of control guidelines (Krolewiecki et al., 2013).

10.1.2 Cost effectiveness of potential control approaches for *Strongyloides stercoralis*

Regarding target groups for control, there are two straightforward options, (i) risk groups already targeted by ongoing control measures, i.e. children and women of child-bearing age and (ii) entire-communities. Besides control delivery practicalities, those two options have another major difference. While the aim of targeting children would be morbidity control, targeting entire communities would be the only way to actually impact transmission.

On the one hand, delivering control to children and women of child bearing age would have the advantage of practicality of using existing delivery networks and could be justified by the additional burden undergone by children through the long-term impact of stunting.

On the other hand, in absence of data relating *S. stercoralis* parasite load and morbidity, the similar reinfection risk across age groups suggest that control should be community-wide. This option onlywould have a substantial impact on transmission sincethe infection can be maintained for decades in absence of treatment (Grove, 1996, Olsen et al., 2009, Toledo et al., 2015). Indeed, in absence of sanitation conditions improvement, and given that almost two third of infections (61.5%) are harboured by adults aged 30 years and above, it is unlikely that treating only children and women would have a substantial impact on transmission, unless parasite loads in children would be disproportionally higher than in adults.

Given the low re-infection rate (15% one year post treatment), another possibility which would depart from current practices might be to treat risk groups already targeted for STH control yearly or biyearly and treat entire communities at some larger time intervals. Again, further studies are needed to investigate the transmission dynamics of *S. stercoralis*.

We estimated that at the current cost of an ivermectin tablet in Cambodia, i.e. 10 USD, the cost to treat one individual would be 28.6 USD with either MDA targeting the risk groups currently reached by STH control, i.e. children and women of child bearing age, or with MDA delivered to entire communities (Forrer et al., in preparation). MDA approaches were the most cost effective in almost all modelled scenarios, but targeted control, i.e. diagnosis followed by delivery of ivermectin only to *S. stercoralis* cases would be the most effective in areas where prevalence is below 20%. Diagnosing *S. stercoralis* using combined Baermann and Koga agar plate on one or two samples would cost 19.5 USD and 12.7 US, respectively.

Twenty per cent is actually the threshold defined by the WHO under which PC control of STH is not recommended. *S. stercoralis* prevalence thresholds in relation to control requirements remain to be estimated. Treatment impact on infection levels, as well as the association between prevalence, the distribution of parasite load among the infected, and transmission should be further investigated.

At the current cost of ivermectin, i.e. be supported by the Cambodian Ministry of Health, which rolling out *S. stercoralis* control in absence of subsidies or donation. Cheaper generics might also help, as experts of the CNM estimated that control could be considered if the cost to treat one individual would drop below 2 USD, which, at the study prevalence rate, i.e. 27.5%, means that the price of one tablet should be 0.7 USD (Virak Khieu, personal communication).

10.2 STH infections in Cambodia

S. stercoralis infection adds up to other STH infections which have been known to be widespread in Cambodia since the late nineties. A national survey conducted in 1997-98 found no less than 51% and 45.4% of school-aged children infected with hookworm and *A. lumbricoides*, respectively, while *T. trichiura* was less common, with a prevalence rate of 8.2% (Urbani et al., 2003). Alternatively, surveys conducted in the period 2000-2004 by the MoH National Centre for Parasitology, Entomology and Malaria Control (CNM) found overall STH prevalence rates over 50% in most areas, with *A. lumbricoides* and hookworm prevalence rates ranging between 24.8% and 61.2%, and 15.3% and 68.9%, respectively (Sinuon et al., 2005, Chesnaye et al., 2011). The public health response was to launch, in 2002, a STH control programme deworming school-aged children twice yearly with mebendazole which reached national coverage in 2004 (Sinuon et al., 2005).

Comparisons with more recent STH prevalence figures are challenging due to the high variability in the diagnosis approaches and assessment conducted at various or unknown time points after deworming (Yong et al., 2014, Khieu et al., 2014a, Khieu et al., 2014b, Khieu et al., 2014c, Moore et al., 2015, Liao et al., 2017). Yet, rates remain globally high in some areas with the peer-reviewed literature and governmental statistics giving prevalence ranges within 0-80%, 0-45% and 3-86%, for *A. lumbricoides*, *T. trichiura* and hookworm, respectively (Jex et al., 2011). Overall, deworming with mebendazole seems to have resulted in a global decrease in *A. lumbricoides* prevalence, while hookworm appears to currently be the most prevalent STH, particularly in the North and Northwest (Yong et al., 2014).

Prevalence remains also high among children despite several years of deworming. In 2009 and 2010 respectively, 45.2% and 49.0% of schoolchildren were found infected with hookworm in Kandal province (South Cambodia) and Preah Vihear province (North Cambodia), respectively (Khieu et al., 2013a, Forrer et al., 2018). In the latter, 4.3% and 3.3% of children harboured moderate or heavy infections, figures that exceed the 1% WHO target for each intensity class despite a decade of biannual deworming. Of note, those high prevalence rates might be partly due to the highly sensitive diagnosis approach used in those sudies. Interestingly, model-based predictions of STH infection over Cambodia using data collected before or after 2000 found that *A. lumbricoides* and *T. trichiura* infections decreased over time, but not hookworm, which national prevalence was estimated after 2000 to be of 28.9% among school-aged children, and ranging between 11.4% in Phnom Penh and 43.3% in Mondulkiri province (Karagiannis-Voules et al., 2015b).

10.3 Limitations

There have been some concerns that coprological methods are not sensitive enough to assess cure and we cannot exclude that some light cases, including with circulating larvae, have been missed (Bisoffi et al., 2011, Requena-Méndez et al., 2013). However, the use of serological diagnosis is limited in endemic settings for cure assessment as they cannot distinguish current from past infection. Indeed, they rely on the detection of parasite-specific antibodies or antigens that can still be present long after contact with the parasite or cure (Requena-Méndez et al., 2013). We used an intensive coprological diagnosis based on the combination of the Baermann method and the KAP, either with an individual sensitivity of 70% on a single test. Combined, and used on two stool samples, the approach has 92.5% sensitivity so we are confident that the cure rate achieved by ivermectin was high, a result which is in line with other findings (Gann et al., 1994, Suputtamongkol et al., 2011, Bisoffi et al., 2011, Barda et al., 2017).

The serological method used in the national mapping has not yet been validated in the field. The main concern of serological diagnosis used for prevalence estimates is cross-reaction with other helminths species (Siddiqui and Berk, 2001, Requena-Méndez et al., 2013). The method we used does not cross-react with *A. lumbricoides* or hookworm, and while there may be cross-reaction with filariasis, this disease is now under surveillance for elimination and is merely absent in Cambodia (WHO, 2016a, Eamudomkarn et al., 2015). Although this is not validation, an interesting comparison suggesting that the serological diagnosis method used in the national survey might achieve similar performances than an intensive coprological approach is that prevalence rates found in the national survey for the provinces of Preah Vihear (40%) and Kandal (19%) were similar to that previously estimated with studies using combined Baermann and KAP on two stool samples, i.e. 44% and 19%, respectively (Khieu et al., 2014b, Khieu et al., 2013a, Forrer et al., Submitted Oct. 10, 2018). The higher prevalence found in the national survey compared to a large scale study conducted in Takeo province, i.e. 35% *vs.* 21%, respectively, might relate to the lower sensitivity of diagnosis performed on one stool sample in the Takeo province study (Khieu et al., 2014c, Forrer et al., Submitted Oct. 10, 2018).

The impact of ivermectin and post-treatment re-infection rates estimated in the present work related to settings with prevalence rate around 30% and cannot be readily generalizable to areas with lower or higher prevalence rates and/or transmission intensities. The question of what happens in areas with high prevalence appears particularly important as the impact of chemotherapy could be different from what was estimated here.

With regard to control implementation, the study design of our cohort study did not allow addressing treatment frequency as participants were treated every year, at baseline and both follow-ups. This question is of importance given the high cost of ivermectin and is also to put in perspective with treatment impact on transmission..

Regarding the cost-effectiveness of potential control strategies, our approach was only based on a hypothetical 1-year programme as no information on reinfection rates across transmission levels is yet available for *S. stercoralis*. It is most likely that studies assessing the cost-effectiveness of *S. stercoralis* control accounting for treatment impact on transmission would find that community-wide treatment is more cost effective than control targeting children and women of child-bearing age only. It has already been estimated that the cost-effectiveness of hookworm control assessed through transmission modelling would be higher if programmes targeted entire communities, a finding that was recently confirmed by a systematic review and meta-analysis of field data (Turner et al., 2015b, Turner et al., 2015a, Clarke et al., 2017, Lo et al., 2015). The main reason is that hookworm and *S. stercoralis* share this feature that adults have higher infection levels due to the maintenance of infection through time (a few years for hookworm and lifelong for *S. stercoralis*). Of note, community-wide control was also more beneficial against *A. lumbricoides* although that parasite has higher infection levels in children. Therefore, as long as adults will not be treated and/or sanitation coverage will be sufficient, adult infections will sustain transmission.

10.4 STH control effectiveness and underlying factors

This within-country local variability of STH infection levels is not specific to Cambodia and is a commonly observed feature of STH or other diseases controlled by PC such as LF. Under an ongoing PC control programme, STH infection levels would be expected to vary within country with local programme effectiveness but also along with political, economic and social contexts, and even at the scale of communities (Humphries et al., 2012, Krentel et al., 2013, Shuford et al., 2016).

STH MDA coverage has been increasing over years since the WHO recommended integrating deworming activities into school-based and primary health care activities in 2001 and in 2009, 73% of the 112 STH endemic countries had deployed STH PC programmes in school-children (WHO, 2012a). Since the onset of preventive chemotherapy control programmes, STH prevalence decreased on all continents (de Silva et al., 2003, Chammartin et al., 2013a, Karagiannis-Voules et al., 2015a, Lai et al., 2013, Pullan et al., 2014, Karagiannis-Voules et al., 2015b, Chammartin et al., 2014a).

Lately, despite the fast rolling-out of STH PC programmes across endemic countries and impressive progresses made, the global coverage remains far below the 2010 target of deworming 75% of children. 38% of pre-SAC and 34% of SAC were reached globally in 2011, with a high variability across WHO regions, between 3% in the Eastern Mediterranean and 57% in the Americas (WHO, 2012a, Barry et al., 2013). Therefore, the schedule for reaching the global 75% coverage of schoolchildren has been reset to 2020 (WHO, 2012a).

Although all remain committed to necessity of MDA for control, some academics have raised various concerns about MDA. Those include widely acknowledged lack of PC sustainability in absence of complementary measures such as health education and WASH improvement, the risk of resistance emergence which may increase with long term mass treatment together with scaling up and increasing treatment pressure on parasites, and overall, the effectiveness of various PC programmes (Parker and Allen, 2011, Humphries et al., 2012, Medley and Hollingsworth, 2015, McCarty et al., 2014).

Factors that can affect MDA effectiveness are manifold and may include programme implementation processes on the supply side and acceptance on the recipient side, the efficacy of various anthelminthic drugs across parasite species and settings, the respective local prevalence and infection intensity of each STH species, as well as WASH and climatic environmental factors, health services –including because some MDA implementation processes are incumbent on health centres–, and socio-economic features (Parker and Allen, 2011, Krentel et al., 2013, McCarty et al., 2014, Nikolay et al., 2015, Shuford et al., 2016).

10.4.1 Drug efficacy

Drug efficacy is responsible for an important share of the variability of STH MDA effectiveness. Let alone the question of strongyloidiasis, as long as only benzimidazoles will be distributed for STH control, and even if the 75% coverage target was reached, the impact of MDA on hookworm will stay suboptimal and the effect on *T. trichiura* infection will remain low, due to the limited efficacy of mebendazole and albendazole on those species (Keiser and Utzinger, 2008, McCarty et al., 2014). The performances of mebendazole against hookworm has been raising concerns in Southeast Asia, where its low efficacy might relate to the presence of the zoonotic *Ancylostoma ceylanicum* that commonly infects humans in the region, including in Cambodia (Traub, 2013, Conlan et al., 2012, Inpankaew et al., 2014, Hotez et al., 2015, McCarty et al., 2014). The efficacy of benzimidazoles against this zoonotic species of hookworm is unknown and needs to be assessed. Depending on the performances achieved by benzimidazoles, the integration of *S. stercoralis* into STH control programmes in Cambodia might have important externalities as ivermectin would improve hookworm and *T. trichiuris* treatment performances. However, mebendazole might need to be replaced by albendazole to improve the treatment of hookworm (Keiser and Utzinger, 2010, Soukhathammavong et al., 2012, Flohr et al., 2007, Levecke et al., 2014, McCarty et al., 2014, Prichard et al., 2012, WHO, 2016b).

The albendazole- ivermectin combination is also more effective against *T. trichiura*, and mathematical models estimated that this combination would result in greater impacts on *T. trichiura* prevalence and infection intensity, as well as in a decrease of heavy infections and therefore on morbidity (Turner et al., 2016a). Yet, the current most effective drug combination against this parasite is albendazole-oxantel palmoate, each of those combinations achieving 27.5% and 68.5% CR and 94.5% and 99.2% ERR, while mebendazole cures 8.4% of infection and achieves an ERR of 58.5 (Speich et al., 2015). Of note, the co-delivery of ivermectin and benzimidazoles should be carefully monitored as this combination might accelerate the emergence of drug resistance to benzimidazole which has already been observed in *Ancylostoma caninum* (Prichard et al., 2012).

In absence of vaccines, it is widely acknowledged that new compounds are needed to face the decreased performances of, and the threat of emerging resistance to, currently used anthelminthic drugs. Two new compounds, moxidectin and tribendimidine, have reached phase II trials. Moxidectin, which is currently considered for validation to be used against onchocerciasis by the US Food and Drug Administration has shown high efficacy against *S. stercoralis* and might be a good candidate to replace ivermectin which cost is an issue in countries where onchocerciasis do not occur (Barda et al., 2017). Tribendimidine has a very broad anti-parasite spectrum, has been proven to be safe in adults and children, and its activity has been documented for 20 species of nematodes, cestodes and trematodes, which is of particular interest for areas where multiparasitic infections are prevalent (Soukhathammavong et al., 2011, Xiao et al., 2013, Xiao et al., 2005). Yet, the need to deliver drug packages both due to the overlapping of NTDs distributions and, in the case of STH, the suboptimal efficacy of single drugs on *T. trichiura* but also hookworms, research is needed to identify safe and optimal dosages for drug combinations (Boatin et al., 2012, Prichard et al., 2012, Keiser and Utzinger, 2008).

10.4.2 Reinfection

Assuming optimal drug efficacy, an important issue of PC is the need to sustain it over time as exposed populations keep getting re-infected, and at high rates, ranging from 57% for hookworm to 94% for *A. lumbricoides* one year post-treatment (Jia et al., 2012).

In the specific case of our study, it seems that high reinfection rate would not be a sufficient explanation to the 50% hookworm prevalence found among SAC. A systematic review including 154 studies estimated that hookworm reinfection rates were 30% 3 months after treatment and reached 50% to 57% 6 to 12 months after deworming (Jia et al., 2012). Deworming campaigns occur in November and May in Cambodia and our survey was conducted between January and May so children participating in the study were diagnosed 2 to 6 months after the deworming round of November. This suggests, assuming the above reinfection rates that 30% to 50% of cases could be due to reinfection. We found a low reinfection rate with *S. stercoralis*, which has the same transmission mode as hookworms, in north Cambodia. Although unknown, hookworm reinfection rates might be lower in our study setting than that estimated in the above cited meta-analysis. However, the low *S. stercoralis* reinfection rates might simply be due to the low larval output of this parasite, i.e. 0-20 larvae per gram of faeces per day (Grove, 1996). Another important issue would be the role played by dogs in hookworm transmission and reinfection rates through environment contamination with *A. ceylanicum*, which is highly prevalent in the region (Inpankaew et al., 2014).

The debate around the feasibility to break STH transmission, which divides the STH research and global health community, will not be opened here (Keenan et al., 2013). Yet, if the low *S. stercoralis* reinfection rates we observed in Preah Vihear were confirmed in other settings, a control approach that would intensively target entire communities might achieve high performances and impact on transmission. There is of course a very long to go from high control effectiveness to breaking transmission, including additional costs and difficulties to walk the "last mile", but learning on experience before addressing a new parasite might achieve higher than expected performances .

10.4.3 Coverage and compliance

STH control programmes in Cambodia report 98.6% and 96.0% national coverage of pre-SAC and SAC in 2015, respectively (WHO, 2015a). A well-known shortcoming of school-based deworming is that children who do not attend school are missed (Chesnaye et al., 2011, Olsen, 2003). School net enrolment ratio (NAR), i.e. the proportion of SAC aged 6-12 years attending primary school, varies greatly among provinces of Cambodia, from 28.7% in Mondulkiri and Rattanakiri provinces to 93.2% in Kampot province (National Institute of Public Health, 2006). A study assessing school-based MDA coverage in Kampot province found that only 48.8% of SAC (21 children) not attending schools were treated, although children attending schools were encouraged to bring along their siblings who do not go to school (Chesnaye et al., 2011). In the particular case of the Preah Vihear province, the net school enrolment ratio of 65.2% might have played a role in the high hookworm infection rate, i.e. 50.8% we found among SAC. The proportion of SAC reached through community-based pre-SAC deworming in unknown and should be assessed.

Coverage can be expected to differ from compliance unless the programme uses a directly-observed treatment approach and thorough recording of treatment uptake (Alexander, 2015, Shuford et al., 2016, Babu and Babu, 2014). The study in Kampot province also estimated that schools tended to over-estimate coverage compared to self-reporting by children (Chesnaye et al., 2011). In addition, the accuracy of self-reporting is known to be questionable, first due to recall bias, particularly after several rounds of MDA, and second, rates reported by children may be overestimated as, as stated by the study authors, "children tend to give answer that are socially expected from them" (Chesnaye et al., 2011, Brieger et al., 2011, Shuford et al., 2016).

Coverage and compliance are key factors in the performance of MDA programmes and should be locally assessed together with their underlying factors, on the supply side and the recipient side, so effectiveness can be improved, where needed.

The most important programme-level factors affecting coverage include methods and time of drug delivery, use of directly-observed therapy, and the motivation of drug distributors as well as the time they may allocate to distribution (Shuford et al., 2016, Krentel et al., 2006, Krentel et al., 2013). Being absent at the moment of drug delivery is a common reason for non-compliance to community-based MDA and might be an important concern (Babu and Babu, 2014, Krentel et al., 2013). Evening home visits might be considered and helped increasing coverage of schistosomiasis control in Cambodia (Sinuon Muth, personal communication).

On the recipient side, the most important and ubiquitous individual-level factors influencing compliance include the knowledge and perceived benefit of PC programme, understanding of personal disease risk, and the fear of side effects (Krentel et al., 2013, Babu and Babu, 2014, Alexander, 2015, Shuford et al., 2016). The fear of side effects, including from recipients who may not have experienced side effects personally, was found to substantially affect compliance to LF control and is also an important discouraging factor of praziquantel uptake (Muhumuza et al., 2013, Knopp et al., 2016, Parker et al., 2008). Since benzimidazoles are generally well tolerated, this aspect may be of lesser importance in compliance to STH control, although this aspect is unknown as most studies on STH have not assessed reasons for non-compliance (Shuford et al., 2016). MDA and treatment acceptance should be included within any programme evaluation.

Systematic non-compliance can be a major threat to MDA effectiveness, scaling-up feasibility and to breaking transmission as large groups of non-compliant individuals could act as reservoirs (Alexander, 2015, Shuford et al., 2016, Dyson et al., 2017). The extent of the impact of systematically untreated individuals on transmission remains non-assessed as their infection levels are mostly unknown, although it appeared in two studies that non-compliers had higher infection levels than compliers (Shuford et al., 2016). Importantly, some groups are systematically not treated due to non-eligibility due to exclusion criteria such as age under 2 years for young children or first trimester pregnancy for benzimidazoles, a restriction that, in absence of other control measures, might be a major threat to transmission interruption (WHO, 1995, Alexander, 2015). Another reason for the need of paediatric dosages of anthelmintic drugs need is that as very young children are the most at risk for stunting due to malnutrition and would greatly benefit from treatment, both at short term and in the long run given the lifetime impact of stunting.

10.4.4 Sanitation

Sanitation coverage impacts the effect of STH treatment as it conditions transmission and reinfection rates (Esrey et al., 1991, Nikolay et al., 2015, Forrer et al., 2016). Community-level improved sanitation coverage was one of the factors explaining the heterogeneity in the impact of the Kenyan deworming programme on hookworm infection (Nikolay et al., 2015). Additionally, we found in Preah Vihear province that *S. stercoralis* reinfection rates one year post treatment decreased in communities with increased improved sanitation coverage.

Most of the evidence supporting the effect of sanitation on STH infection levels and diarrheal diseases originates from meta-analyses of observational studies and relates to individual access to, and use of, improved sanitation facilities, which consist in adequate indicators when assessing the association between sanitation and STH prevalence (Esrey et al., 1991, Asaolu and Ofoezie, 2003, Clasen et al., 2010, Ziegelbauer et al., 2012, Strunz et al., 2014, Wolf et al., 2014). Hard evidence supporting the impact of sanitation on diarrhea, child growth or STH infection levels is scarce, with only a handful of RCTs conducted and very few concluding to an effect of sanitation coverage on STH infection levels, growth, or morbidity (Freeman et al., 2013a, Clasen et al., 2014, Patil et al., 2014, Dreibelbis et al., 2014, Pickering et al., 2015).

Yet, assessing the effectiveness of sanitation improvement measures does not equate to measuring the impact of sanitation coverage per se (Freeman et al., 2013a, Dreibelbis et al., 2014, Patil et al., 2014, Clasen et al., 2014, Pickering et al., 2015). Because parasites eggs and larvae slowly accumulate in the environment through time, prevalence studies would not be expected to capture the impact of sanitation coverage on infection levels. But post-treatment longitudinal assessments of reinfection rates might consist in one of the best opportunities to measure the impact of sanitation coverage on infection. However, longitudinal studies investigating the effect of community-level sanitation coverage on the risk of incident (re-)infection are extremely rare (Ziegelbauer et al., 2012, Strunz et al., 2014, Nikolay et al., 2015). Studies assessing the impact of treatment among communities with various existing -i.e. already established and not resulting from a sanitation intervention under assessment- sanitation coverage rates might provide valuable insight into the effect of sanitation on STH re-infection and transmission. This would be even more informative if conducted across settings with various transmission levels.

10.5 Control impact assessment, programme monitoring and evaluation

MDA programme effectiveness may also highly vary within countries. Programmes should be tailored to local circumstances, and may need to account for geographical, ecological, economic, as well as social and cultural differences so they are locally effective (Parker and Allen, 2011, Prichard et al., 2012, Krentel et al., 2013, Medley and Hollingsworth, 2015).

Programme monitoring and evaluation (M&E), which should ideally include measurements of their impact on STH infection levels and morbidity, are needed both from an international and global health standpoint and for individual countries programme managers and decision makers (Kabatereine et al., 2010, Parker and Allen, 2011, Humphries et al., 2012, Boatin et al., 2012, Medley and Hollingsworth, 2015).

First, the lack of hard evidence on deworming impact on STH infection levels and morbidity results in the underestimation of their burden and is a major obstacle in obtaining funding for basic and operational research (Boatin et al., 2012). In the case of *S. stercoralis*, these challenges may result in complete neglect. A strong case needs to be built to get subsidized or donated ivermectin, or start the production of generics, for *S. stercoralis* control. Second, at country level, monitoring would provide the necessary information to adapt programmes to increase effectiveness as and where needed (Kabatereine et al., 2010, Boatin et al., 2012). In the specific case of Cambodia and the perspective of *S. stercoralis* control, identifying the strengths and weaknesses of the STH control programme might help overcoming some additional challenges due to the integration of a new treatment, and to the potential expansion of control to additional risk groups if community-based control was to be deployed.

A first step of M&E assessments should encompass coverage and compliance on the one hand, and drug efficacy on the different STH species on the other hand so their respective contribution to programme effectiveness can be estimated and whenever possible, adequate adjustments can be made. Yet, ad-hoc monitoring, i.e. an ongoing process estimating whether programme outputs are reached and goals met and allowing for adjustment of programme activities are extremely rare, and even more so is the use of rigorous and unbiased monitoring approaches (Kabatereine et al., 2010). M&E surveys should be conducted in a consistent way with large enough samples to reflect the actual situation in entire evaluated areas and not be biased towards compliers (Parker and Allen, 2011). Although more challenging, concomitant monitoring of several diseases is feasible, an example being the successful use of LF transmission assessment surveys to assess STH and malaria in Haiti (Knipes et al., 2017).

Additionally, regular treatment generates a dynamic system with transmission and infection incidence changing over time so indicators allowing to measure changes in infection levels and morbidity should be regularly collected (Kabatereine et al., 2010, Boatin et al., 2012). Mathematical models can help the impact of deworming control measures on infection levels and transmission elimination scenarios (Boatin et al., 2012, Basanez et al., 2012, Truscott et al., 2015, Anderson et al., 2015). Yet this will necessitate the use of new diagnostic tools that are sensitive enough to detect light intensity infections (Boatin et al., 2012, Lustigman et al., 2012a).

Both the World Health Assembly resolution 66.12 and the London declaration 2012 on neglected tropical diseases have (re-)affirmed the importance of, and commitment to, increasing NTDs control coverage (NTDs, 2012, World-Health-Assembly, 2013). Given the high geographical variability of MDA effectiveness at large and small scale, assessing factors associated with local programme performance appears as a necessary step to efficiently scale up MDA. Despite the 2012 London Declaration commitments, a major issue in monitoring and evaluation (M&E) is funding availability in resource-limited countries, in addition to the lack of will from funders to grant programme activities other than drug delivery (Kabatereine et al., 2010).Yet, investing in M&E to improve programme output quality, .i.e. to increase effectiveness and quality rather than increase coverage and quantity, might turn out to be more cost-effective and beneficial to populations than scaling up programmes where they might not yet be sufficiently effective.

10.6 Challenges in measuring STH control impact on morbidity

PC has reduced STH infection levels in many countries, an impact that has been mostly measured in terms of case number estimates and marginally in infection levels and impact on severe morbidity (de Silva et al., 2003, Knopp et al., 2009a, Humphries et al., 2012, Chammartin et al., 2013a, Lai et al., 2013, Pullan et al., 2014, Karagiannis-Voules et al., 2015b, Karagiannis-Voules et al., 2015a).

There is a large body of literature documenting the variability of health impacts, drug efficacy and local relative distribution of the three major STH species. Yet, the evidence supporting the impact of deworming on STH morbidity is limited. Strengthening this evidence is a challenge currently faced by the scientific community mostly due to the non-specificity and multiplicity of symptoms and the issue of excluding co-infections, but also due to the difficulty to capture and assess subtle and long term health impacts (Campbell et al., 2016). Similar issues will be faced when attempting to assess the burden of *S. stercoralis* and the impact of treatment on the health problems it causes.

Cochrane reviews and meta-analysis stand as the highest quality of available evidence, and their results are used to determine morbidities in Global Burden of Disease assessments (Campbell et al., 2016). However, the use of meta-analyses aiming at assessing the impact of deworming on morbidity might be of concern when pooling results over all STH species, drugs used and regardless of infection status as was recently done in a Cochrane review that, unsurprisingly, did not find any effect of STH deworming on morbidity indicators (Taylor-Robinson et al., 2015).

A Cochrane review concluding to an absence of effect of STH deworming on health status, would have important implications since it would contribute to minimizing STH morbidity, as concluding to a lack of effect is not the same as a lack of evidence. The appropriateness of such an approach to investigate health impacts of affections as heterogeneous as those caused by *A. lumbricoides*, T. *trichiura* and hookworms should be critically assessed before use (Campbell et al., 2016).

Other approaches, including the use of long-term longitudinal studies which may be used to assess causal relationships, should be considered to provide evidence of STH-related morbidity, and studies should be sufficiently powered to allow species-specific assessments. Typically, while RCTs are needed to assess short-term effects of deworming, health impacts in children such as that of malnutrition might be difficult to assess with such designs as long-term effects might be better reflected in long-term longitudinal studies (Medley and Hollingsworth, 2015, Lo et al., 2015, Campbell et al., 2016). Such issues should be considered upon the investigation and assessment of *S. stercoralis* burden, as it is also probably associated with malnutrition and might as well cause long term morbidity.

10.7 Sanitation improvement for sustainable control

The major drawback of MDA is its lack of sustainability, mostly due to reinfection, relying on drug donation in the long run, programme fatigue, and, emergence of resistance to anthelminthic drugs. The need for improving sanitation as a sustainable control measure against diarrheal diseases and STH infections, and as part of the Millennium Sustainable Goals (SDGs) for sustainable development and poverty alleviation, is widely acknowledged by the scientific and public health community (WHO, 2010, Gazzinelli et al., 2012, Freeman et al., 2013b, WHO, 2013, Campbell et al., 2014).

The benefits of sanitation go beyond its effect on STH infections by impacting diarrheal diseases which are responsible for 29% of deaths of under five children, and are a major cause of burden, accounting for 3.6% of global DALYs in 2010 (as a comparison this proportion was 3.3% both for malaria and HIV/AIDS), while protein-energy malnutrition accounted for 1.4% of all DALYs (Murray et al., 2012, Prüss-Ustün A, 2016). It has been estimated that 58% of all diarrhea cases occurring in low- and middle-income countries are attributable to unsafe water, sanitation and hygiene, while 100% of STH infections are due to environmental conditions that are manageable, i.e. sanitation, which universal coverage would interrupt transmission (Prüss-Ustün et al., 2014, Prüss-Ustün A, 2016).

The two major challenges in the implementation of effective sanitation measures are the high costs for households and the lack of perceived need by communities. In turn, the two main approaches to improve sanitation conditions are to provide subsidies or hardware for latrine construction, or promoting behaviour change (Freeman et al., 2013b, Campbell et al., 2014, Gertler P, 2015).

The most widely used approach to address the lack of demand is the community-led total sanitation (CLTS), that has been adopted as the main sanitation improvement measure by many governments and non-governmental organizations (NGOs) and is now applied in 66 countries worldwide including Cambodia and Lao PDR (UNICEF, 2013, Sigler et al., 2015). CLTS is a participatory approach that aims at mobilizing communities for latrine construction by using emotional drivers such as disgust, shame, and fear of illness to trigger the need for latrines and strictly prohibits the use of subsidies or latrine provision. The goal is to end open defecation, with 100% of households owning latrines and all villagers having stopped defecating in the environment (Kar and Chambers, 2008, Sigler et al., 2015). Many programmes implementing the CLTS intervention have more or less departed from it and developed country- or context-specific CLTS-like programmes including locally tailored modifications or combinations with other activities (Sigler et al., 2015, Garn et al., 2017). A recent meta-analysis estimated that the achieved increased coverage among 13 CLTS and CLTS-like interventions in 8 countries was only 12%. The most striking result was the high variability of achieved coverage rates across settings, which ranged between -3% to 65% (Garn et al., 2017). This high variability of CLTS-derived interventions provide an opportunity to identify factors associated with low or high effectiveness, and which of them would be generalizable (Sigler et al., 2015, Gertler P, 2015).

A major challenge in the CLTS approach is its sustainability. Coverage rates decrease after programme implementation, even in communities that achieved high or full coverage. The two main reasons that have so far been identified are the quasi-absence of follow-up post implementation and the substantial proportion of built dry-pit latrines which are not durable. The lack of M&E is a major barrier to achieve full coverage as follow-up visits help keeping the population aware, motivated and participating (UNICEF, 2013, Sigler et al., 2015, Belizario et al., 2015).

Importantly, the evidence shows that CLTS interventions can be highly effective if implemented with a particularly strong behaviour change component and intensive post-implementation follow-up as it was done in Mali, where the intervention resulted in increased childhood growth and decreased stunting, as assessed by a RCT (Pickering et al., 2015).

Another limitation is that CLTS promotes building unimproved latrines to attain its objective. For the most poor households this results in the construction of dry-pit latrines which are fragile, non-satisfactory, and as a result, nor constantly used or sustainable (Pickering et al., 2015).

CLTS has had so far a limited impact among the Cambodian population who have a strong preference for pour-flush latrines and would rather have no latrine than any other type of facility (Kunthy, 2009, Pedi D, 2012, UNICEF, 2013, Pedi D, 2014). Two observations from a programme implemented in Kampong Speu Province suggest difficulties in conveying objective reasons for latrine adoption: 28% of respondents gave "someone told me I had to" as the major reason for latrine construction, while 11% of respondents only cited "personal awareness of the importance of having a toilet".

The CLTS technique strictly prohibits the use of subsidies. Yet, a combined approach of CLTS and subsidies for households living under the poverty line was effective in a poor setting of India where cost was identified as the main obstacle to build latrines, and was the only approach that resulted in increased sanitation coverage and decreased open defecation in a cluster-randomized trial conducted in the poorest area of Bangladesh (Guiteras et al., 2015). Combining CLTS programmes with subsidies or hardware provision for the poorest households so they could build latrines in accordance with their preferences could turn out to be an effective measure in Cambodia.

Research should be conducted to assess the strengths and weaknesses of CLTS-derived programmes so successful approaches can help improving programmes with lower impact. Yet, overcoming some difficulties might be particularly challenging as factors impacting programme effectiveness may not all be of human origin and may also encompass environmental and climatic aspects, including access to water, which absence is a major obstacle in access to improved pour-flush facilities.

Although highly challenging, achieving sustained universal sanitation coverage in rural areas might be feasible in some settings, provided that schools are also equipped. However, the goal of stopping open defecation in rural environments might be over challenging as most of the population works outside their village. It seems unlikely that they would go back home to use latrines, even more so if open defecation is culturally acceptable.

Yet even imperfect sanitation coverage might substantially impact transmission and enhance the effect of chemotherapy-based STH control. The literature mentions that sanitation coverage needs to be over a given –high, about 90%- threshold to protect from infection, but we could not find any hard evidence about that claim (Albonico et al., 2006). Moreover, our findings about *S. stercoralis*, which should be generalizable to other STH and at least to hookworm that has the same transmission mode, suggest that community-level sanitation coverage might be protective below that 90% threshold. Further studies are needed to investigate this aspect. Confirming that increased sanitation coverage benefits not only to those who own facilities and use them but to entire populations by decreasing the risk of contact with STH eggs of larvae on the soil would have important implications for control, cost-effectiveness of sanitation improvement measures, and equity.

10.8 Integration of *Strongyloides stercoralis* into STH control in Cambodia

In Cambodia, the primary health care system already works through outreach services to deliver various activities including immunization, antenatal care, health education, family planning, and Vitamin A distribution. Deworming already targets pre-school children and their mothers through community-based delivery of immunization and Vitamin A distribution (WHO, 2005, National Center for Parasitology, 2014).

Although integration should go beyond chemotherapy and encompass health education, hygiene, sanitation and housing conditions improvement, in the context of NTD control, the concept of integration usually relates to distributing drug packages (Lammie et al., 2006, Molyneux et al., 2005, Hotez, 2009). Let alone the question of the effectiveness of health education and sanitation improvement measures, control activities other than chemotherapy tend to be more expensive and difficult to scale up and obtaining funding for such activities is challenging (Kabatereine et al., 2010). In areas of co-endemicities, the integrated delivery of combined anthelmintic drugs rather than running parallel programmes is highly cost-effective and can help saving up to 50% of resources (Molyneux et al., 2005, Lammie et al., 2006, Hotez, 2009, Evans et al., 2011, Leslie et al., 2013, Shuford et al., 2016).

However, some concerns have been raised about integration which may exacerbate difficulties (Parker and Allen, 2011). The co-delivery of several treatments might increase logistic difficulties and temporarily decrease coverage (Kabatereine et al., 2010, Parker and Allen, 2011, Prichard et al., 2012). The experience of integrating the control of five diseases in Uganda pointed out the additional difficulties for community drug distributors to grasp the complex instructions to deliver several treatments, which might result in less time spent communicating with recipients, an aspect that is crucial for compliance (Kabatereine, 2009, Parker and Allen, 2011, Krentel et al., 2013, Shuford et al., 2016). Additionally, self-reporting and recall of treatment uptake is more challenging when several drugs are delivered (Prichard et al., 2012, Shuford et al., 2016). Finally, there is lack of safety data for drug combinations and optimal dosages (Boatin et al., 2012, Prichard et al., 2012).

An important aspect is the capacity of the delivery system in place to uptake the additional workload and ensuring that health workers are properly trained to face the additional challenges that integration may pose (Kabatereine, 2009, Kabatereine et al., 2010, Parker and Allen, 2011). Integration can weaken programmes that are already struggling to implement ongoing measures while it should be no problem for systems that are not overwhelmed (Kabatereine et al., 2010).

Regarding the specific case of the integration of *S. stercoralis* to the STH control programme in Cambodia, adding the delivery of ivermectin to mebendazole would be expected to have a good feasibility if control was to target risk groups already reached, i.e. children and women of child bearing age. *S stercoralis* is also a STH so current health education messages and material would remain valid. Challenges would involve explaining the population that this “new” worm needs to be treated with a different drug, and to weigh recipients since ivermectin posology depends on body weight. The deployment of community-wide *S. stercoralis* control would imply extending community-based control from pre-school children to men and women aged 50 years and above. The CNM has the experience of implementing community based control which has been ongoing since 1996 for schistosomiasis, targeting a population at risk of 80,000 individuals in two and five districts of Kratie and Stung Treng province, respectively (Sinuon et al., 2007).

This experience could provide important insight for the potential introduction of *S. stercoralis* community-wide control, including in areas with difficult access such as communities that have to be reached by boat (Virak Khieu, personal communication). Logistic issues due to remoteness would also be reflected in programme costs.

There is a lack of M&E of STH control activities in Cambodia, where the effectiveness and impact of control measures on infection levels need to be assessed (Dunn et al., 2016). The CNM is aware of this and wishes to proceed to M&E but is currently facing challenges to obtain funding, an issue that is not rare in STH control as funders prefer to grant drug delivery activities (Kabatereine et al., 2010). The capacity of the current control delivery network to implement community-wide control should be investigated, and the local health staff in charge of MDA might also be asked about their opinion, concerns and perception of the integration of an additional treatment.

Cambodia has demonstrated its ability to address helminthic infections. STH control started in 2002 with 11 provinces, was scaled-up to the entire primary schoolchildren population by early 2004, and was among the first countries to reach the 75% coverage target of the WHO (Sinuon et al., 2005, Montresor et al., 2008, WHO, 2012a). Community-wide schistosomiasis control has been delivered yearly since 2001 to 80,000 villagers (Sinuon et al., 2007, Montresor et al., 2008). Prevalence dramatically decreased and no severe case of schistosomiasis has been observed since 2005 (Khieu, 2016). LF control with combined albendazole and diethylcarbamazine was initiated in 2005 and targeted almost half a million individuals in the four provinces where the filarial disease is endemic. The programme was monitored all along and prevalence dropped to 0% after two MDA rounds and remained null thereafter, leading to the interruption of MDA in 2010. By 2016, LF was eliminated as a public health problem, and is now under surveillance for elimination by 2020 (WHO, 2016a). Cambodia appears to have the clear capacity to integrate *S. stercoralis* into STH control although additional control activities might be preceded with monitoring and evaluation of ongoing procedures, and an assessment of financial and manpower resource needs for integration, particularly if control is deployed community-wide, might help avoiding some difficulties inherent to the integration of large-scale programmes.

Regardless of when control will be implemented, new generations of laboratory technicians and health workers should be trained to perform the Baermann and KAP methods so *S. stercoralis* can be diagnosed in hospitals and primary health care facilities. Along the same line, medical studies should include strongyloidiasis in their curriculum, including hyperinfection. Such an approach might help appraising the incidence of hyperinfection in endemic settings, which is crucial to document the burden of *S. stercoralis*.

10.9 Next steps to have *Strongyloides stercoralis* recognized as a major STH

Within the NTD control framework, preventive chemotherapy control is used against diseases for which an efficacious and safe drug is available and a control strategy exists. Currently, seven diseases or groups of diseases, i.e. STH infections, schistosomiasis, foodborne trematode infections, lymphatic filariasis, onchocerciasis, cysticercosis, and dracunculiasis are targeted by PC (WHO, 2013).

Strongyloidiasis belongs to that list but no defined control strategy exists for this parasite. Three major obstacles currently preclude having *S. stercoralis* recognized as a global public health problem and addressed by a control strategy: the lack of readily available and affordable diagnostic technique, the cost of ivermectin, and the lack of data documenting the impact of ivermectin on *S. stercoralis* infection, which also involves documenting *S. stercoralis* burden (Albonico et al., 2016).

The public health community is aware that strongyloidiasis is likely to be a public health problem in numerous LMICs, as well as among specific populations of high-income countries such as Australia, but as long as global or at least national burden estimates (case number and morbidity) will not available, it seems unlikely that *S. stercoralis* will join the list of major STH and will be recognized as a public health problem by the WHO (Jex et al., 2011, McCarty et al., 2014).

The minimum required to efficiently advocate for donation or generic production of ivermectin would be first to document *S. stercoralis* case number estimates at least at region level. A major hindrance to obtain large-scale estimates is the absence of a diagnostic test easily applicable in the field that would ease large-scale prevalence surveys. Our cost-effectiveness study clearly reflected this issue, with the cost of diagnosing one individual with a sensitive approach reaching almost 20 USD. The cost of the serological diagnostic used for the Cambodian national survey was of the same magnitude (Virak Khieu, personal communication). Until a readily usable and affordable diagnostic approach is available, the large-scale assessment of *S. Stercoralis* infection will remain costly, time- and labour-consuming, and will necessitate skilled staff specifically trained for *S. stercoralis* diagnosis (Albonico et al., 2016).

Ivermectin is highly efficacious at a single oral dose of 200 µg/kg body weight and can be used for large-scale control (Gann et al., 1994, Suputtamongkol et al., 2011, Forrer et al., 2016, Albonico et al., 2016, Barda et al., 2017). *S. stercoralis* prevalence dramatically declined in Ecuador and Tanzania, in areas where ivermectin has been distributed for years for the control of onchocerciasis (Knopp et al., 2009a, Anselmi et al., 2015, Albonico et al., 2016). The results of our cohort study in Preah Vihear province, where ivermectin achieved a high cure rate over 95% and *S. stercoralis* reinfection rates one year post treatment were below 15%, confirm those findings (Forrer et al., 2016). However further cohort studies documenting the impact of a single oral dose of ivermectin on *S. stercoralis*, including in settings of various transmission intensities, are needed to strengthen evidence and assess the impact of control on infection levels and transmission.

Ivermectin has been donated since 1987 for onchocerciasis and in 2008 for lymphatic filariasis control, respectively, but is not listed as an essential medicine for STH control and is not donated or subsidized for *S. stercoralis* treatment (WHO, 2015b). A market analysis, i.e. the number of ivermectin tablets needed to treat *S. stercoralis* cases worldwide would help pharmaceutical companies, funding agencies and other stakeholders appraising the considerable market opened by the control of this widespread parasite (Albonico et al., 2016). *S. stercoralis* can be expected to be widespread in regions with warm climate, poor sanitation conditions that are not currently addressed by onchocerciasis control. Additionally, the prevalence of *S. stercoralis* will most likely reverse to its original levels in a few years in regions where successful control of onchocerciasis will result in the interruption if ivermectin distribution (Anselmi et al., 2015). Of note, assessing the impact of ivermectin PC should account for its additional effect on scabies, as well as hookworm and *T. trichiura* when combined to albendazole (Knopp et al., 2010a, Keiser and Utzinger, 2010). Cost-effectiveness studies assessing the impact of ivermectin combined to either benzimidazole on all four STH are needed.

Finally, documenting the burden of *S. stercoralis* infection will be instrumental to the definition of a control strategy and deployment of PC. *S. stercoralis* morbidity is certainly largely underestimated, both in terms of disability and mortality. First, because very few studies investigating clinical signs of strongyloidiasis in endemic settings have been conducted and more are needed to strengthen evidence (Becker et al., 2011, Khieu et al., 2013b, Forrer et al., 2017). Second, a crucial and to our knowledge completely undocumented aspect of strongyloidiasis morbidity in LMICs, is the risk of hyperinfection, which in absence of detection and treatment, is 100% fatal (Keiser and Nutman, 2004, Marcos et al., 2008). Hyperinfection occurs in case of immunosuppression, mostly due to treatment with corticosteroids. The availability and increasing use of over-the-counter drug cocktails containing corticosteroids in LMICs raised a serious concern about the risks of developing hyperinfection in endemic settings (Olsen et al., 2009). Indeed, low-dose and short-duration prednisone therapies, i.e. 20-30 mg daily for 6-17 days can trigger fatal hyperinfection cases in individuals with competent immune functions and without any underlying immunosuppressive condition (Nutman, 2016, Ghosh and Ghosh, 2007). The risk of hyperinfection in endemic settings appears far from negligible and should be estimated.

To better guide control, the transmission dynamics of *S. stercoralis* need to be documented. Investigating reinfection rates in settings with various prevalence rates would help appraising the relationship between transmission and prevalence. Such investigations should account for community-level sanitation coverage which most likely will impact reinfection rates whatever the transmission intensity. A necessary step is to assess the relationship between transmission dynamics and infection intensity. Because *S. stercoralis* infection intensity is believed to change within individual over time, studies documenting this aspect of infection are also required. Transmission dynamics models are precious tools to guide control efforts as they can also be used to identify the most effective and cost-effective control approaches. Upon the availability of meaningful infection intensity thresholds, i.e. which are able to reflect morbidity, mapping *S. stercoralis* infection intensity would also provide crucial information for control implementation. Ideally, mathematical transmission dynamics models would be coupled to geostatistical models to appraise infection dynamics across low, moderate and high-risk areas.

There is a long way to go for *S. stercoralis* to join the WHO list of major STH and be attributed a global control strategy. Hopefully, Cambodia will soon be joined by other countries in the assessment of *S. stercoralis* prevalence and burden. At the beginning of STH control, there were 112 countries requiring PC for STH. It is therefore likely that *S. stercoralis* infections are also common in most of those countries with the exception of areas where onchocerciasis control is ongoing, i.e. 19 countries of Africa and 2 foci in South America, one in Brazil, the other in Venezuela (WHO, 2017a, WHO, 2017c).

The example of Cambodia shows that producing if not national, at least province-level estimates, for *S. stercoralis* is feasible, even in countries where resources are scarce. Collaborations between research entities and institutions in charge of parasitic disease control might help raising funds to conduct such studies and start producing up to date *S. stercoralis* prevalence estimates. This would consist in a first crucial step in raising awareness about this highly neglected parasite, and would hopefully trigger interest from the research and public health community. The next greatest need would be a cheap and readily usable diagnostic method that could accelerate the documentation of the parasite prevalence and burden, which in turn might ease access to treatment.

Chapter 11

Further research needs and recommendations

11.1 Global needs for *S. stercoralis* research

Three main inputs would help raising awareness about *S. stercoralis*, advocating for access to treatment and ease the parasite management in endemic countries: the availability of an easy-to-use and cheap diagnostic method, providing burden estimates including morbidity and national-level prevalence rates, while maps of infection distribution and transmission dynamics models would help guiding control efforts.

- ⇨ Develop a cheap diagnostic tool that would be readily available (i) for large-scale prevalence studies to document *S. stercoralis* prevalence and burden to advocate for access to treatment, and (ii) in primary health care facilities for the management of infection in endemic settings; this would also help detecting hyperinfection cases, avoid mortality, and document the burden of *S. stercoralis*

- ⇨ Conduct community-based and/or hospital based studies to document infection intensity and establish infection intensity thresholds that reflect morbidity to guide control efforts.

- ⇨ Assess whether infection intensity varies within-host through time and if this variation affects morbidity and transmission through the quantity of excreted larvae. This information is crucial to understand the parasite transmission dynamics.

- ⇨ Conduct RCTs to assess the association between *S. stercoralis* and malnutrition/growth retardation at short term accounting for co-infections with other helminths and protozoan parasites, as well as nutritional uptake.

- ⇨ Use before-after treatment studies accounting for co-infections with other helminths and protozoan parasites to further document clinical signs associated with chronic infection in endemic settings

- ⇨ Conduct hospital-based studies to document hyperinfection in endemic countries. Retrospective identification of cases using hospital logs might be useful to locate patients who could then reach either at home or upon another hospital visit.

- ⇨ Develop *S. stercoralis* transmission dynamics models to appraise infection dynamics and assess the most effective and cost-effective control approaches.

11.2 Research needs in Cambodia

With regard to *S. stercoralis* infection in Cambodia, the next steps for research would include:

- ⇨ Documenting re-infection rates across settings with low, moderate and high (in absence of infection intensity estimates/thresholds) prevalence rates, accounting for community-level sanitation coverage

- ⇨ Assessing available *vs.* needed financial and manpower resources for the integration of *S. stercoralis* control

- ⇨ Assessing the cost-effectiveness of integrated *S. stercoralis* control, i.e. including effects of the ivermectin-mebendazole (or albendazole) on the three other STH

Additionally, public health policy might be modified to include the management of *S. stercoralis*.

- ⇨ Train the primary health care laboratory staff to perform the Baermann and KAP methods to diagnose *S. stercoralis*

- ⇨ Add *S. stercoralis* to medical studies curriculum so new generations of doctors are doable to diagnose infection. Specific training for *S. stercoralis* should also be provided to doctors of the primary health care system.

Research on STH control effectiveness and modalities should also be conducted.

- ⇨ Assess the efficacy of mebendazole and albendazole, alone or in combination with ivermectin on hookworms, including the zoonotic *A. ceylanicum*

- ⇨ Monitor ongoing STH control programme and assess coverage and compliance, including on school-aged children not enrolled in schools, and identify programme-level and recipient-related factors impacting control effectiveness.

- ⇨ Document the burden of *A. ceylanicum* infection in humans, starting with the identification of clinical signs associated with *A. ceylanicum* infections in humans. If *A. ceylanicum* causes significant morbidity in humans a "one health" approach also targeting dogs might be considered

  Any attempts to document STH morbidity should be conducted using a highly sensitive diagnostic approach (combined methods on 2 stool samples) for helminths and protozoan parasites and possibly use an infection status definition that would maximize diagnostic specificity (e.g. as done in our work, include only negatives with all diagnostic results available).

Chapter 12

Conclusion

S. stercoralis is widespread in Cambodia with almost one in three individuals infected. This parasite is responsible for troublesome gastrointestinal and dermatological morbidity, and might be associated with growth retardation in children. Additionally, *S. stercoralis* infection is potentially fatal in immunocompromised individuals but that aspect of infection remains undocumented in endemic settings.

S. stercoralis has been recognized as a public health problem in Cambodia where it needs to be addressed by preventive-chemotherapy control programmes. However the onset of control is currently hindered by the high cost of ivermectin.

Cambodia is the first country to produce a national estimate of *S. stercoralis* prevalence. Yet, the parasite is likely to be widespread in LMIC with warm climate and poor sanitation conditions where ivermectin is not delivered for the control on onchocerciasis. Conducting large-scale surveys is challenging as currently available diagnostic techniques with satisfactory sensitivity are costly, and/or time- and labour-consuming. Next steps include documenting the burden due to both *S. stercoralis* chronic and potentially fatal hyperinfection. A readily usable diagnostic tool would greatly help documenting *S. stercoralis* infection, while up to date prevalence estimates would in turn, help advocating to ivermectin donation or generic production.

The example of Cambodia shows that *S. stercoralis* specific surveys can be conducted even in countries with limited resources, where the contribution of research grants might help in starting the process. The production of prevalence estimates in other STH endemic countries is crucial to help raising awareness and get access to treatment for this highly neglected parasite, which belongs to the WHO list of major STH.

Bibliography

ALBONICO, M., BECKER, S. L., ODERMATT, P., ANGHEBEN, A., ANSELMI, M., AMOR, A., BARDA, B., BUONFRATE, D., COOPER, P., GETAZ, L., KEISER, J., KHIEU, V., MONTRESOR, A., MUNOZ, J., REQUENA-MENDEZ, A., SAVIOLI, L., SPEARE, R., STEINMANN, P., VAN LIESHOUT, L., UTZINGER, J., BISOFFI, Z. & STRONGNET WORKING, G. 2016. StrongNet: An International Network to Improve Diagnostics and Access to Treatment for Strongyloidiasis Control. *PLoS Negl Trop Dis,* 10**,** e0004898.

ALBONICO, M., MONTRESOR, A., CROMPTON, D. W. & SAVIOLI, L. 2006. Intervention for the control of soil-transmitted helminthiasis in the community. *Adv Parasitol,* 61**,** 311-48.

ALEXANDER, N. D. 2015. Are we nearly there yet? Coverage and compliance of mass drug administration for lymphatic filariasis elimination. *Trans R Soc Trop Med Hyg,* 109**,** 173-4.

ALLEN, A. V. & RIDLEY, D. S. 1970. Further observations on the formol-ether concentration technique for faecal parasites. *J Clin Pathol,* 23**,** 545-6.

ANAMNART, W., PATTANAWONGSA, A., INTAPAN, P. M., MORAKOTE, N., JANWAN, P. & MALEEWONG, W. 2013. Detrimental effect of water submersion of stools on development of *Strongyloides stercoralis*. *PLoS One,* 8**,** e82339.

ANDERSON, R., TRUSCOTT, J. & HOLLINGSWORTH, T. D. 2014. The coverage and frequency of mass drug administration required to eliminate persistent transmission of soil-transmitted helminths. *Philos Trans R Soc Lond B Biol Sci,* 369**,** 20130435.

ANDERSON, R. M., TRUSCOTT, J. E., PULLAN, R. L., BROOKER, S. J. & HOLLINGSWORTH, T. D. 2013. How effective is school-based deworming for the community-wide control of soil-transmitted helminths? *PLoS Negl Trop Dis,* 7**,** e2027.

ANDERSON, R. M., TURNER, H. C., TRUSCOTT, J. E., HOLLINGSWORTH, T. D. & BROOKER, S. J. 2015. Should the Goal for the Treatment of Soil Transmitted Helminth (STH) Infections Be Changed from Morbidity Control in Children to Community-Wide Transmission Elimination? *PLoS Negl Trop Dis,* 9**,** e0003897.

ANSELMI, M., BUONFRATE, D., GUEVARA ESPINOZA, A., PRANDI, R., MARQUEZ, M., GOBBO, M., MONTRESOR, A., ALBONICO, M., RACINES ORBE, M., MARTIN MOREIRA, J. & BISOFFI, Z. 2015. Mass Administration of Ivermectin for the Elimination of Onchocerciasis Significantly Reduced and Maintained Low the Prevalence of *Strongyloides stercoralis* in Esmeraldas, Ecuador. *PLoS Negl Trop Dis,* 9**,** e0004150.

ASAOLU, S. O. & OFOEZIE, I. E. 2003. The role of health education and sanitation in the control of helminth infections. *Acta Trop,* 86**,** 283-94.

ASIAN-DEVELOPMENT-BANK 2014. Cambodia: Country poverty analysis 2014.

ASSELIN, L. M. & TUAN ANH, V. 2008. Multidimensional poverty and multiple correspondance analysis. *Quantitative approaches to multidimensional poverty measurement.* London: Palgrave Macmillan.

BABU, B. V. & BABU, G. R. 2014. Coverage of, and compliance with, mass drug administration under the programme to eliminate lymphatic filariasis in India: a systematic review. *Trans R Soc Trop Med Hyg,* 108**,** 538-49.

BAERMANN, G. 1917. A simple method for the detection of *Ankylostomum* (nematode) larvae in soil tests. 41-47.

BAKIRI, A. H. & MINGOMATAJ, E. C. 2010. Parasites induced skin allergy: a strategic manipulation of the host immunity. *J Clin Med Res,* 2**,** 247-55.

BARDA, B., SAYASONE, S., PHONGLUXA, K., XAYAVONG, S., KEODUANGSY, K., ODERMATT, P., PUCHKOV, M., HUWYLER, J., HATTENDORF, J. & KEISER, J. 2017. Efficacy of moxidectin versus ivermectin against *Strongyloides stercoralis* infections: a randomized controlled non-inferiority trial. *Clin Infect Dis*.

BARRY, M. A., SIMON, G. G., MISTRY, N. & HOTEZ, P. J. 2013. Global trends in neglected tropical disease control and elimination: impact on child health. *Arch Dis Child,* 98**,** 635-41.

BASANEZ, M. G., MCCARTHY, J. S., FRENCH, M. D., YANG, G. J., WALKER, M., GAMBHIR, M., PRICHARD, R. K. & CHURCHER, T. S. 2012. A research agenda for helminth diseases of humans: modelling for control and elimination. *PLoS Negl Trop Dis,* 6**,** e1548.

BECKER, S. L., SIETO, B., SILUE, K. D., ADJOSSAN, L., KONE, S., HATZ, C., KERN, W. V., N'GORAN, E. K. & UTZINGER, J. 2011. Diagnosis, clinical features, and self-reported morbidity of *Strongyloides stercoralis* and hookworm infection in a co-endemic setting. *PLoS Negl Trop Dis,* 5**,** e1292.

BELIZARIO, V. Y., JR., LIWANAG, H. J., NAIG, J. R., CHUA, P. L., MADAMBA, M. I. & DAHILDAHIL, R. O. 2015. Parasitological and nutritional status of school-age and preschool-age children in four villages in Southern Leyte, Philippines: Lessons for monitoring the outcome of Community-Led Total Sanitation. *Acta Trop,* 141**,** 16-24.

BETHONY, J., BROOKER, S., ALBONICO, M., GEIGER, S. M., LOUKAS, A., DIEMERT, D. & HOTEZ, P. J. 2006. Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. *Lancet,* 367**,** 1521-1532.

BETHONY, J., CHEN, J., LIN, S., XIAO, S., ZHAN, B., LI, S., XUE, H., XING, F., HUMPHRIES, D., YAN, W., CHEN, G., FOSTER, V., HAWDON, J. M. & HOTEZ, P. J. 2002. Emerging patterns of hookworm infection: influence of aging on the intensity of *Necator* infection in Hainan province, People's Republic of China. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America,* 35**,** 1336-1344.

BISOFFI, Z., BUONFRATE, D., ANGHEBEN, A., BOSCOLO, M., ANSELMI, M., MAROCCO, S., MONTEIRO, G., GOBBO, M., BISOFFI, G. & GOBBI, F. 2011. Randomized clinical trial on ivermectin versus thiabendazole for the treatment of strongyloidiasis. *PLoS Negl Trop Dis,* 5**,** e1254.

BISOFFI, Z., BUONFRATE, D., MONTRESOR, A., REQUENA-MÉNDEZ, A., MUÑOZ, J., KROLEWIECKI, A. J., GOTUZZO, E., MENA, M. A., CHIODINI, P. L., ANSELMI, M., MOREIRA, J. & ALBONICO, M. 2013. *Strongyloides stercoralis*: a plea for action. *PLoS Negl Trop Dis,* 7**,** e2214.

BISOFFI, Z., BUONFRATE, D., SEQUI, M., MEJIA, R., CIMINO, R. O., KROLEWIECKI, A. J., ALBONICO, M., GOBBO, M., BONAFINI, S., ANGHEBEN, A., REQUENA-MENDEZ, A., MUNOZ, J. & NUTMAN, T. B. 2014. Diagnostic accuracy of five serologic tests for *Strongyloides stercoralis* infection. *PLoS Negl Trop Dis,* 8**,** e2640.

BOATIN, B. A., BASANEZ, M. G., PRICHARD, R. K., AWADZI, K., BARAKAT, R. M., GARCIA, H. H., GAZZINELLI, A., GRANT, W. N., MCCARTHY, J. S., N'GORAN, E. K., OSEI-ATWENEBOANA, M. Y., SRIPA, B., YANG, G. J. & LUSTIGMAN, S. 2012. A research agenda for helminth diseases of humans: towards control and elimination. *PLoS Negl Trop Dis,* 6**,** e1547.

BOOTH, M., VOUNATSOU, P., N'GORAN, E. K., TANNER, M. & UTZINGER, J. 2003. The influence of sampling effort and the performance of the Kato-Katz technique in diagnosing *Schistosoma mansoni* and hookworm co-infections in rural Côte d'Ivoire. *Parasitology,* 127**,** 525-531.

BOOYSEN, F., VAN DER BERG, S., BURGER, R., VON MALTITZ, M. & DU RAND, G. 2008. Using an asset index to assess trends in poverty in seven Sub-Saharan African countries. *World Development,* 36**,** 1113-1130.

BRIEGER, W. R., OKEIBUNOR, J. C., ABIOSE, A. O., WANJI, S., ELHASSAN, E., NDYOMUGYENYI, R. & AMAZIGO, U. V. 2011. Compliance with eight years of annual ivermectin treatment of onchocerciasis in Cameroon and Nigeria. *Parasit Vectors,* 4**,** 152.

BROOKER, S. 2010. Estimating the global distribution and disease burden of intestinal nematode infections: adding up the numbers--a review. *Int J Parasitol,* 40**,** 1137-44.

BROOKER, S., ALEXANDER, N., GEIGER, S., MOYEED, R. A., STANDER, J., FLEMING, F., HOTEZ, P. J., CORREA-OLIVEIRA, R. & BETHONY, J. 2006. Contrasting patterns in the small-scale heterogeneity of human helminth infections in urban and rural environments in Brazil. *International journal for parasitology,* 36**,** 1143-1151.

BROOKER, S., BETHONY, J. & HOTEZ, P. J. 2004a. Human hookworm infection in the 21st century. *Advances in Parasitology,* 58**,** 197-288.

BROOKER, S. & CLEMENTS, A. C. A. 2009. Spatial heterogeneity of parasite co-infection: determinants and geostatistical prediction at regional scales. *International journal for parasitology,* 39**,** 591-597.

BROOKER, S., HOTEZ, P. J. & BUNDY, D. A. P. 2008. Hookworm-related anaemia among pregnant women: a systematic review. *PLoS.Negl.Trop.Dis.,* 2**,** e291.

BROOKER, S., KABATEREINE, N. B., SMITH, J. L., MUPFASONI, D., MWANJE, M. T., NDAYISHIMIYE, O., LWAMBO, N. J., MBOTHA, D., KARANJA, P., MWANDAWIRO, C., MUCHIRI, E., CLEMENTS, A. C., BUNDY, D. A. & SNOW, R. W. 2009. An updated atlas of human helminth infections: the example of East Africa. *Int J Health Geogr,* 8**,** 42.

BROOKER, S., KABATEREINE, N. B., TUKAHEBWA, E. M. & KAZIBWE, F. 2004b. Spatial analysis of the distribution of intestinal nematode infections in Uganda. *Epidemiol Infect,* 132**,** 1065-71.

BROOKER, S., SINGHASIVANON, P., WAIKAGUL, J., SUPAVEJ, S., KOJIMA, S., TAKEUCHI, T., LUONG, T. V. & LOOAREESUWAN, S. 2003. Mapping soil-transmitted helminths in Southeast Asia and implications for parasite control. *Southeast Asian J Trop Med Public Health,* 34**,** 24-36.

BROOKER, S. J., NIKOLAY, B., BALABANOVA, D. & PULLAN, R. L. 2015. Global feasibility assessment of interrupting the transmission of soil-transmitted helminths: a statistical modelling study. *Lancet Infect Dis,* 15**,** 941-50.

BUONFRATE, D., FORMENTI, F., PERANDIN, F. & BISOFFI, Z. 2015a. Novel approaches to the diagnosis of *Strongyloides stercoralis* infection. *Clin Microbiol Infect,* 21**,** 543-552.

BUONFRATE, D., SEQUI, M., MEJIA, R., CIMINO, R. O., KROLEWIECKI, A. J., ALBONICO, M., DEGANI, M., TAIS, S., ANGHEBEN, A., REQUENA-MENDEZ, A., MUNOZ, J., NUTMAN, T. B. & BISOFFI, Z. 2015b. Accuracy of five serologic tests for the follow up of Strongyloides stercoralis infection. *PLoS Negl Trop Dis,* 9**,** e0003491.

CAMPBELL, S. J., NERY, S. V., DOI, S. A., GRAY, D. J., SOARES MAGALHAES, R. J., MCCARTHY, J. S., TRAUB, R. J., ANDREWS, R. M. & CLEMENTS, A. C. 2016. Complexities and Perplexities: A Critical Appraisal of the Evidence for Soil-Transmitted Helminth Infection-Related Morbidity. *PLoS Negl Trop Dis,* 10**,** e0004566.

CAMPBELL, S. J., SAVAGE, G. B., GRAY, D. J., ATKINSON, J. A., SOARES MAGALHAES, R. J., NERY, S. V., MCCARTHY, J. S., VELLEMAN, Y., WICKEN, J. H., TRAUB, R. J., WILLIAMS, G. M., ANDREWS, R. M. & CLEMENTS, A. C. 2014. Water, Sanitation, and Hygiene (WASH): a critical component for sustainable soil-transmitted helminth and schistosomiasis control. *PLoS Negl Trop Dis,* 8**,** e2651.

CARABALLO, L., ZAKZUK, J., LEE, B. W., ACEVEDO, N., SOH, J. Y., SÁNCHEZ-BORGES, M., HOSSNY, E., GARCÍA, E., ROSARIO, N., ANSOTEGUI, I., PUERTA, L., SÁNCHEZ, J. & CARDONA, V. 2016. Particularities of allergy in the Tropics. *World Allergy Organ J,* 9**,** 20.

CARVALHO, E. M. & DA FONSECA PORTO, A. 2004. Epidemiological and clinical interaction between HTLV-1 and Strongyloides stercoralis. *Parasite Immunol,* 26**,** 487-97.

CARVALHO, L. & PARISE, E. R. 2006. Evaluation of nutritional status of nonhospitalized patients with liver cirrhosis. *Arq Gastroenterol,* 43**,** 269-74.

CHAI, J. Y., HAN, E. T., GUK, S. M., SHIN, E. H., SOHN, W. M., YONG, T. S., EOM, K. S., LEE, K. H., JEONG, H. G., RYANG, Y. S., HOANG, E. H., PHOMMASACK, B., INSISIENGMAY, B., LEE, S. H. & RIM, H. J. 2007. High prevalence of liver and intestinal fluke infections among residents of Savannakhet province in Laos. *The Korean Journal of Parasitology,* 45**,** 213-218.

CHAI, J. Y., PARK, J. H., HAN, E. T., GUK, S. M., SHIN, E. H., LIN, A., KIM, J. L., SOHN, W. M., YONG, T. S., EOM, K. S., MIN, D. Y., HWANG, E. H., PHOMMMASACK, B., INSISIENGMAY, B. & RIM, H. J. 2005. Mixed infections with *Opisthorchis viverrini* and intestinal flukes in residents of Vientiane municipality and Saravane province in Laos. *Journal of Helminthology,* 79**,** 283-289.

CHAI, J. Y., YONG, T. S., EOM, K. S., MIN, D. Y., JEON, H. K., KIM, T. Y., JUNG, B. K., SISABATH, L., INSISIENGMAY, B., PHOMMASACK, B. & RIM, H. J. 2013. Hyperendemicity of *Haplorchis taichui* infection among riparian people in Saravane and Champasak province, Lao PDR. *Korean J Parasitol,* 51**,** 305-11.

CHAMMARTIN, F., GUIMARAES, L. H., SCHOLTE, R. G., BAVIA, M. E., UTZINGER, J. & VOUNATSOU, P. 2014a. Spatio-temporal distribution of soil-transmitted helminth infections in Brazil. *Parasit Vectors,* 7**,** 440.

CHAMMARTIN, F., HOUNGBEDJI, C. A., HÜRLIMANN, E., YAPI, R. B., SILUE, K. D., SORO, G., KOUAME, F. N., EK, N. G., UTZINGER, J., RASO, G. & VOUNATSOU, P. 2014b. Bayesian risk mapping and model-based estimation of *Schistosoma haematobium-Schistosoma mansoni* co-distribution in Côte d'Ivoire. *PLoS Negl Trop Dis,* 8**,** e3407.

CHAMMARTIN, F., SCHOLTE, R. G., GUIMARAES, L. H., TANNER, M., UTZINGER, J. & VOUNATSOU, P. 2013a. Soil-transmitted helminth infection in South America: a systematic review and geostatistical meta-analysis. *Lancet Infect Dis,* 13**,** 507-18.

CHAMMARTIN, F., SCHOLTE, R. G., MALONE, J. B., BAVIA, M. E., NIETO, P., UTZINGER, J. & VOUNATSOU, P. 2013b. Modelling the geographical distribution of soil-transmitted helminth infections in Bolivia. *Parasit Vectors,* 6**,** 152.

CHESNAYE, N., SINUON, M., SOCHEAT, D., KOPORC, K. & MATHIEU, E. 2011. Treatment coverage survey after a school-based mass distribution of mebendazole: Kampot Province, Cambodia. *Acta Trop,* 118**,** 21-6.

CHU, B. & PROJECT, G. H. T. A. 2011. Cambodia: NTD Cost and Funding Gap Analysis *In:* USAID (ed.).

CIMINO, R. O. & KROLEWIECKI, A. 2014. The Epidemiology of Human Strongyloidiasis. *Current Tropical Medicine Reports,* 1**,** 216-222.

CLARKE, N. E., CLEMENTS, A. C., DOI, S. A., WANG, D., CAMPBELL, S. J., GRAY, D. & NERY, S. V. 2017. Differential effect of mass deworming and targeted deworming for soil-transmitted helminth control in children: a systematic review and meta-analysis. *Lancet,* 389**,** 287-297.

CLASEN, T., BOISSON, S., ROUTRAY, P., TORONDEL, B., BELL, M., CUMMING, O., ENSINK, J., FREEMAN, M., JENKINS, M., ODAGIRI, M., RAY, S., SINHA, A., SUAR, M. & SCHMIDT, W. P. 2014. Effectiveness of a rural sanitation programme on diarrhoea, soil-transmitted helminth infection, and child malnutrition in Odisha, India: a cluster-randomised trial. *Lancet Glob Health,* 2**,** e645-53.

CLASEN, T. F., BOSTOEN, K., SCHMIDT, W. P., BOISSON, S., FUNG, I. C., JENKINS, M. W., SCOTT, B., SUGDEN, S. & CAIRNCROSS, S. 2010. Interventions to improve disposal of human excreta for preventing diarrhoea. *Cochrane Database Syst Rev***,** CD007180.

CLEMENTS, A. C., DEVILLE, M. A., NDAYISHIMIYE, O., BROOKER, S. & FENWICK, A. 2010. Spatial co-distribution of neglected tropical diseases in the east African great lakes region: revisiting the justification for integrated control. *Trop Med Int Health,* 15**,** 198-207.

CLEMENTS, A. C., MOYEED, R. & BROOKER, S. 2006a. Bayesian geostatistical prediction of the intensity of infection with *Schistosoma mansoni* in East Africa. *Parasitology,* 133**,** 711-9.

CLEMENTS, A. C. A., LWAMBO, N. J. S., BLAIR, L., NYANDINDI, U., KAATANO, G., KINUNG'HI, S., WEBSTER, J. P., FENWICK, A. & BROOKER, S. 2006b. Bayesian spatial analysis and disease mapping: tools to enhance planning and implementation of a schistosomiasis control programme in Tanzania. *Tropical Medicine & International Health: TM & IH,* 11**,** 490-503.

CNM 2004. *National Policy and Guideline for Helminth Control in Cambodia,* Phnom Penh, Cambodia, National Center for Parasitology, Entomology and Malaria Control, Ministry of Health.

CONCHA, R., HARRINGTON, W., JR. & ROGERS, A. I. 2005. Intestinal strongyloidiasis: recognition, management, and determinants of outcome. *Journal of clinical gastroenterology,* 39**,** 203-211.

CONLAN, J. V., KHAMLOME, B., VONGXAY, K., ELLIOT, A., PALLANT, L., SRIPA, B., BLACKSELL, S. D., FENWICK, S. & THOMPSON, R. C. 2012. Soil-transmitted helminthiasis in Laos: a community-wide cross-sectional study of humans and dogs in a mass drug administration environment. *Am J Trop Med Hyg,* 86**,** 624-34.

DE OLIVEIRA, L. C., RIBEIRO, C. T., MENDES DDE, M., OLIVEIRA, T. C. & COSTA-CRUZ, J. M. 2002. Frequency of Strongyloides stercoralis infection in alcoholics. *Mem Inst Oswaldo Cruz,* 97**,** 119-21.

DE ONIS M, O. A., BORGHI E, SIYAM A, NISHIDA C, SIEKMANN J 2007. Development of a WHO growth reference for school-aged children and adolescents. *Bulletin of the World Health Organization,* 85**,** 649-732.

DE SILVA, N. R. 2003. Impact of mass chemotherapy on the morbidity due to soil-transmitted nematodes. *Acta Trop,* 86**,** 197-214.

DE SILVA, N. R., BROOKER, S., HOTEZ, P. J., MONTRESOR, A., ENGELS, D. & SAVIOLI, L. 2003. Soil-transmitted helminth infections: updating the global picture. *Trends Parasitol,* 19**,** 547-51.

DIGGLE, P. J., TAWN, J. A. & MOYEED, R. A. 1998. Model-based geostatistics. *Journal of the Royal Statistical Society: Series C (Applied Statistics),* 47**,** 299-350.

DOWDLE, W. R. 1998. The principles of disease elimination and eradication. *Bull World Health Organ,* 76 Suppl 2**,** 22-5.

DREIBELBIS, R., FREEMAN, M. C., GREENE, L. E., SABOORI, S. & RHEINGANS, R. 2014. The impact of school water, sanitation, and hygiene interventions on the health of younger siblings of pupils: a cluster-randomized trial in Kenya. *Am J Public Health,* 104**,** e91-7.

DREYER, G., FERNANDES-SILVA, E., ALVES, S., ROCHA, A., ALBUQUERQUE, R. & ADDISS, D. 1996. Patterns of detection of *Strongyloides stercoralis* in stool specimens: implications for diagnosis and clinical trials. *Journal of clinical microbiology,* 34**,** 2569-2571.

DUNN, J. C., TURNER, H. C., TUN, A. & ANDERSON, R. M. 2016. Epidemiological surveys of, and research on, soil-transmitted helminths in Southeast Asia: a systematic review. *Parasit Vectors,* 9**,** 31.

DUVIGNAUD, A., PISTONE, T. & MALVY, D. 2016. Strongyloidiasis in a young French woman raises concern about possible ongoing autochthonous transmission in Spain. *Int J Infect Dis,* 42**,** 43-4.

DYSON, L., STOLK, W. A., FARRELL, S. H. & HOLLINGSWORTH, T. D. 2017. Measuring and modelling the effects of systematic non-adherence to mass drug administration. *Epidemics,* 18**,** 56-66.

EAMUDOMKARN, C., SITHITHAWORN, P., KAMAMIA, C., YAKOVLEVA, A., SITHITHAWORN, J., KAEWKES, S., TECHASEN, A., LOILOME, W., YONGVANIT, P., WANGBOON, C., SAICHUA, P., ITOH, M. & J, M. B. 2018. Diagnostic performance of urinary IgG antibody detection: A novel approach for population screening of strongyloidiasis. *PLoS One,* 13**,** e0192598.

EAMUDOMKARN, C., SITHITHAWORN, P., SITHITHAWORN, J., KAEWKES, S., SRIPA, B. & ITOH, M. 2015. Comparative evaluation of Strongyloides ratti and S. stercoralis larval antigen for diagnosis of strongyloidiasis in an endemic area of opisthorchiasis. *Parasitol Res,* 114**,** 2543-51.

ECHAZÚ, A., BONANNO, D., JUAREZ, M., CAJAL, S. P., HEREDIA, V., CAROPRESI, S., CIMINO, R. O., CARO, N., VARGAS, P. A., PAREDES, G. & KROLEWIECKI, A. J. 2015. Effect of Poor Access to Water and Sanitation As Risk Factors for Soil-Transmitted Helminth Infection: Selectiveness by the Infective Route. *PLoS Negl Trop Dis,* 9**,** e0004111.

ECHAZÚ, A., JUAREZ, M., VARGAS, P. A., CAJAL, S. P., CIMINO, R. O., HEREDIA, V., CAROPRESI, S., PAREDES, G., ARIAS, L. M., ABRIL, M., GOLD, S., LAMMIE, P. & KROLEWIECKI, A. J. 2017. Albendazole and ivermectin for the control of soil-transmitted helminths in an area with high prevalence of Strongyloides stercoralis and hookworm in northwestern Argentina: A community-based pragmatic study. *PLoS Negl Trop Dis,* 11**,** e0006003.

ESREY, S. A., POTASH, J. B., ROBERTS, L. & SHIFF, C. 1991. Effects of improved water supply and sanitation on ascariasis, diarrhoea, dracunculiasis, hookworm infection, schistosomiasis, and trachoma. *Bull World Health Organ,* 69**,** 609-21.

EVANS, D., MCFARLAND, D., ADAMANI, W., EIGEGE, A., MIRI, E., SCHULZ, J., PEDE, E., UMBUGADU, C., OGBU-PEARSE, P. & RICHARDS, F. O. 2011. Cost-effectiveness of triple drug administration (TDA) with praziquantel, ivermectin and albendazole for the prevention of neglected tropical diseases in Nigeria. *Ann Trop Med Parasitol,* 105**,** 537-47.

EVANS, W. D., PATTANAYAK, S. K., YOUNG, S., BUSZIN, J., RAI, S. & BIHM, J. W. 2014. Social marketing of water and sanitation products: a systematic review of peer-reviewed literature. *Soc Sci Med,* 110**,** 18-25.

EZEAMAMA, A. E., FRIEDMAN, J. F., OLVEDA, R. M., ACOSTA, L. P., KURTIS, J. D., MOR, V. & MCGARVEY, S. T. 2005. Functional significance of low-intensity polyparasite helminth infections in anemia. *J Infect Dis,* 192**,** 2160-70.

FAO 2014. Country fact sheet on food and agriculture policy trends.

FARDET, L., GENEREAU, T., POIROT, J. L., GUIDET, B., KETTANEH, A. & CABANE, J. 2007. Severe strongyloidiasis in corticosteroid-treated patients: case series and literature review. *J Infect,* 54**,** 18-27.

FEWTRELL, L. & COLFORD, J. M., JR. 2005. Water, sanitation and hygiene in developing countries: interventions and diarrhoea--a review. *Water Sci Technol,* 52**,** 133-42.

FITZPATRICK, C., FLEMING, F. M., MADIN-WARBURTON, M., SCHNEIDER, T., MEHEUS, F., ASIEDU, K., SOLOMON, A. W., MONTRESOR, A. & BISWAS, G. 2016. Benchmarking the Cost per Person of Mass Treatment for Selected Neglected Tropical Diseases: An Approach Based on Literature Review and Meta-regression with Web-Based Software Application. *PLoS Negl Trop Dis,* 10**,** e0005037.

FLOHR, C., TUYEN, L. N., LEWIS, S., MINH, T. T., CAMPBELL, J., BRITTON, J., WILLIAMS, H., HIEN, T. T., FARRAR, J. & QUINNELL, R. J. 2007. Low efficacy of mebendazole against hookworm in Vietnam: two randomized controlled trials. *Am J Trop Med Hyg,* 76**,** 732-6.

FOREMAN, E. B., ABRAHAM, P. J. & GARLAND, J. L. 2006. Not your typical strongyloides infection: a literature review and case study. *Southern medical journal,* 99**,** 847-852.

FORRER, A., KHIEU, V., SCHAR, F., HATTENDORF, J., MARTI, H., NEUMAYR, A., CHAR, M. C., HATZ, C., MUTH, S. & ODERMATT, P. 2017. Strongyloides stercoralis is associated with significant morbidity in rural Cambodia, including stunting in children. *PLoS Negl Trop Dis,* 11**,** e0005685.

FORRER, A., KHIEU, V., SCHAR, F., VOUNATSOU, P., CHAMMARTIN, F., MARTI, H., MUTH, S. & ODERMATT, P. 2018. Strongyloides stercoralis and hookworm co-infection: spatial distribution and determinants in Preah Vihear Province, Cambodia. *Parasit Vectors,* 11**,** 33.

FORRER, A., KHIEU, V., SCHINDLER, C., SCHÄR, F., MARTI, H., CHAR, M. C., MUTH, S. & ODERMATT, P. 2016. Ivermectin Treatment and Sanitation Effectively Reduce *Strongyloides stercoralis* Infection Risk in Rural Communities in Cambodia. *PLoS Negl Trop Dis,* 10**,** e0004909.

FORRER, A., KHIEU, V., VOUNATSOU, P., SITHITHAWORN, J., MUTH, S. & ODERMATT, P. Submitted Oct. 10, 2018. *Strongyloides stercoralis*: mapping of a highly prevalent helminth in Cambodia *PLoS Negl Trop Dis*.

FORRER, A., SAYASONE, S., VOUNATSOU, P., VONGHACHACK, Y., BOUAKHASITH, D., VOGT, S., GLASER, R., UTZINGER, J., AKKHAVONG, K. & ODERMATT, P. 2012. Spatial distribution of, and risk factors for, *Opisthorchis viverrini* infection in southern Lao PDR. *PLoS Negl Trop Dis,* 6**,** e1481.

FORRER, A., SUTHERLAND, C. S., KHIEU, V., SCHÄR, F., MARTI, H., MUTH, S. & ODERMATT, P. in preparation. Cost-effectiveness analysis for potential control strategies for *Strongyloides stercoralis* in Cambodia *PLoS Negl Trop Dis*.

FORRER, A., VOUNATSOU, P., SAYASONE, S., VONGHACHACK, Y., BOUAKHASITH, D., UTZINGER, J., AKKHAVONG, K. & ODERMATT, P. 2015. Risk profiling of hookworm infection and intensity in southern Lao People's Democratic Republic using Bayesian models. *PLoS Negl Trop Dis,* 9**,** e0003486.

FREEMAN, M. C., CHARD, A. N., NIKOLAY, B., GARN, J. V., OKOYO, C., KIHARA, J., NJENGA, S. M., PULLAN, R. L., BROOKER, S. J. & MWANDAWIRO, C. S. 2015. Associations between school- and household-level water, sanitation and hygiene conditions and soil-transmitted helminth infection among Kenyan school children. *Parasit Vectors,* 8**,** 412.

FREEMAN, M. C., CLASEN, T., BROOKER, S. J., AKOKO, D. O. & RHEINGANS, R. 2013a. The impact of a school-based hygiene, water quality and sanitation intervention on soil-transmitted helminth reinfection: a cluster-randomized trial. *Am J Trop Med Hyg,* 89**,** 875-83.

FREEMAN, M. C., CLASEN, T., DREIBELBIS, R., SABOORI, S., GREENE, L. E., BRUMBACK, B., MUGA, R. & RHEINGANS, R. 2014. The impact of a school-based water supply and treatment, hygiene, and sanitation programme on pupil diarrhoea: a cluster-randomized trial. *Epidemiol Infect,* 142**,** 340-51.

FREEMAN, M. C., GARN, J. V., SCLAR, G. D., BOISSON, S., MEDLICOTT, K., ALEXANDER, K. T., PENAKALAPATI, G., ANDERSON, D., MAHTANI, A. G., GRIMES, J. E. T., REHFUESS, E. A. & CLASEN, T. F. 2017. The impact of sanitation on infectious disease and nutritional status: A systematic review and meta-analysis. *Int J Hyg Environ Health,* 220**,** 928-949.

FREEMAN, M. C., OGDEN, S., JACOBSON, J., ABBOTT, D., ADDISS, D. G., AMNIE, A. G., BECKWITH, C., CAIRNCROSS, S., CALLEJAS, R., COLFORD, J. M., JR., EMERSON, P. M., FENWICK, A., FISHMAN, R., GALLO, K., GRIMES, J., KARAPETYAN, G., KEENE, B., LAMMIE, P. J., MACARTHUR, C., LOCHERY, P., PETACH, H., PLATT, J., PRABASI, S., ROSENBOOM, J. W., ROY, S., SAYWELL, D., SCHECHTMAN, L., TANTRI, A., VELLEMAN, Y. & UTZINGER, J. 2013b. Integration of water, sanitation, and hygiene for the prevention and control of neglected tropical diseases: a rationale for inter-sectoral collaboration. *PLoS Negl Trop Dis,* 7**,** e2439.

GANN, P. H., NEVA, F. A. & GAM, A. A. 1994. A randomized trial of single- and two-dose ivermectin versus thiabendazole for treatment of strongyloidiasis. *J Infect Dis,* 169**,** 1076-9.

GARN, J. V., SCLAR, G. D., FREEMAN, M. C., PENAKALAPATI, G., ALEXANDER, K. T., BROOKS, P., REHFUESS, E. A., BOISSON, S., MEDLICOTT, K. O. & CLASEN, T. F. 2017. The impact of sanitation interventions on latrine coverage and latrine use: A systematic review and meta-analysis. *Int J Hyg Environ Health,* 220**,** 329-340.

GAZZINELLI, A., CORREA-OLIVEIRA, R., YANG, G. J., BOATIN, B. A. & KLOOS, H. 2012. A research agenda for helminth diseases of humans: social ecology, environmental determinants, and health systems. *PLoS Negl Trop Dis,* 6**,** e1603.

GELFAND, A. E. & SMITH, A. F. M. 1990. Sampling-based approaches to calculating marginal densities. *Journal of the American Statistical Association,* 85**,** 398-409.

GENTA, R. M. 1989. Global prevalence of strongyloidiasis: critical review with epidemiologic insights into the prevention of disseminated disease. *Rev Infect Dis,* 11**,** 755-67.

GEORGE, E. I. & MCCULLOCH, R. E. 1993. Variable selection via Gibbs sampling. *Journal of the American Statistical Association,* 88**,** 881-889.

GERTLER P, S. M., ALZUA ML, CAMERON L, MARTINEZ S, PATIL S 2015. How does health promotion work? Evidence from the dirty business of eliminating open defecation - Working paper 20997. National bureau of economic research, Cambridge, MA USA.

GHOSH, K. & GHOSH, K. 2007. Strongyloides stercoralis septicaemia following steroid therapy for eosinophilia: report of three cases. *Trans R Soc Trop Med Hyg,* 101**,** 1163-5.

GIACOMETTI, A., CIRIONI, O., ANTONICELLI, L., D'AMATO, G., SILVESTRI, C., DEL PRETE, M. S. & SCALISE, G. 2003. Prevalence of intestinal parasites among individuals with allergic skin diseases. *J Parasitol,* 89**,** 490-2.

GIARDINA, F., GOSONIU, L., KONATE, L., DIOUF, M. B., PERRY, R., GAYE, O., FAYE, O. & VOUNATSOU, P. 2012. Estimating the burden of malaria in Senegal: Bayesian zero-inflated binomial geostatistical modeling of the MIS 2008 data. *PLoS One,* 7**,** e32625.

GLIZE, B. & MALVY, D. 2014. Autochthonous strongyloidiasis, Bordeaux area, South-Western France. *Travel Med Infect Dis,* 12**,** 106-8.

GLOBAL-FOREST-WATCH. 2016. *Country profile: Cambodia, Preah Vihear Province* [Online]. Available: http://www.globalforestwatch.org/country/KHM/18 [Accessed 07.04.2017].

GREFFEUILLE, V., SOPHONNEARY, P., LAILLOU, A., GAUTHIER, L., HONG, R., HONG, R., POIROT, E., DIJKHUIZEN, M., WIERINGA, F. & BERGER, J. 2016. Persistent Inequalities in Child Undernutrition in Cambodia from 2000 until Today. *Nutrients,* 8.

GROUP, W. M. G. R. 2006. Child Growth Standards: Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: methods and development. *In:* WORLD HEALTH ORGANIZATION, G. (ed.).

GROVE, D. I. 1996. Human strongyloidiasis. *Adv Parasitol,* 38**,** 251-309.

GUITERAS, R., LEVINSOHN, J. & MOBARAK, A. M. 2015. Sanitation subsidies. Encouraging sanitation investment in the developing world: a cluster-randomized trial. *Science,* 348**,** 903-6.

HASLETT, S., JONES, G. T. & A, S. 2013. Small-area Estimation of Poverty and Malnutrition in Cambodia. April 2013. *In:* NATIONAL INSTITUTE OF STATISTICS, M. O. P., ROYAL GOVERNMENT OF CAMBODIA AND THE UNITED NATIONS WORLD FOOD PROGRAMME (ed.). National Institute of Statistics, Ministry of Planning, Royal Government of Cambodia and the United Nations World Food Programme

HAWDON, J. M. & HOTEZ, P. J. 1996. Hookworm: developmental biology of the infectious process. *Curr Opin Genet Dev,* 6**,** 618-23.

HAY, R. J., STEER, A. C., ENGELMAN, D. & WALTON, S. 2012. Scabies in the developing world--its prevalence, complications, and management. *Clin Microbiol Infect,* 18**,** 313-23.

HENRIQUEZ-CAMACHO, C., GOTUZZO, E., ECHEVARRIA, J., WHITE, A. C., JR., TERASHIMA, A., SAMALVIDES, F., PEREZ-MOLINA, J. A. & PLANA, M. N. 2016. Ivermectin versus albendazole or thiabendazole for *Strongyloides stercoralis* infection. *Cochrane Database Syst Rev,* 1**,** CD007745.

HERBERT, D. R., LEE, J. J., LEE, N. A., NOLAN, T. J., SCHAD, G. A. & ABRAHAM, D. 2000. Role of IL-5 in innate and adaptive immunity to larval Strongyloides stercoralis in mice. *J Immunol,* 165**,** 4544-51.

HIRATA, T., NAKAMURA, H., KINJO, N., HOKAMA, A., KINJO, F., YAMANE, N. & FUJITA, J. 2007. Prevalence of Blastocystis hominis and Strongyloides stercoralis infection in Okinawa, Japan. *Parasitol Res,* 101**,** 1717-9.

HOTEZ, P., DE SILVA N, BROOKER S, BETHONY J 2003. Soil Transmitted Helminth Infections: the nature, causes and burden of the condition. *In:* PROJECT, D. C. P. (ed.). Bethesda, Maryland, USA: Fogarty International Center, National Institutes of Health.

HOTEZ, P. J. 2009. Mass drug administration and integrated control for the world's high-prevalence neglected tropical diseases. *Clin Pharmacol Ther,* 85**,** 659-64.

HOTEZ, P. J., ALVARADO, M., BASANEZ, M. G., BOLLIGER, I., BOURNE, R., BOUSSINESQ, M., BROOKER, S. J., BROWN, A. S., BUCKLE, G., BUDKE, C. M.,

CARABIN, H., COFFENG, L. E., FEVRE, E. M., FURST, T., HALASA, Y. A., JASRASARIA, R., JOHNS, N. E., KEISER, J., KING, C. H., LOZANO, R., MURDOCH, M. E., O'HANLON, S., PION, S. D., PULLAN, R. L., RAMAIAH, K. D., ROBERTS, T., SHEPARD, D. S., SMITH, J. L., STOLK, W. A., UNDURRAGA, E. A., UTZINGER, J., WANG, M., MURRAY, C. J. & NAGHAVI, M. 2014. The global burden of disease study 2010: interpretation and implications for the neglected tropical diseases. *PLoS Negl Trop Dis,* 8**,** e2865.

HOTEZ, P. J., BOTTAZZI, M. E., STRYCH, U., CHANG, L. Y., LIM, Y. A., GOODENOW, M. M. & ABUBAKAR, S. 2015. Neglected tropical diseases among the Association of Southeast Asian Nations (ASEAN): overview and update. *PLoS Negl Trop Dis,* 9**,** e0003575.

HOTEZ, P. J., BRINDLEY, P. J., BETHONY, J. M., KING, C. H., PEARCE, E. J. & JACOBSON, J. 2008. Helminth infections: the great neglected tropical diseases. *The Journal of clinical investigation,* 118**,** 1311-1321.

HOTEZ, P. J., BROOKER, S., BETHONY, J. M., BOTTAZZI, M. E., LOUKAS, A. & XIAO, S. 2004. Hookworm infection. *The New England journal of medicine,* 351**,** 799-807.

HOTEZ, P. J., FENWICK, A., SAVIOLI, L. & MOLYNEUX, D. H. 2009. Rescuing the bottom billion through control of neglected tropical diseases. *Lancet,* 373**,** 1570-1575.

HOUWELING, T., KUNST, A. & MACKENBACH, J. 2003. Measuring health inequality among children in developing countries: does the choice of the indicator of economic status matter? *International Journal for Equity in Health,* 2.

HUMPHRIES, D., NGUYEN, S., BOAKYE, D., WILSON, M. & CAPPELLO, M. 2012. The promise and pitfalls of mass drug administration to control intestinal helminth infections. *Curr Opin Infect Dis,* 25**,** 584-9.

IGUAL-ADELL, R., OLTRA-ALCARAZ, C., SOLER-COMPANY, E., SÁNCHEZ-SÁNCHEZ, P., MATOGO-OYANA, J. & RODRÍGUEZ-CALABUIG, D. 2004. Efficacy and safety of ivermectin and thiabendazole in the treatment of strongyloidiasis. *Expert Opinion on Pharmacotherapy,* 5**,** 2615-2619.

INPANKAEW, T., SCHÄR, F., DALSGAARD, A., KHIEU, V., CHIMNOI, W., CHHOUN, C., SOK, D., MARTI, H., MUTH, S., ODERMATT, P. & TRAUB, R. J. 2014. High prevalence of *Ancylostoma ceylanicum* hookworm infections in humans, Cambodia, 2012. *Emerg Infect Dis,* 20**,** 976-82.

INTERNATIONAL-FOOD-POLICY-RESEARCH-INSTITUTE 2016. Global Nutrition Report 2016: From Promise to Impact: Ending Malnutrtion by 2030. Washington, DC.

JEX, A. R., LIM, Y. A., BETHONY, J. M., HOTEZ, P. J., YOUNG, N. D. & GASSER, R. B. 2011. Soil-transmitted helminths of humans in Southeast Asia-towards integrated control. *Adv Parasitol,* 74**,** 231-65.

JIA, T. W., MELVILLE, S., UTZINGER, J., KING, C. H. & ZHOU, X. N. 2012. Soil-transmitted helminth reinfection after drug treatment: a systematic review and meta-analysis. *PLoS Negl Trop Dis,* 6**,** e1621.

JOHNSTON, F. H., MORRIS, P. S., SPEARE, R., MCCARTHY, J., CURRIE, B., EWALD, D., PAGE, W. & DEMPSEY, K. 2005. Strongyloidiasis: a review of the evidence for Australian practitioners. *Aust J Rural Health,* 13**,** 247-54.

KABATEREINE, N. 2009. The unsung hero of neglected tropical diseases: interview with Narcis Kabatereine by Gavin Yamey. *PLoS Negl Trop Dis,* 3**,** e546.

KABATEREINE, N. B., MALECELA, M., LADO, M., ZARAMBA, S., AMIEL, O. & KOLACZINSKI, J. H. 2010. How to (or not to) integrate vertical programmes for the control of major neglected tropical diseases in sub-Saharan Africa. *PLoS Negl Trop Dis,* 4**,** e755.

KABATEREINE, N. B., TUKAHEBWA, E. M., KAZIBWE, F., TWA-TWA, J. M., BARENZI, J. F., ZARAMBA, S., STOTHARD, J. R., FENWICK, A. & BROOKER, S. 2005. Soil-transmitted helminthiasis in Uganda: epidemiology and cost of control. *Trop Med Int Health,* 10**,** 1187-9.

KAR, K. & CHAMBERS, R. 2008. Handbook on community-led total sanitation.

KARAGIANNIS-VOULES, D. A., BIEDERMANN, P., EKPO, U. F., GARBA, A., LANGER, E., MATHIEU, E., MIDZI, N., MWINZI, P., POLDERMAN, A. M., RASO, G., SACKO, M.,

TALLA, I., TCHUENTE, L. A., TOURE, S., WINKLER, M. S., UTZINGER, J. & VOUNATSOU, P. 2015a. Spatial and temporal distribution of soil-transmitted helminth infection in sub-Saharan Africa: a systematic review and geostatistical meta-analysis. *Lancet Infect Dis,* 15**,** 74-84.

KARAGIANNIS-VOULES, D. A., ODERMATT, P., BIEDERMANN, P., KHIEU, V., SCHAR, F., MUTH, S., UTZINGER, J. & VOUNATSOU, P. 2015b. Geostatistical modelling of soil-transmitted helminth infection in Cambodia: do socioeconomic factors improve predictions? *Acta Trop,* 141**,** 204-12.

KATZ, N., CHAVES, A. & PELLEGRINO, J. 1972. A simple device for quantitative stool thick-smear technique in *Schistosomiasis mansoni*. *Rev Inst Med Trop Sao Paulo,* 14**,** 397-400.

KEENAN, J. D., HOTEZ, P. J., AMZA, A., STOLLER, N. E., GAYNOR, B. D., PORCO, T. C. & LIETMAN, T. M. 2013. Elimination and eradication of neglected tropical diseases with mass drug administrations: a survey of experts. *PLoS Negl Trop Dis,* 7**,** e2562.

KEISER, J. & UTZINGER, J. 2008. Efficacy of current drugs against soil-transmitted helminth infections: systematic review and meta-analysis. *JAMA,* 299**,** 1937-1948.

KEISER, J. & UTZINGER, J. 2010. The drugs we have and the drugs we need against major helminth infections. *Adv Parasitol,* 73**,** 197-230.

KEISER, P. B. & NUTMAN, T. B. 2004. Strongyloides stercoralis in the Immunocompromised Population. *Clin Microbiol Rev,* 17**,** 208-17.

KHIEU, V. 2016. Helminth control in Cambodia. Ministry of Health, National Centre for Parasitology, Entomology and Malaria Control

KHIEU, V., HATTENDORF, J., SCHÄR, F., MARTI, H., CHAR, M. C., MUTH, S. & ODERMATT, P. 2014a. *Strongyloides stercoralis* infection and re-infection in a cohort of children in Cambodia. *Parasitol Int,* 63**,** 708-12.

KHIEU, V., OR, V., TEP, C., ODERMATT, P., TSUYUOKA, R., CHAR, M. C., BRADY, M. A., SIDWELL, J., YAJIMA, A., HUY, R., RAMAIAH, K. D. & MUTH, S. 2018. How elimination of lymphatic filariasis as a public health problem in the Kingdom of Cambodia was achieved. *Infect Dis Poverty,* 7**,** 15.

KHIEU, V., SCHÄR, F., FORRER, A., HATTENDORF, J., MARTI, H., DUONG, S., VOUNATSOU, P., MUTH, S. & ODERMATT, P. 2014b. High prevalence and spatial distribution of *Strongyloides stercoralis* in rural Cambodia. *PLoS Negl Trop Dis,* 8**,** e2854.

KHIEU, V., SCHÄR, F., MARTI, H., BLESS, P. J., CHAR, M. C., MUTH, S. & ODERMATT, P. 2014c. Prevalence and risk factors of *Strongyloides stercoralis* in Takeo Province, Cambodia. *Parasit Vectors,* 7**,** 221.

KHIEU, V., SCHÄR, F., MARTI, H., SAYASONE, S., DUONG, S., MUTH, S. & ODERMATT, P. 2013a. Diagnosis, treatment and risk factors of *Strongyloides stercoralis* in schoolchildren in Cambodia. *PLoS neglected tropical diseases,* 7.

KHIEU, V., SREY, S., SCHÄR, F., MUTH, S., MARTI, H. & ODERMATT, P. 2013b. *Strongyloides stercoralis* is a cause of abdominal pain, diarrhea and urticaria in rural Cambodia. *BMC research notes,* 6.

KIM, D. A., HWONG, A. R., STAFFORD, D., HUGHES, D. A., O'MALLEY, A. J., FOWLER, J. H. & CHRISTAKIS, N. A. 2015a. Social network targeting to maximise population behaviour change: a cluster randomised controlled trial. *Lancet,* 386**,** 145-53.

KIM, Y. E., SICURI, E. & TEDIOSI, F. 2015b. Financial and Economic Costs of the Elimination and Eradication of Onchocerciasis (River Blindness) in Africa. *PLoS Negl Trop Dis,* 9**,** e0004056.

KING, C. H. & BERTINO, A. M. 2008. Asymmetries of poverty: why global burden of disease valuations underestimate the burden of neglected tropical diseases. *PLoS Negl Trop Dis,* 2**,** e209.

KNIPES, A. K., LEMOINE, J. F., MONESTIME, F., FAYETTE, C. R., DIRENY, A. N., DESIR, L., BEAU DE ROCHARS, V. E., STREIT, T. G., RENNEKER, K., CHU, B. K., CHANG, M. A., MACE, K. E., WON, K. Y. & LAMMIE, P. J. 2017. Partnering for impact: Integrated transmission assessment surveys for lymphatic filariasis, soil transmitted helminths and malaria in Haiti. *PLoS Negl Trop Dis,* 11**,** e0005387.

KNOPP, S., MGENI, A. F., KHAMIS, I. S., STEINMANN, P., STOTHARD, J. R., ROLLINSON, D., MARTI, H. & UTZINGER, J. 2008a. Diagnosis of soil-transmitted helminths in the era of preventive chemotherapy: effect of multiple stool sampling and use of different diagnostic techniques. *PLoS Negl Trop Dis,* 2**,** e331.

KNOPP, S., MOHAMMED, K. A., ROLLINSON, D., STOTHARD, J. R., KHAMIS, I. S., UTZINGER, J. & MARTI, H. 2009a. Changing patterns of soil-transmitted helminthiases in Zanzibar in the context of national helminth control programmes. *Am J Trop Med Hyg,* 81**,** 1071-8.

KNOPP, S., MOHAMMED, K. A., SIMBA KHAMIS, I., MGENI, A. F., STOTHARD, J. R., ROLLINSON, D., MARTI, H. & UTZINGER, J. 2008b. Spatial distribution of soil-transmitted helminths, including Strongyloides stercoralis, among children in Zanzibar. *Geospat Health,* 3**,** 47-56.

KNOPP, S., MOHAMMED, K. A., SPEICH, B., HATTENDORF, J., KHAMIS, I. S., KHAMIS, A. N., STOTHARD, J. R., ROLLINSON, D., MARTI, H. & UTZINGER, J. 2010a. Albendazole and mebendazole administered alone or in combination with ivermectin against *Trichuris trichiura*: a randomized controlled trial. *Clin Infect Dis,* 51**,** 1420-8.

KNOPP, S., MOHAMMED, K. A., STOTHARD, J. R., KHAMIS, I. S., ROLLINSON, D., MARTI, H. & UTZINGER, J. 2010b. Patterns and risk factors of helminthiasis and anemia in a rural and a peri-urban community in Zanzibar, in the context of helminth control programmes. *PLoS Negl Trop Dis,* 4**,** e681.

KNOPP, S., PERSON, B., AME, S. M., ALI, S. M., MUHSIN, J., JUMA, S., KHAMIS, I. S., RABONE, M., BLAIR, L., FENWICK, A., MOHAMMED, K. A. & ROLLINSON, D. 2016. Praziquantel coverage in schools and communities targeted for the elimination of urogenital schistosomiasis in Zanzibar: a cross-sectional survey. *Parasit Vectors,* 9**,** 5.

KNOPP, S., RINALDI, L., KHAMIS, I. S., STOTHARD, J. R., ROLLINSON, D., MAURELLI, M. P., STEINMANN, P., MARTI, H., CRINGOLI, G. & UTZINGER, J. 2009b. A single FLOTAC is more sensitive than triplicate Kato-Katz for the diagnosis of low-intensity soil-transmitted helminth infections. *Trans R Soc Trop Med Hyg,* 103**,** 347-54.

KOGA, K., KASUYA, S., KHAMBOONRUANG, C., SUKHAVAT, K., IEDA, M., TAKATSUKA, N., KITA, K. & OHTOMO, H. 1991. A modified agar plate method for detection of *Strongyloides stercoralis*. *Am J Trop Med Hyg,* 45**,** 518-21.

KOLKHIR, P., BALAKIRSKI, G., MERK, H. F., OLISOVA, O. & MAURER, M. 2016. Chronic spontaneous urticaria and internal parasites - a systematic review. *Allergy,* 71**,** 308-22.

KOSKI, K. G. & SCOTT, M. E. 2001. Gastrointestinal nematodes, nutrition and immunity: breaking the negative spiral. *Annu Rev Nutr,* 21**,** 297-321.

KOUNNAVONG, S., VONGLOKHAM, M., HOUAMBOUN, K., ODERMATT, P. & BOUPHA, B. 2011. Soil-transmitted helminth infections and risk factors in preschool children in southern rural Lao People's Democratic Republic. *Trans.R.Soc.Trop Med Hyg,* 105**,** 160-166.

KRENTEL, A., FISCHER, P., MANOEMPIL, P., SUPALI, T., SERVAIS, G. & RUCKERT, P. 2006. Using knowledge, attitudes and practice (KAP) surveys on lymphatic filariasis to prepare a health promotion campaign for mass drug administration in Alor District, Indonesia. *Trop Med Int Health,* 11**,** 1731-40.

KRENTEL, A., FISCHER, P. U. & WEIL, G. J. 2013. A review of factors that influence individual compliance with mass drug administration for elimination of lymphatic filariasis. *PLoS Negl Trop Dis,* 7**,** e2447.

KROLEWIECKI, A. J., LAMMIE, P., JACOBSON, J., GABRIELLI, A. F., LEVECKE, B., SOCIAS, E., ARIAS, L. M., SOSA, N., ABRAHAM, D., CIMINO, R., ECHAZU, A., CRUDO, F., VERCRUYSSE, J. & ALBONICO, M. 2013. A public health response against *Strongyloides stercoralis*: time to look at soil-transmitted helminthiasis in full. *PLoS Negl Trop Dis,* 7**,** e2165.

KROTNEVA, S. P., COFFENG, L. E., NOMA, M., ZOURE, H. G., BAKONE, L., AMAZIGO, U. V., DE VLAS, S. J. & STOLK, W. A. 2015. African Program for Onchocerciasis Control 1995-2010: Impact of Annual Ivermectin Mass Treatment on Off-Target Infectious Diseases. *PLoS Negl Trop Dis,* 9**,** e0004051.

KUNTHY, S. C., RNF 2009. Community-Led Total Sanitation (CLTS) in Cambodia. *In:* UNICEF (ed.).

KUONG, K., FIORENTINO, M., PERIGNON, M., CHAMNAN, C., BERGER, J., SINUON, M., MOLYDEN, V., BURJA, K., PARKER, M., LY, S. C., FRIIS, H., ROOS, N. & WIERINGA, F. T. 2016. Cognitive Performance and Iron Status are Negatively Associated with Hookworm Infection in Cambodian Schoolchildren. *Am J Trop Med Hyg,* 95**,** 856-863.

LAI, Y. S., ZHOU, X. N., UTZINGER, J. & VOUNATSOU, P. 2013. Bayesian geostatistical modelling of soil-transmitted helminth survey data in the People's Republic of China. *Parasit Vectors,* 6**,** 359.

LAMMIE, P. J., FENWICK, A. & UTZINGER, J. 2006. A blueprint for success: integration of neglected tropical disease control programmes. *Trends Parasitol,* 22**,** 313-21.

LEIGHTON, P. M. & MACSWEEN, H. M. 1990. Strongyloides stercoralis. The cause of an urticarial-like eruption of 65 years' duration. *Arch Intern Med,* 150**,** 1747-8.

LEPCZYNSKA, M., CHEN, W. C. & DZIKA, E. 2015. Mysterious chronic urticaria caused by *Blastocystis* spp.? *Int J Dermatol*.

LESLIE, J., GARBA, A., BOUBACAR, K., YAYE, Y., SEBONGOU, H., BARKIRE, A., FLEMING, F. M., MOUNKAILA, I., ADAMOU, S. & JACKOU, M. L. 2013. Neglected tropical diseases: comparison of the costs of integrated and vertical preventive chemotherapy treatment in Niger. *Int Health,* 5**,** 78-84.

LEVECKE, B., MONTRESOR, A., ALBONICO, M., AME, S. M., BEHNKE, J. M., BETHONY, J. M., NOUMEDEM, C. D., ENGELS, D., GUILLARD, B., KOTZE, A. C., KROLEWIECKI, A. J., MCCARTHY, J. S., MEKONNEN, Z., PERIAGO, M. V., SOPHEAK, H., TCHUEM-TCHUENTE, L. A., DUONG, T. T., HUONG, N. T., ZEYNUDIN, A. & VERCRUYSSE, J. 2014. Assessment of anthelmintic efficacy of mebendazole in school children in six countries where soil-transmitted helminths are endemic. *PLoS Negl Trop Dis,* 8**,** e3204.

LEVENHAGEN, M. A. & COSTA-CRUZ, J. M. 2014. Update on immunologic and molecular diagnosis of human strongyloidiasis. *Acta Trop,* 135C**,** 33-43.

LIAO, C. W., CHIU, K. C., CHIANG, I. C., CHENG, P. C., CHUANG, T. W., KUO, J. H., TU, Y. H. & FAN, C. K. 2017. Prevalence and Risk Factors for Intestinal Parasitic Infection in Schoolchildren in Battambang, Cambodia. *Am J Trop Med Hyg*.

LO, N. C., BOGOCH, II, BLACKBURN, B. G., RASO, G., N'GORAN, E. K., COULIBALY, J. T., BECKER, S. L., ABRAMS, H. B., UTZINGER, J. & ANDREWS, J. R. 2015. Comparison of community-wide, integrated mass drug administration strategies for schistosomiasis and soil-transmitted helminthiasis: a cost-effectiveness modelling study. *Lancet Glob Health,* 3**,** e629-38.

LO, N. C., LAI, Y. S., KARAGIANNIS-VOULES, D. A., BOGOCH, II, COULIBALY, J. T., BENDAVID, E., UTZINGER, J., VOUNATSOU, P. & ANDREWS, J. R. 2016. Assessment of global guidelines for preventive chemotherapy against schistosomiasis and soil-transmitted helminthiasis: a cost-effectiveness modelling study. *Lancet Infect Dis,* 16**,** 1065-75.

LUNN, D., THOMAS, A., BEST, N. & SPIEGELHALTER, D. 2000. WinBUGS - A Bayesian modelling framework: Concepts, structure, and extensibility. *Statistics and Computing,* 10**,** 325-337.

LUSTIGMAN, S., GELDHOF, P., GRANT, W. N., OSEI-ATWENEBOANA, M. Y., SRIPA, B. & BASANEZ, M. G. 2012a. A research agenda for helminth diseases of humans: basic research and enabling technologies to support control and elimination of helminthiases. *PLoS Negl Trop Dis,* 6**,** e1445.

LUSTIGMAN, S., PRICHARD, R. K., GAZZINELLI, A., GRANT, W. N., BOATIN, B. A., MCCARTHY, J. S. & BASANEZ, M. G. 2012b. A research agenda for helminth diseases of humans: the problem of helminthiases. *PLoS Negl Trop Dis,* 6**,** e1582.

MAHMOUD, A. A. 1996. Strongyloidiasis. *Clin Infect Dis,* 23**,** 949-52; quiz 953.

MARCOS, L. A. & GOTUZZO, E. 2013. Intestinal protozoan infections in the immunocompromised host. *Curr Opin Infect Dis,* 26**,** 295-301.

MARCOS, L. A., TERASHIMA, A., DUPONT, H. L. & GOTUZZO, E. 2008. *Strongyloides* hyperinfection syndrome: an emerging global infectious disease. *Trans R Soc Trop Med Hyg,* 102**,** 314-8.

MARQUES, C. C., DA PENHA ZAGO-GOMES, M., GONCALVES, C. S. & PEREIRA, F. E. 2010. Alcoholism and Strongyloides stercoralis: daily ethanol ingestion has a positive correlation with the frequency of Strongyloides larvae in the stools. *PLoS Negl Trop Dis,* 4**,** e717.

MARTI, H. & ESCHER, E. 1990. SAF--an alternative fixation solution for parasitological stool specimens. *Schweizerische medizinische Wochenschrift,* 120**,** 1473-1476.

MARTI, H. & KOELLA, J. C. 1993. Multiple stool examinations for ova and parasites and rate of false-negative results. *Journal of clinical microbiology,* 31**,** 3044-3045.

MCCARTHY, J. S., LUSTIGMAN, S., YANG, G. J., BARAKAT, R. M., GARCIA, H. H., SRIPA, B., WILLINGHAM, A. L., PRICHARD, R. K. & BASANEZ, M. G. 2012. A research agenda for helminth diseases of humans: diagnostics for control and elimination programmes. *PLoS Negl Trop Dis,* 6**,** e1601.

MCCARTY, T. R., TURKELTAUB, J. A. & HOTEZ, P. J. 2014. Global progress towards eliminating gastrointestinal helminth infections. *Curr Opin Gastroenterol,* 30**,** 18-24.

MEDLEY, G. F. & HOLLINGSWORTH, T. D. 2015. MDA helminth control: more questions than answers. *Lancet Glob Health,* 3**,** e583-4.

MEJIA, R. & NUTMAN, T. B. 2012. Screening, prevention, and treatment for hyperinfection syndrome and disseminated infections caused by Strongyloides stercoralis. *Curr Opin Infect Dis,* 25**,** 458-63.

MIETTINEN, O. 1976. Estimability and estimation in case-referent studies. *Am J Epidemiol,* 103**,** 226-35.

MOH 2004. Diagnosis and treatment at the district. A diagnosis and treatment guideline for the district hospital in Lao PDR. Vientiane: Ministry of Health.

MOHAMMED, K. A., DEB, R. M., STANTON, M. C. & MOLYNEUX, D. H. 2012. Soil transmitted helminths and scabies in Zanzibar, Tanzania following mass drug administration for lymphatic filariasis--a rapid assessment methodology to assess impact. *Parasit Vectors,* 5**,** 299.

MOLYNEUX, D. H., HOTEZ, P. J. & FENWICK, A. 2005. "Rapid-impact interventions": how a policy of integrated control for Africa's neglected tropical diseases could benefit the poor. *PLoS Med,* 2**,** e336.

MONTES, M., SAWHNEY, C. & BARROS, N. 2010. *Strongyloides stercoralis*: there but not seen. *Curr Opin Infect Dis,* 23**,** 500-4.

MONTRESOR, A. 2011. Cure rate is not a valid indicator for assessing drug efficacy and impact of preventive chemotherapy interventions against schistosomiasis and soil-transmitted helminthiasis. *Trans R Soc Trop Med Hyg,* 105**,** 361-3.

MONTRESOR, A., CONG, D. T., SINUON, M., TSUYUOKA, R., CHANTHAVISOUK, C., STRANDGAARD, H., VELAYUDHAN, R., CAPUANO, C. M., LE ANH, T. & TEE DATO, A. S. 2008. Large-scale preventive chemotherapy for the control of helminth infection in Western Pacific countries: six years later. *PLoS Negl Trop Dis,* 2**,** e278.

MONTRESOR, A., GABRIELLI, A. F., DIARRA, A. & ENGELS, D. 2010. Estimation of the cost of large-scale school deworming programmes with benzimidazoles. *Trans R Soc Trop Med Hyg,* 104**,** 129-32.

MOORE, C. E., NGET, P., SAROEUN, M., KUONG, S., CHANTHOU, S., KUMAR, V., BOUSFIELD, R., NADER, J., BAILEY, J. W., BEECHING, N. J., DAY, N. P. & PARRY, C. M. 2015. Intestinal parasite infections in symptomatic children attending hospital in Siem Reap, cambodia. *PLoS One,* 10**,** e0123719.

MUHUMUZA, S., OLSEN, A., KATAHOIRE, A. & NUWAHA, F. 2013. Uptake of preventive treatment for intestinal schistosomiasis among school children in Jinja district, Uganda: a cross sectional study. *PLoS One,* 8**,** e63438.

MURRAY, C. J., VOS, T., LOZANO, R., NAGHAVI, M., FLAXMAN, A. D., MICHAUD, C., EZZATI, M., SHIBUYA, K., SALOMON, J. A., ABDALLA, S., ABOYANS, V., ABRAHAM, J., ACKERMAN, I., AGGARWAL, R., AHN, S. Y., ALI, M. K., ALVARADO, M., ANDERSON, H. R., ANDERSON, L. M., ANDREWS, K. G., ATKINSON, C., BADDOUR, L. M., BAHALIM, A. N., BARKER-COLLO, S., BARRERO, L. H., BARTELS, D. H., BASANEZ, M. G., BAXTER, A., BELL, M. L., BENJAMIN, E. J., BENNETT, D., BERNABE, E., BHALLA, K., BHANDARI, B., BIKBOV, B., BIN ABDULHAK, A., BIRBECK, G., BLACK, J. A., BLENCOWE, H., BLORE, J. D., BLYTH, F., BOLLIGER, I., BONAVENTURE, A., BOUFOUS, S., BOURNE, R., BOUSSINESQ, M., BRAITHWAITE, T., BRAYNE, C., BRIDGETT, L., BROOKER, S., BROOKS, P., BRUGHA, T. S., BRYAN-HANCOCK, C., BUCELLO, C., BUCHBINDER, R., BUCKLE, G., BUDKE, C. M., BURCH, M., BURNEY, P., BURSTEIN, R., CALABRIA, B., CAMPBELL, B., CANTER, C. E., CARABIN, H., CARAPETIS, J., CARMONA, L., CELLA, C., CHARLSON, F., CHEN, H., CHENG, A. T., CHOU, D., CHUGH, S. S., COFFENG, L. E., COLAN, S. D., COLQUHOUN, S., COLSON, K. E., CONDON, J., CONNOR, M. D., COOPER, L. T., CORRIERE, M., CORTINOVIS, M., DE VACCARO, K. C., COUSER, W., COWIE, B. C., CRIQUI, M. H., CROSS, M., DABHADKAR, K. C., DAHIYA, M., DAHODWALA, N., DAMSERE-DERRY, J., DANAEI, G., DAVIS, A., DE LEO, D., DEGENHARDT, L., DELLAVALLE, R., DELOSSANTOS, A., DENENBERG, J., DERRETT, S., DES JARLAIS, D. C., DHARMARATNE, S. D., et al. 2012. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet,* 380**,** 2197-223.

MUTH, S., SAYASONE, S., ODERMATT-BIAYS, S., PHOMPIDA, S., DUONG, S. & ODERMATT, P. 2010. *Schistosoma mekongi* in Cambodia and Lao People's Democratic Republic. *Advances in parasitology,* 72**,** 179-203.

NAHSHONI, A., BAUM, S., BARZILAI, A. & SCHWARTZ, E. 2016. Chronic Urticaria in Returning Travellers: The Role of Anthelmintic Treatment. *Dermatology,* 232**,** 468-71.

NASA-EARTH-OBSERVATORY. 2017. *Cambodia's Forests Are Disappearing* [Online]. Available: https://earthobservatory.nasa.gov/IOTD/view.php?id=89413 [Accessed 07.04.2017].

NATIONAL CENTER FOR PARASITOLOGY, E. A. M. C. 2014. Summarized guidelines for deworming target risk groups in Cambodia. Ministry of Health. Phnom Pehn, Cambodia.

NATIONAL INSTITUTE OF PUBLIC HEALTH, N. I. O. S. C. A. O. M. 2006. Cambodia Demographic and Health Survey 2005. *In:* NATIONAL INSTITUTE OF PUBLIC HEALTH, N. I. O. S. A. O. M. (ed.). Phnom Penh, Cambodia and Calverton, Maryland, USA.

NATIONAL INSTITUTE OF STATISTICS, M. O. P. 2013a. Cambodia - General Population Census of Cambodia 2008.

NATIONAL INSTITUTE OF STATISTICS, M. O. P. 2013b. *Cambodia inter-censal population survey 2013, final report* [Online]. [Accessed].

NATIONAL STATISTICS CENTRE OF THE LAO, P. D. R. 2005. *Lao PDR Census* [Online]. Available: http://www.nsc.gov.la/Products/Populationcensus2005/PopulationCensus2005_chapter1.htm [Accessed].

NEWTON, R. C., LIMPUANGTHIP, P., GREENBERG, S., GAM, A. & NEVA, F. A. 1992. Strongyloides stercoralis hyperinfection in a carrier of HTLV-I virus with evidence of selective immunosuppression. *Am J Med,* 92**,** 202-8.

NGUI, R., LIM, Y. A., TRAUB, R., MAHMUD, R. & MISTAM, M. S. 2012. Epidemiological and genetic data supporting the transmission of *Ancylostoma ceylanicum* among human and domestic animals. *PLoS Negl Trop Dis,* 6**,** e1522.

NIKOLAY, B., BROOKER, S. J. & PULLAN, R. L. 2014. Sensitivity of diagnostic tests for human soil-transmitted helminth infections: a meta-analysis in the absence of a true gold standard. *Int J Parasitol,* 44**,** 765-74.

NIKOLAY, B., MWANDAWIRO, C. S., KIHARA, J. H., OKOYO, C., CANO, J., MWANJE, M. T., SULTANI, H., ALUSALA, D., TURNER, H. C., TETI, C., GARN, J., FREEMAN, M. C., ALLEN, E., ANDERSON, R. M., PULLAN, R. L., NJENGA, S. M. & BROOKER, S. J. 2015. Understanding Heterogeneity in the Impact of National Neglected Tropical Disease Control Programmes: Evidence from School-Based Deworming in Kenya. *PLoS Negl Trop Dis,* 9**,** e0004108.

NONTASUT, P., MUENNOO, C., SA-NGUANKIAT, S., FONGSRI, S. & VICHIT, A. 2005. Prevalence of *Strongyloides* in Northern Thailand and treatment with ivermectin vs albendazole. *The Southeast Asian journal of tropical medicine and public health,* 36**,** 442-444.

NTDS, U. T. C. 2012. *London Declaration on Neglected Tropical Diseases* [Online]. Available:

http://unitingtocombatntds.org/downloads/press/ntd_event_london_declaration_on_ntds.pdf [Accessed].

NTZOUFRAS, I. 2009. *Bayesian Modeling Using WinBUGS*, John Wiley & Sons, Inc., Hoboken, NJ, USA.

NUTMAN, T. B. 2016. Human infection with *Strongyloides stercoralis* and other related *Strongyloides* species. *Parasitology***,** 1-11.

OLSEN, A. 2003. Experience with school-based interventions against soil-transmitted helminths and extension of coverage to non-enrolled children. *Acta Trop,* 86**,** 255-66.

OLSEN, A. 2007. Efficacy and safety of drug combinations in the treatment of schistosomiasis, soil-transmitted helminthiasis, lymphatic filariasis and onchocerciasis. *Trans R Soc Trop Med Hyg,* 101**,** 747-58.

OLSEN, A., VAN LIESHOUT, L., MARTI, H., POLDERMAN, T., POLMAN, K., STEINMANN, P., STOTHARD, R., THYBO, S., VERWEIJ, J. J. & MAGNUSSEN, P. 2009. Strongyloidiasis--the most neglected of the neglected tropical diseases? *Trans R Soc Trop Med Hyg,* 103**,** 967-72.

OPEN-DEVELOPMENT-CAMBODIA. 2016. *Forest cover* [Online]. Available: https://opendevelopmentcambodia.net/profiles/forest-cover [Accessed 07.04.2017].

OPHDI 2016. "Cambodia Country Briefing", Multidimensional Poverty Index Data Bank. *In:* OXFORD, O. P. A. H. D. I.-U. O. (ed.).

PALMEIRIM, M. S., OUATTARA, M., ESSE, C., KOFFI, V. A., ASSARE, R. K., HURLIMANN, E., COULIBALY, J. T., DIAKITE, N. R., DONGO, K., BONFOH, B., UTZINGER, J., N'GORAN, E. K. & RASO, G. 2018. Are schoolchildren less infected if they have good knowledge about parasitic worms? A case study from rural Cote d'Ivoire. *BMC Public Health,* 18**,** 951.

PARKER, M. & ALLEN, T. 2011. Does mass drug administration for the integrated treatment of neglected tropical diseases really work? Assessing evidence for the control of schistosomiasis and soil-transmitted helminths in Uganda. *Health Res Policy Syst,* 9**,** 3.

PARKER, M., ALLEN, T. & HASTINGS, J. 2008. Resisting control of neglected tropical diseases: dilemmas in the mass treatment of schistosomiasis and soil-transmitted helminths in north-west Uganda. *J Biosoc Sci,* 40**,** 161-81.

PATIL, S. R., ARNOLD, B. F., SALVATORE, A. L., BRICENO, B., GANGULY, S., COLFORD, J. M., JR. & GERTLER, P. J. 2014. The effect of India's total sanitation campaign on defecation behaviors and child health in rural Madhya Pradesh: a cluster randomized controlled trial. *PLoS Med,* 11**,** e1001709.

Characteristics of household sanitation use and demain in CLTS and non-CLTS villages: a comparative study from rural Cambodia, 2012. Directed by PEDI D, J. M., MCLENNAN L, AUN H, REVELL G.

PEDI D, S. M., SOPHEA P, JENKINS M 2014. Rural consumer sanitation adoption study - An analysis of rural consumers in the emerging sanitation market in Cambodia. *In:* USAID, T. S. F. F., WATERSHED (ed.).

PHOMMASACK, B., SAKLOKHAM, K., CHANTHAVISOUK, C., NAKHONESID-FISH, V., STRANDGAARD, H., MONTRESOR, A., SHUEY, D. A. & EHRENBERG, J. 2008. Coverage and costs of a school deworming programme in 2007 targeting all primary schools in Lao PDR. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 102**,** 1201-1206.

PHONGLUXA, K., XAYASENG, V., VONGHACHACK, Y., AKKHAVONG, K., VAN EEUWIJK, P. & ODERMATT, P. 2013. Helminth infection in southern Laos: high prevalence and low awareness. *Parasit Vectors,* 6**,** 328.

PICKERING, A. J., DJEBBARI, H., LOPEZ, C., COULIBALY, M. & ALZUA, M. L. 2015. Effect of a community-led sanitation intervention on child diarrhoea and child growth in rural Mali: a cluster-randomised controlled trial. *Lancet Glob Health,* 3**,** e701-11.

PRICHARD, R. K., BASANEZ, M. G., BOATIN, B. A., MCCARTHY, J. S., GARCIA, H. H., YANG, G. J., SRIPA, B. & LUSTIGMAN, S. 2012. A research agenda for helminth diseases of humans: intervention for control and elimination. *PLoS Negl Trop Dis,* 6**,** e1549.

PRÜSS-USTÜN, A., BARTRAM, J., CLASEN, T., COLFORD, J. M., JR., CUMMING, O., CURTIS, V., BONJOUR, S., DANGOUR, A. D., DE FRANCE, J., FEWTRELL, L., FREEMAN, M. C., GORDON, B., HUNTER, P. R., JOHNSTON, R. B., MATHERS, C., MAUSEZAHL, D., MEDLICOTT, K., NEIRA, M., STOCKS, M., WOLF, J. & CAIRNCROSS, S. 2014. Burden of disease from inadequate water, sanitation and hygiene in low- and middle-income settings: a retrospective analysis of data from 145 countries. *Trop Med Int Health,* 19**,** 894-905.

PRÜSS-USTÜN A, W. J., CORVALÁN C, BOS R, NEIRA M 2016. Preventing disease through healthy environments: a global assessment of the burden of disease from environmental risks. *In:* WHO (ed.).

PULLAN, R. L., BETHONY, J. M., GEIGER, S. M., CORREA-OLIVEIRA, R., BROOKER, S. & QUINNELL, R. J. 2010a. Human helminth co-infection: no evidence of common genetic control of hookworm and *Schistosoma mansoni* infection intensity in a Brazilian community. *Int J Parasitol,* 40**,** 299-306.

PULLAN, R. L., BETHONY, J. M., GEIGER, S. M., CUNDILL, B., CORREA-OLIVEIRA, R., QUINNELL, R. J. & BROOKER, S. 2008. Human helminth co-infection: analysis of spatial patterns and risk factors in a Brazilian community. *PLoS Negl Trop Dis,* 2**,** e352.

PULLAN, R. L., KABATEREINE, N. B., QUINNELL, R. J. & BROOKER, S. 2010b. Spatial and genetic epidemiology of hookworm in a rural community in Uganda. *PLoS Negl Trop Dis,* 4**,** e713.

PULLAN, R. L., SMITH, J. L., JASRASARIA, R. & BROOKER, S. J. 2014. Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. *Parasit Vectors,* 7**,** 37.

RAMANATHAN, R. & NUTMAN, T. 2008. Strongyloides stercoralis infection in the immunocompromised host. *Curr Infect Dis Rep,* 10**,** 105-10.

RAMPUR, L., JARIWALA, S. P., HUDES, G., ROSENSTREICH, D. L. & DE VOS, G. 2016. Effect of ivermectin on allergy-type manifestations in occult strongyloidiasis. *Ann Allergy Asthma Immunol,* 117**,** 423-428.

RASO, G., MATTHYS, B., N'GORAN, E. K., TANNER, M., VOUNATSOU, P. & UTZINGER, J. 2005. Spatial risk prediction and mapping of *Schistosoma mansoni* infections among schoolchildren living in western Côte d'Ivoire. *Parasitology,* 131**,** 97-108.

RASO, G., VOUNATSOU, P., GOSONIU, L., TANNER, M., N'GORAN, E. K. & UTZINGER, J. 2006a. Risk factors and spatial patterns of hookworm infection among schoolchildren in a rural area of western Côte d'Ivoire. *International Journal for Parasitology,* 36**,** 201-210.

RASO, G., VOUNATSOU, P., SINGER, B. H., N'GORAN, E. Z. K., TANNER, M. & UTZINGER, J. 2006b. An integrated approach for risk profiling and spatial prediction of Schistosoma mansoni-hookworm coinfection. *Proceedings of the National Academy of Sciences of the United States of America,* 103**,** 6934-6939.

RCORETEAM 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

REDDY, M., GILL, S. S., KALKAR, S. R., WU, W., ANDERSON, P. J. & ROCHON, P. A. 2007. Oral drug therapy for multiple neglected tropical diseases: a systematic review. *JAMA,* 298**,** 1911-24.

REQUENA-MÉNDEZ, A., CHIODINI, P., BISOFFI, Z., BUONFRATE, D., GOTUZZO, E. & MUNOZ, J. 2013. The laboratory diagnosis and follow up of strongyloidiasis: a systematic review. *PLoS Negl Trop Dis,* 7**,** e2002.

RIM, H. J., CHAI, J. Y., MIN, D. Y., CHO, S. Y., EOM, K. S., HONG, S. J., SOHN, W. M., YONG, T. S., DEODATO, G., STANDGAARD, H., PHOMMASACK, B., YUN, C. H. & HOANG, E. H. 2003. Prevalence of intestinal parasite infections on a national scale among primary schoolchildren in Laos. *Parasitology Research,* 91**,** 267-272.

ROBINSON, R. D., LINDO, J. F., NEVA, F. A., GAM, A. A., VOGEL, P., TERRY, S. I. & COOPER, E. S. 1994. Immunoepidemiologic studies of Strongyloides stercoralis and human T lymphotropic virus type I infections in Jamaica. *J Infect Dis,* 169**,** 692-6.

RODRIGUEZ CALABUIG, D., IGUAL ADELL, R., OLTRA ALCARAZ, C., SANCHEZ SANCHEZ, P., BUSTAMANTE BALEN, M., PARRA GODOY, F. & NAGORE ENGUIDANOS, E. 2001. [Agricultural occupation and strongyloidiasis. A case-control study]. *Rev Clin Esp,* 201**,** 81-4.

SANCHEZ, P. R., GUZMAN, A. P., GUILLEN, S. M., ADELL, R. I., ESTRUCH, A. M., GONZALO, I. N. & OLMOS, C. R. 2001. Endemic strongyloidiasis on the Spanish Mediterranean coast. *QJM,* 94**,** 357-63.

SANDERS, N. L. & MISHRA, A. 2016. Role of interleukin-18 in the pathophysiology of allergic diseases. *Cytokine Growth Factor Rev,* 32**,** 31-39.

SATO, Y., KOBAYASHI, J., TOMA, H. & SHIROMA, Y. 1995. Efficacy of stool examination for detection of *Strongyloides* infection. *Am J Trop Med Hyg,* 53**,** 248-50.

SAYASONE, S., MAK, T. K., VANMANY, M., RASPHONE, O., VOUNATSOU, P., UTZINGER, J., AKKHAVONG, K. & ODERMATT, P. 2011. Helminth and intestinal protozoa infections, multiparasitism and risk factors in Champasack province, Lao People's Democratic Republic. *PLoS Negl Trop Dis,* 5**,** e1037.

SAYASONE, S., ODERMATT, P., PHOUMINDR, N., VONGSARAVANE, X., SENSOMBATH, V., PHETSOUVANH, R., CHOULAMANY, X. & STROBEL, M. 2007. Epidemiology of Opisthorchis viverrini in a rural district of southern Lao PDR. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 101**,** 40-47.

SAYASONE, S., UTZINGER, J., AKKHAVONG, K. & ODERMATT, P. 2014. Multiparasitism and intensity of helminth infections in relation to symptoms and nutritional status among children: A cross-sectional study in southern Lao People's Democratic Republic. *Acta Trop*.

SAYASONE, S., VONGHAJACK, Y., VANMANY, M., RASPHONE, O., TESANA, S., UTZINGER, J. Ç., AKKHAVONG, K. & ODERMATT, P. 2009. Diversity of human intestinal helminthiasis in Lao PDR. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 103**,** 247-254.

SCHÄR, F., GIARDINA, F., KHIEU, V., MUTH, S., VOUNATSOU, P., MARTI, H. & ODERMATT, P. 2015. Occurrence of and risk factors for *Strongyloides stercoralis* infection in South-East Asia. *Acta Trop*.

SCHÄR, F., HATTENDORF, J., KHIEU, V., MUTH, S., CHAR, M. C., MARTI, H. P. & ODERMATT, P. 2014. *Strongyloides stercoralis* larvae excretion patterns before and after treatment. *Parasitology***,** 1-6.

SCHÄR, F., TROSTDORF, U., GIARDINA, F., KHIEU, V., MUTH, S., MARTI, H., VOUNATSOU, P. & ODERMATT, P. 2013. *Strongyloides stercoralis*: Global Distribution and Risk Factors. *PLoS neglected tropical diseases,* 7.

SHUFORD, K. V., TURNER, H. C. & ANDERSON, R. M. 2016. Compliance with anthelmintic treatment in the neglected tropical diseases control programmes: a systematic review. *Parasit Vectors,* 9**,** 29.

SIDDIQUI, A. A. & BERK, S. L. 2001. Diagnosis of *Strongyloides stercoralis* infection. *Clin Infect Dis,* 33**,** 1040-7.

SIGLER, R., MAHMOUDI, L. & GRAHAM, J. P. 2015. Analysis of behavioral change techniques in community-led total sanitation programmes. *Health Promot Int,* 30**,** 16-28.

SINUON, M., TSUYUOKA, R., SOCHEAT, D., MONTRESOR, A. & PALMER, K. 2005. Financial costs of deworming children in all primary schools in Cambodia. *Trans R Soc Trop Med Hyg,* 99**,** 664-8.

SINUON, M., TSUYUOKA, R., SOCHEAT, D., ODERMATT, P., OHMAE, H., MATSUDA, H., MONTRESOR, A. & PALMER, K. 2007. Control of *Schistosoma mekongi* in Cambodia: results of eight years of control activities in the two endemic provinces. *Trans R Soc Trop Med Hyg,* 101**,** 34-9.

SMITH, J. L. & BROOKER, S. 2010. Impact of hookworm infection and deworming on anaemia in non-pregnant populations: a systematic review. *Trop Med Int Health,* 15**,** 776-795.

SOARES MAGALHÃES, R. J., BIRITWUM, N. K., GYAPONG, J. O., BROOKER, S., ZHANG, Y., BLAIR, L., FENWICK, A. & CLEMENTS, A. C. 2011a. Mapping helminth co-infection and co-intensity: geostatistical prediction in ghana. *PLoS Negl Trop Dis,* 5**,** e1200.

SOARES MAGALHÃES, R. J., BIRITWUM, N. K., GYAPONG, J. O., BROOKER, S., ZHANG, Y., BLAIR, L., FENWICK, A. & CLEMENTS, A. C. A. 2011b. Mapping Helminth Co-Infection and Co-Intensity: Geostatistical Prediction in Ghana. *PLoS Negl Trop Dis,* 5.

SOARES MAGALHÃES, R. J., SALAMAT, M. S., LEONARDO, L., GRAY, D. J., CARABIN, H., HALTON, K., MCMANUS, D. P., WILLIAMS, G. M., RIVERA, P., SANIEL, O., HERNANDEZ, L., YAKOB, L., MCGARVEY, S. T. & CLEMENTS, A. C. 2015. Mapping the Risk of Soil-Transmitted Helminthic Infections in the Philippines. *PLoS Negl Trop Dis,* 9, e0003915.

SOUKHATHAMMAVONG, P., ODERMATT, P., SAYASONE, S., VONGHACHACK, Y., VOUNATSOU, P., HATZ, C., AKKHAVONG, K. & KEISER, J. 2011. 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,* 11**,** 110-8.

SOUKHATHAMMAVONG, P. A., SAYASONE, S., PHONGLUXA, K., XAYASENG, V., UTZINGER, J., VOUNATSOU, P., HATZ, C., AKKHAVONG, K., KEISER, J. & ODERMATT, P. 2012. Low efficacy of single-dose albendazole and mebendazole against hookworm and effect on concomitant helminth infection in Lao PDR. *PLoS Negl Trop Dis,* 6**,** e1417.

SPEICH, B., ALI, S. M., AME, S. M., BOGOCH, II, ALLES, R., HUWYLER, J., ALBONICO, M., HATTENDORF, J., UTZINGER, J. & KEISER, J. 2015. Efficacy and safety of albendazole plus ivermectin, albendazole plus mebendazole, albendazole plus oxantel pamoate, and mebendazole alone against Trichuris trichiura and concomitant soil-transmitted helminth infections: a four-arm, randomised controlled trial. *Lancet Infect Dis,* 15**,** 277-84.

SPIEGELHALTER, D. J., BEST, N. G., CARLIN, B. P. & VAN DER LINDE, A. 2002. Bayesian measures of model complexity and fit. *Journal of the Royal Statistical Society: Series B (Statistical Methodology),* 64**,** 583-639.

STEINMANN, P., UTZINGER, J., DU, Z. W., JIANG, J. Y., CHEN, J. X., HATTENDORF, J., ZHOU, H. & ZHOU, X. N. 2011. Efficacy of single-dose and triple-dose albendazole and mebendazole against soil-transmitted helminths and *Taenia spp.*: a randomized controlled trial. *PLoS One,* 6**,** e25003.

STEINMANN, P., UTZINGER, J., DU, Z. W. & ZHOU, X. N. 2010. Multiparasitism a neglected reality on global, regional and local scale. *Advances in parasitology,* 73**,** 21-50.

STEINMANN, P., ZHOU, X. N., DU, Z. W., JIANG, J. Y., WANG, L. B., WANG, X. Z., LI, L. H., MARTI, H. & UTZINGER, J. 2007a. Occurrence of *Strongyloides stercoralis* in Yunnan Province, China, and comparison of diagnostic methods. *PLoS Negl Trop Dis,* 1**,** e75.

STEINMANN, P., ZHOU, X. N., LI, Y. L., LI, H. J., CHEN, S. R., YANG, Z., FAN, W., JIA, T. W., LI, L. H., VOUNATSOU, P. & UTZINGER, J. 2007b. Helminth infections and risk factor analysis among residents in Eryuan county, Yunnan province, China. *Acta Tropica,* 104**,** 38-51.

STEPHENSON, L. S., LATHAM, M. C. & OTTESEN, E. A. 2000a. Global malnutrition. *Parasitology,* 121 Suppl**,** S5-22.

STEPHENSON, L. S., LATHAM, M. C. & OTTESEN, E. A. 2000b. Malnutrition and parasitic helminth infections. *Parasitology,* 121 Suppl**,** S23-38.

STRUNZ, E. C., ADDISS, D. G., STOCKS, M. E., OGDEN, S., UTZINGER, J. & FREEMAN, M. C. 2014. Water, sanitation, hygiene, and soil-transmitted helminth infection: a systematic review and meta-analysis. *PLoS Med,* 11**,** e1001620.

SUPUTTAMONGKOL, Y., PREMASATHIAN, N., BHUMIMUANG, K., WAYWA, D., NILGANUWONG, S., KARUPHONG, E., ANEKTHANANON, T., WANACHIWANAWIN, D. & SILPASAKORN, S. 2011. Efficacy and safety of single and double doses of ivermectin versus 7-day high dose albendazole for chronic strongyloidiasis. *PLoS Negl Trop Dis,* 5**,** e1044.

TANAKA, T., HIRATA, T., PARROTT, G., HIGASHIARAKAWA, M., KINJO, T., KINJO, T., HOKAMA, A. & FUJITA, J. 2016. Relationship Among Strongyloides stercoralis Infection, Human T-Cell Lymphotropic Virus Type 1 Infection, and Cancer: A 24-Year Cohort Inpatient Study in Okinawa, Japan. *Am J Trop Med Hyg,* 94**,** 365-70.

TARAFDER, M. R., CARABIN, H., JOSEPH, L., BALOLONG, E., JR., OLVEDA, R. & MCGARVEY, S. T. 2010. Estimating the sensitivity and specificity of Kato-Katz stool examination technique for detection of hookworms, Ascaris lumbricoides and Trichuris trichiura infections in humans in the absence of a 'gold standard'. *Int J Parasitol,* 40**,** 399-404.

TAYLOR-ROBINSON, D. C., MAAYAN, N., SOARES-WEISER, K., DONEGAN, S. & GARNER, P. 2015. Deworming drugs for soil-transmitted intestinal worms in children: effects on nutritional indicators, haemoglobin, and school performance. *Cochrane Database Syst Rev***,** CD000371.

TOLEDO, R., MUÑOZ-ANTOLI, C. & ESTEBAN, J. G. 2015. Strongyloidiasis with emphasis on human infections and its different clinical forms. *Adv Parasitol,* 88**,** 165-241.

TOMCZYK, S., DERIBE, K., BROOKER, S. J., CLARK, H., RAFIQUE, K., KNOPP, S., UTZINGER, J. & DAVEY, G. 2014. Association between footwear use and neglected tropical diseases: a systematic review and meta-analysis. *PLoS Negl Trop Dis,* 8**,** e3285.

TRAUB, R. J. 2013. *Ancylostoma ceylanicum*, a re-emerging but neglected parasitic zoonosis. *International journal for parasitology,* 43**,** 1009-1015.

TRAUB, R. J., INPANKAEW, T., SUTTHIKORNCHAI, C., SUKTHANA, Y. & THOMPSON, R. C. A. 2008. PCR-based coprodiagnostic tools reveal dogs as reservoirs of zoonotic ancylostomiasis caused by *Ancylostoma ceylanicum* in temple communities in Bangkok. *Veterinary parasitology,* 155**,** 67-73.

TRUSCOTT, J. E., HOLLINGSWORTH, T. D., BROOKER, S. J. & ANDERSON, R. M. 2014. Can chemotherapy alone eliminate the transmission of soil transmitted helminths? *Parasit Vectors,* 7**,** 266.

TRUSCOTT, J. E., TURNER, H. C. & ANDERSON, R. M. 2015. What impact will the achievement of the current World Health Organisation targets for anthelmintic treatment coverage in children have on the intensity of soil transmitted helminth infections? *Parasit Vectors,* 8**,** 551.

TURNER, H. C., TRUSCOTT, J. E., BETTIS, A. A., HOLLINGSWORTH, T. D., BROOKER, S. J. & ANDERSON, R. M. 2016a. Analysis of the population-level impact of co-administering ivermectin with albendazole or mebendazole for the control and elimination of Trichuris trichiura. *Parasite Epidemiol Control,* 1**,** 177-187.

TURNER, H. C., TRUSCOTT, J. E., BETTIS, A. A., SHUFORD, K. V., DUNN, J. C., HOLLINGSWORTH, T. D., BROOKER, S. J. & ANDERSON, R. M. 2015a. An economic evaluation of expanding hookworm control strategies to target the whole community. *Parasit Vectors,* 8**,** 570.

TURNER, H. C., TRUSCOTT, J. E., FLEMING, F. M., HOLLINGSWORTH, T. D., BROOKER, S. J. & ANDERSON, R. M. 2016b. Cost-effectiveness of scaling up mass drug administration for the control of soil-transmitted helminths: a comparison of cost function and constant costs analyses. *Lancet Infect Dis,* 16**,** 838-46.

TURNER, H. C., TRUSCOTT, J. E., HOLLINGSWORTH, T. D., BETTIS, A. A., BROOKER, S. J. & ANDERSON, R. M. 2015b. Cost and cost-effectiveness of soil-transmitted helminth treatment programmes: systematic review and research needs. *Parasit Vectors,* 8**,** 355.

UNDP 2015. Country analysis report: Lao PDR. *In:* NATIONS, U. (ed.).

UNDP 2016a. Briefing note for countries on the 2016 Human Development Report - Cambodia. *In:* NATIONS, U. (ed.).

UNDP. 2016b. *Human Development Index* [Online]. Available: http://hdr.undp.org/en/composite/HDI [Accessed 07.04.2017].

UNICEF 2013. Community-Led Total Sanitation in East Asia and Pacific.

UNICEF 2016. The State if the World's Children 2016 Statistical Tables.

UNICEF, W. H. O. 2015. Progress on Sanitation and Drinking Water: 2015 Update and MDG Assessment. *In:* WHO PRESS, W. H. O. (ed.).

UNITED-NATIONS-POPULATION-FUND 2014. Urbanization and its linkage to socio-economic and environmental issues.

UPARANUKRAW, P., PHONGSRI, S. & MORAKOTE, N. 1999. Fluctuations of larval excretion in *Strongyloides stercoralis* infection. *Am J Trop Med Hyg,* 60**,** 967-73.

URBANI, C. & ALBONICO, M. 2003. Anthelminthic drug safety and drug administration in the control of soil-transmitted helminthiasis in community campaigns. *Acta Trop,* 86**,** 215-21.

URBANI, C., ODERMATT, P., SOCHEAT, D., SINUON, M., HOYER, S. & HATZ, C. 2003. Control of soil-transmitted helminth infections in schoolchildren in Cambodia: implications for an integrated approach. *In:* CROMPTON, D. W. T., MONTRESOR, A., NESHEIM, M. C. & SAVIOLI, L. (eds.) *Controlling Disease Due to Helminth Infections.* WHO.

UTZINGER, J., BECKER, S. L., KNOPP, S., BLUM, J., NEUMAYR, A. L., KEISER, J. & HATZ, C. F. 2012. Neglected tropical diseases: diagnosis, clinical management, treatment and control. *Swiss Med Wkly,* 142**,** w13727.

VADLAMUDI, R. S., CHI, D. S. & KRISHNASWAMY, G. 2006. Intestinal strongyloidiasis and hyperinfection syndrome. *Clinical and molecular allergy: CMA,* 4.

VANNACHONE, B., KOBAYASHI, J., NAMBANYA, S., MANIVONG, K., INTHAKONE, S. & SATO, Y. 1998. An epidemiological survey on intestinal parasite infection in Khammouane Province, Lao PDR, with special reference to *Strongyloides* infection. *Southeast Asian J Trop Med Public Health,* 29**,** 717-22.

VERCRUYSSE, J., BEHNKE, J. M., ALBONICO, M., AME, S. M., ANGEBAULT, C., BETHONY, J. M., ENGELS, D., GUILLARD, B., NGUYEN, T. V., KANG, G., KATTULA, D., KOTZE, A. C., MCCARTHY, J. S., MEKONNEN, Z., MONTRESOR, A., PERIAGO, M. V., SUMO, L., TCHUENTE, L. A., DANG, T. C., ZEYNUDIN, A. & LEVECKE, B. 2011. Assessment of the anthelmintic efficacy of albendazole in school children in seven countries where soil-transmitted helminths are endemic. *PLoS Negl Trop Dis,* 5**,** e948.

VONGHACHACK, Y., SAYASONE, S., BOUAKHASITH, D., TAISAYAVONG, K., AKKAVONG, K. & ODERMATT, P. 2014. Epidemiology of Strongyloides stercoralis on Mekong islands in Southern Laos. *Acta Trop*.

VOS, T., FLAXMAN, A. D., NAGHAVI, M., LOZANO, R., MICHAUD, C., EZZATI, M., SHIBUYA, K., SALOMON, J. A., ABDALLA, S., ABOYANS, V., ABRAHAM, J., ACKERMAN, I., AGGARWAL, R., AHN, S. Y., ALI, M. K., ALVARADO, M., ANDERSON, H. R., ANDERSON, L. M., ANDREWS, K. G., ATKINSON, C., BADDOUR, L. M., BAHALIM, A. N., BARKER-COLLO, S., BARRERO, L. H., BARTELS, D. H., BASANEZ, M. G., BAXTER, A., BELL, M. L., BENJAMIN, E. J., BENNETT, D., BERNABE, E., BHALLA, K., BHANDARI, B., BIKBOV, B., BIN ABDULHAK, A., BIRBECK, G., BLACK, J. A., BLENCOWE, H., BLORE, J. D., BLYTH, F., BOLLIGER, I., BONAVENTURE, A., BOUFOUS, S., BOURNE, R., BOUSSINESQ, M., BRAITHWAITE, T., BRAYNE, C., BRIDGETT, L., BROOKER, S., BROOKS, P., BRUGHA, T. S., BRYAN-HANCOCK, C., BUCELLO, C., BUCHBINDER, R., BUCKLE, G., BUDKE, C. M., BURCH, M., BURNEY, P., BURSTEIN, R., CALABRIA, B., CAMPBELL, B., CANTER, C. E., CARABIN, H., CARAPETIS, J., CARMONA, L., CELLA, C., CHARLSON, F., CHEN, H., CHENG, A. T., CHOU, D., CHUGH, S. S., COFFENG, L. E., COLAN, S. D., COLQUHOUN, S., COLSON, K. E., CONDON, J., CONNOR, M. D., COOPER, L. T., CORRIERE, M., CORTINOVIS, M., DE VACCARO, K. C., COUSER, W., COWIE, B. C., CRIQUI, M. H., CROSS, M., DABHADKAR, K. C., DAHIYA, M., DAHODWALA, N., DAMSERE-DERRY, J., DANAEI, G., DAVIS, A., DE LEO, D., DEGENHARDT, L., DELLAVALLE, R., DELOSSANTOS, A., DENENBERG, J., DERRETT, S., DES JARLAIS, D. C., DHARMARATNE, S. D., DHERANI, M., et al. 2012. Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet,* 380**,** 2163-96.

VOUNATSOU, P., RASO, G., TANNER, M., N'GORAN, E. K. & UTZINGER, J. 2009. Bayesian geostatistical modelling for mapping schistosomiasis transmission. *Parasitology,* 136**,** 1695-1705.

VYAS, S. & KUMARANAYAKE, L. 2006. Constructing socio-economic status indices: how to use principal components analysis. *Health Policy Plan,* 21**,** 459-468.

WHO 1995. WHO Model Prescribing Information. Drugs used in parasitic diseases. Second edition.

WHO. 2002. *Prevention and control of schistosomiasis and soil-transmitted helminthiasis: a report of a WHO expert committee* [Online]. [Accessed].

WHO 2003. World Health Organization (WHO). Making choices in health: WHO guide to cost-effectiveness analysis. . *In:* GENEVA: WORLD HEALTH ORGANIZATION (ed.).

WHO 2005. Deworming for health and development - Report of the third global meeting of the partners for parasite control.

WHO 2009. *WHO Model formulary 2008,* Geneva, WHO.

WHO 2010. First WHO report on neglected tropical diseases: "Working to overcome the global impact of negleted tropical diseases". Geneva: World Health Organization.

WHO 2012a. Eliminating soil-transmitted helminthiases as a public health problem in children. Progress report 2001-2010 and strategic plan 2011-2020.

WHO 2012b. Soil-transmitted helminthiases: number of children treated in 2010. *Weekly epidemiological Record,* 87**,** 225-232.

WHO 2013. Sustaining the drive to overcome the global impact of neglected tropical diseases : second WHO report on neglected tropical diseases. *In:* CROMPTON, D. W. (ed.).

WHO 2015a. PCT databank - soil-transmitted helminths.

WHO 2015b. WHO Model List of Essential Medicines. *In:* WHO (ed.).

WHO 2016a. Cambodia, Cook Islands, Niue and Vanuatu eliminate lymphatic filariasis as a public health problem.

WHO 2016b. Strategic and technical advisory group for neglected tropical diseases subgroup on disease-specific indicators: integrating national programmes to eliminate lymphatic filariasis and onchocerciasis: report of a meeting, Geneva, 7–8 February 2015.

WHO 2016c. The work of WHO in the Western Pacific Region. 1 July 2015 - 30 June 2016. Report of the regional director. *In:* WHO (ed.).

WHO. 2017a. *African Programme for Onchocerciasis control* [Online]. Available: http://www.who.int/blindness/partnerships/APOC/en/ [Accessed 25.05.2017].

WHO. 2017b. *Cambodia and the Lao People's Democratic Republic wipe out trachoma—leading infectious cause of blindness* [Online]. Available: http://www.wpro.who.int/mediacentre/releases/2017/20170919/en/ [Accessed 04.11.2017].

WHO 2017c. Onchocerciasis Elimination Program for the Americas (OEPA).

WHO & CROMPTON, D. W. 2006. *Preventive chemotherapy in human helminthiasis: coordinated use of anthelminthic drugs in control interventions: a manual for health professionals and programme managers. Geneva: World Health Organization*

WHO/UNICEF-JOINT-MONITORING-PROGRAMME-(JMP)-FOR-WATER-SUPPLY-AND-SANITATION. 2015. *Cambodia: estimates on the use of water sources and sanitation facilities (1980 - 2015)* [Online]. Available: https://www.wssinfo.org/documents/?tx_displaycontroller[type]=country_files [Accessed].

WOLF, J., PRÜSS-USTÜN, A., CUMMING, O., BARTRAM, J., BONJOUR, S., CAIRNCROSS, S., CLASEN, T., COLFORD, J. M., JR., CURTIS, V., DE FRANCE, J., FEWTRELL, L., FREEMAN, M. C., GORDON, B., HUNTER, P. R., JEANDRON, A., JOHNSTON, R. B., MAUSEZAHL, D., MATHERS, C., NEIRA, M. & HIGGINS, J. P. 2014. Assessing the impact of drinking water and sanitation on diarrhoeal disease in low- and middle-income settings: systematic review and meta-regression. *Trop Med Int Health,* 19**,** 928-42.

WORLD-HEALTH-ASSEMBLY 2001. WHA 54.19 Schistosomiasis and soil-transmitted helminth infections. *In:* WHO (ed.).

WORLD-HEALTH-ASSEMBLY 2013. WHA Resolution 66.12.

WORLDBANK. *Poverty & Equity - Country dashboard: Lao PDR* [Online]. Available: http://povertydata.worldbank.org/poverty/country/LAO [Accessed 2017].

WORLDBANK. 2015. *Country indicators* [Online]. Available: http://data.worldbank.org/indicator/NY.GDP.PCAP.CD?locations=KH [Accessed 08.05.2017 2017].

WORLDBANK. 2016. *Human Development Indicators* [Online]. Available: http://hdr.undp.org/en/countries/profiles/KHM [Accessed 23.03.2017].

WORLDBANK. 2017a. *Cambodia - overview* [Online]. Available: http://www.worldbank.org/en/country/cambodia/overview#3 [Accessed 07.04.2017].

WORLDBANK 2017b. Poverty & Equity - Country dashboard: Cambodia.

XIAO, S. H., HUI-MING, W., TANNER, M., UTZINGER, J. & CHONG, W. 2005. Tribendimidine: a promising, safe and broad-spectrum anthelmintic agent from China. *Acta tropica,* 94**,** 1-14.

XIAO, S. H., UTZINGER, J., TANNER, M., KEISER, J. & XUE, J. 2013. Advances with the Chinese anthelminthic drug tribendimidine in clinical trials and laboratory investigations. *Acta tropica,* 126**,** 115-126.

YAP, P., DU, Z. W., WU, F. W., JIANG, J. Y., CHEN, R., ZHOU, X. N., HATTENDORF, J., UTZINGER, J. & STEINMANN, P. 2013. Rapid re-infection with soil-transmitted helminths after triple-dose albendazole treatment of school-aged children in Yunnan, People's Republic of China. *The American journal of tropical medicine and hygiene,* 89**,** 23-31.

YAP, P., UTZINGER, J., HATTENDORF, J. & STEINMANN, P. 2014. Influence of nutrition on infection and re-infection with soil-transmitted helminths: a systematic review. *Parasit Vectors,* 7**,** 229.

YONG, T. S., CHAI, J. Y., SOHN, W. M., EOM, K. S., JEOUNG, H. G., HOANG, E. H., YOON, C. H., JUNG, B. K., LEE, S. H., SINUON, M. & SOCHEAT, D. 2014.

Prevalence of intestinal helminths among inhabitants of Cambodia (2006-2011). *Korean J Parasitol,* 52**,** 661-6.

ZAGO-GOMES, M. P., AIKAWA, K. F., PERAZZIO, S. F., GONCALVES, C. S. & PEREIRA, F. E. 2002. Prevalence of intestinal nematodes in alcoholic patients. *Rev Soc Bras Med Trop,* 35**,** 571-4.

ZIEGELBAUER, K., SPEICH, B., MAUSEZAHL, D., BOS, R., KEISER, J. & UTZINGER, J. 2012. Effect of sanitation on soil-transmitted helminth infection: systematic review and meta-analysis. *PLoS Med,* 9**,** e1001162.