EPIDEMIOLOGY AND CLINICAL ASPECTS OF *STRONGYLOIDES STERCORALIS* INFECTION IN CAMBODIA

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Prof. Dr. Jörg Schibler

Dekan

To my beloved family

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SUMMARY

Background: The threadworm *Strongyloides stercoralis* is endemic in settings where sanitary conditions are poor and where the climate is warm and humid. More than 70 tropical countries in Southeast Asia, Sub-Saharan Africa, West Indies and Latin America are considered as high endemic settings. However, *S. stercoralis* is also prevalent in subtropical and temperate regions including Australia, Japan, Canada, United States and Europe. The global prevalence of *S. stercoralis* is heterogeneous. It is believed that about 30 - 100 million people worldwide are infected with *S. stercoralis*. But the true number and the global burden of infection remain unknown and most probably are today underestimating in many areas of the tropical resource poor countries. The low sensitivity of the currently available diagnostic tools and a scarcity of specialized survey are most important factors for that. Moreover, many epidemiological aspects of *S. stercoralis* infection are poorly understood or unknown. It is not known in detail where *S. stercoralis* is endemic, which infection rates and intensities can typically be expected in different settings and populations, and when an individual was infected at first-time and how quickly the re-infection can occur after successful treatment. Epidemiological information on *S. stercoralis* such as large-scale prevalence, re-infection, risk factors, clinical features and treatment efficacy are unknown in Cambodia and many parts of Southeast Asia.

Aim and objectives: This PhD thesis aimed to understand the importance of *S. stercoralis* infection in Cambodia by pursuing four main objectives: (i) assess *S. stercoralis* infection and risk factors, validate diagnostic methods and determine treatment efficacy among schoolchildren, (ii) determine large-scale prevalence and risk factors in two socioeconomic and ecological distinctly different settings, (iii) determine re-infection rates among schoolchildren, and (iv) document clinical aspects of patients with high intensity of *S. stercoralis* infection in rural communities.

Methods: School- and community-based studies were carried out in four primary schools and 120 villages of three provinces (Kandal, Preah Vihear and Takeo) in Cambodia, from 2009 to 2011. After obtaining the written informed consent from participants, an individual questionnaire was administrated to obtain demographic, risk-perception and behavioral data. The head of household was interviewed with a household questionnaire on socioeconomic indicators of the household such as house type, household assets, latrine and livestock. After the interview, each participant was given a pre-labeled plastic container (ID code, name, sex, age and date) for stool sample collection. In case a multiple stool samples analysis, another stool container was distributed upon collection of the first or second sample. The fecal materials were analyzed by Baermann method and Koga-agar plate (KAP) culture for diagnosing *S. stercoralis* and Kato-Katz method for helminth co-infections.

Two school-based studies were performed in four primary schools in Kandal province. In 2009, a cross-sectional study was carried out among 458 children, examining three fecal samples per child, to assess risk factors, diagnostic methods and treatment efficacy after three weeks of ivermectin treatment (100µg/kg/day for two days). A two-year cohort study was conducted among 302 schoolchildren from 2009 to 2011, analyzing two stool samples per child, to determine re-infection and risk factors of *S. stercoralis*.

Two large-scale cross-sectional community-based studies were conducted in 2010 and 2011 to assess infection prevalence and risk factors in two provinces (2396 participants from 60 villages of Preah Vihear province, analyzed two stool samples per participant; and 2861 participants from 60 villages of Takeo province, examined one stool sample per participant). Bayesian kriging was used to predict risk at non-surveyed locations in Preah Vihear province. A case-series study, nested in the survey in Preah Vihear province in 2010, was carried out to document the clinical features of 21 *S. stercoralis* cases, with high numbers of *S. stercoralis* larvae in their fecal specimen detected by Baermann technique.

Principal findings: A cross-sectional school-based survey in 2009 found that 24.4% of 458 schoolchildren were infected with *S. stercoralis*. The prevalence of *S. stercoralis* infection increased considerably (from 18.6% to 24.4%) when three stool samples were examined. The sensitivity of KAP culture and Baermann technique was 88.4% and 75.0%, respectively. Clinical features such as itchy skin and diarrheal episodes were significantly associated with *S. stercoralis* infection. Children who reported defecating in latrines were significantly less infected with *S. stercoralis* than those who did not use latrines (OR: 0.4; 95% CI: 0.2 – 0.6; $P<0.001$). Almost three-quarters of the infections could have been reduced by proper sanitation (PAR: 0.7; 95% CI: 0.5– 0.9). Ivermectin (200 µg/kg BW, PO, over 2 days) was highly efficacious against *S. stercoralis* infection, with a cure rate of 98.3% three weeks after treatment.

In Preah Vihear and Takeo provinces, *S. stercoralis* infection prevalence among general population was 44.7% and 21.0%, respectively. In both areas found that the male participants were significantly more infected than females ($P<0.001$) in all age classes. In Preah Vihear province, northern Cambodia, *S. stercoralis* infection statistically increased with age, starting at 31.4% in children less than 6 years to a peak of at 51.2% in participants older than 50 years. Participants defecating in latrines were significantly less infected with *S. stercoralis* than those who did not use latrine (OR: 0.5; 95% CI: 0.4 - 0.8; $P<0.001$). *S. stercoralis* infection exhibited almost no tendency to spatial clustering in this province. Infection risk significantly decreased with increasing rainfall and soil organic carbon content and to increase in lands occupied by rice fields. In Takeo province, southern Cambodia, *S. stercoralis* infection prevalence reached 14.5% in children under or equal to 5 years and 28.0% in participants aged between 56 and 60 years. Participants who reported having a latrine were statistically less infected with *S. stercoralis* infection than those who did not possess latrine at home (OR: 0.7; 95% CI: 04 - 0.8; P: 0.003). Muscle pain and urticaria were significantly associated with *S. stercoralis* infection.

A two-year cohort study among 302 schoolchildren revealed a prevalence rate of

24.2% and 22.5% at baseline (2009) and follow-up (2011), respectively. Almost one-third (31.5%) of 73 treated *S. stercoralis* cases at baseline were re-infected at follow-up. But, almost 70% of children infected at baseline and treated remained free of re-infection for the period of two years. Children reported having shoes and defecating in toilet were statistically less infected with *S. stercoralis* than those who did not possess shoes (OR: 0.3; 95% CI: 0.1 – 0.5; *P*: 0.031) and use latrine (OR: 0.4; 95% CI: 0.2 – 0.9; *P*<0.001) at follow-up. None of the reported clinical symptom was significantly associated with *S. stercoralis* infection at follow-up.

Clinical symptoms of 21 *S. stercoralis* patients with high intensity infection (more than 250 larvae in Baermann test) from Preah Vihear province were documented in 2010. The median age of the patients was 11 years (range: 5 - 67); 23.8% were females. Eleven patients (52.4%) were younger than 16 years. Out of 21 patients, 20 (95.2%), 18 (85.7%) and 14 (66.7%) reported frequent abdominal pain, diarrhea and periods of sensation of itching, respectively, during the previous six months. Five patients (23.8%) reported having experienced urticaria the week preceding the examination. One patient suffered from extended urticaria. Three weeks after ivermectin treatment (200µg/kg BW, single oral dose), most symptoms (diarrhea, abdominal pain and urticaria) almost entirely resolved.

Conclusions: *S. stercoralis* infection is highly prevalent in rural communities of Cambodia where appropriate diagnosis and treatment do not exist. The re-infection rate of *S. stercoralis* among schoolchildren after two years of ivermectin treatment is considerable, but more than two-third remains free of infection for at least 2 years. Preschool- and school-aged children are highly affected. Personal hygiene and sanitation including wearing shoes, possession and use of latrines, are significant predictors of *S. stercoralis* infection. Gastrointestinal and cutaneous symptoms are associated with *S. stercoralis* infection and resolve almost entirely after ivermectin treatment. Thus, *S. stercoralis* infection should no longer be neglected in Cambodia and elsewhere in tropical resource poor countries. Access to adequate diagnosis and treatment of *S. stercoralis* infection is an urgent need in Cambodia.

SUMMARY IN KHMER

សារចាន: ដង្កូវព្រូនអង្គ្គីលីស "*Strongyloides stercoralis*" កើតមាននៅក្នុងតំបន់ដែលមានអនាម័យមិនសូវល្អ និងអាកាសធាតុក្តៅហើយសើម។ ប្រទេសត្រូពិកជាង៧០ នៅក្នុងតំបន់អាស៊ីអាគ្នេយ៍ អនុ-តំបន់អាហ្វ្រិកសាហារ៉ា ឥណ្ឌាខាងលិច និងអាមេរិកឡាទីន ត្រូវបានគេចាត់ទុកថាជាតំបន់ដែលមានការចំលងខ្ពស់។ ទោះជាយ៉ាងណាក៏ដោយ ក៏គេឃើញដង្កូវព្រូនអង្គ្គីលីសកើតមាននៅក្នុងអនុតំបន់ត្រូពិច និងនៅប្រទេសត្រជាក់មួយចំនួនរួមមាន ប្រទេសអូស្ត្រាលី,ជប៉ុន,កាណាដា,សហរដ្ឋអាមេរិច និងអឺរ៉ុប។ អត្រាប្រេវ៉ាឡង់សកលរបស់ដង្កូវព្រូនអង្គ្គីលីសមានភាពវិសភាគ។ គេជឿថាមានមនុស្សទូទាំងពិភពលោកប្រហែលពី ៣០ ទៅ១០០លាននាក់ បានឆ្លងដង្កូវព្រូនអង្គ្គីលីសនេះ។ ប៉ុន្តែចំនួនអ្នកកើតជំងឺព្រូនអង្គ្គីលីស និងទំហំបន្ទុកជាសកលនៃជំងឺនេះពិតប្រាកដ នៅមិនទាន់បានដឹងច្បាស់នៅឡើយទេ ហើយនេះក៏ប្រហែលជាមូលហេតុដែលនាំអោយ ការប៉ាន់ប្រមានពីអ្នកកើតជំងឺនេះនាពេលបច្ចុប្បន្ន មានភាពពុំសូវសុក្រិត ជាពិសេសនៅក្នុងតំបន់ជាច្រើននៃបណ្តាប្រទេសត្រូពិកដែលក្រីក្រ។ កត្តាសំខាន់ដែលនាំអោយមានការប៉ាន់ប្រមាណពុំសូវសុក្រិតនេះរួមមាន ឧបករណ៍ធ្វើរោគវិនិច្ឆ័យ ដែលប្រើប្រាស់បច្ចុប្បន្នមានភាពពុំសូវសុក្រិតក្នុងការធ្វើរោគវិនិច្ឆ័យ និងកង្វះខាតការសិក្សាស្រាវជ្រាវឯកទេសលើជំងឺនេះ។ លើសពីនេះទៅទៀតមានទិដ្ឋភាពអេពីដេមីសាស្ត្ររបស់ជំងឺនេះជាច្រើន ដែលគេមិនសូវបានយល់ដឹង ឬសឹងតែមិនយល់ដឹង។ គេមិនបានដឹងបានលំអិតសោះថានៅកន្លែងដែលមានការចំលងជំងឺដង្កូវព្រូនអង្គ្គីលីសនេះតើមានអត្រាចំលងជំងឺនិងអាំងតង់ស៊ីតេដែលរំពឹងថានឹងអាចមានជាធម្មតា ក្នុងតំបន់និងប្រជាជនដែលរស់នៅផ្សេងគ្នានោះប៉ុណ្ណា ហើយថាតើនៅពេលណាដែលបុគ្គលម្នាក់ បានឆ្លងជំងឺជាលើកដំបូង និងថាតើការឆ្លងជំងឺឡើងវិញ មានរយៈពេលយូរប៉ុន្មានដែរ បន្ទាប់ពីបានទទួលការព្យាបាលដែលមានប្រសិទ្ធ-ភាពនោះ។ យើងមិនដឹងពីព័ត៌មានអេពីដេមីសាស្ត្ររបស់ជំងឺព្រូនអង្គ្គីលីនេះដូចជា អត្រាប្រេវ៉ាឡង់ទ្រង់ជាទ្រាយធំ, ការឆ្លងជំងឺឡើងវិញ, កត្តាហានិភ័យ, ទិដ្ឋភាពរោគសញ្ញាគ្លីនិក និង ប្រសិទ្ធភាពនៃការព្យា-បាលនៅប្រទេសកម្ពុជា ក៏ដូចនៅប្រទេសជាច្រើនទៀត នៃតំបន់អាស៊ីអាគ្នេយ៍ ឡើយ។

គោលបំណង: និក្ខេបបទបណ្ឌិតនេះ មានគោលដៅស្វែងយល់ពី ទំហំជំងឺដង្កូវព្រូនអង្គ្គីលីសនៅក្នុងប្រទេសកម្ពុជា តាមរយៈគោលបំណងសំខាន់ៗចំនួនបួន រួមមាន: (១) ប៉ាន់ប្រមានពីជំងឺដង្កូវព្រូនអង្គ្គីលីសនិងកត្តាហានិភ័យនានា ព្រមទាំងកំណត់ពីប្រសិទ្ធិភាពរបស់ឧបករណ៍ធ្វើរោគវិនិច្ឆ័យ និងកំណត់ពីប្រសិទ្ធភាពនៃការព្យាបាលក្នុងចំណោមសិស្សសាលាបឋមសិក្សា, (២) កំណត់ពីអត្រាប្រេវ៉ាឡង់ជាទ្រង់ទ្រាយធំ និងកត្តាហានិភ័យនានា ក្នុងខេត្ត២ ដែលមានស្ថានភាពសេដ្ឋកិច្ចសង្គម និងអេកូឡូស៊ីខុសគ្នា,

(៣)កំណត់ពីអត្រាការឆ្លងជំងឺឡើងវិញ ក្នុងចំណោមសិស្សសាលាបឋមសិក្សា និង (៤) កត់ត្រាទុកជាឯកសារពីទិដ្ឋភាពរោគសញ្ញាគ្លីនិកនៃអ្នកជំងឺដែលមានអាំងតង់ស៊ីតេដង្កូវព្រូនអង្គីលីសខ្ពស់ ដែលរស់នៅតាមសហគមន៍។

វិធីសាស្ត្រ: ការសិក្សាស្រាវជ្រាវត្រូវបានអនុវត្ត ពីឆ្នាំ២០០៩ ដល់ឆ្នាំ២០១១ នៅតាមសាលារៀន និងនៅតាមសហគមន៍។ សាលារៀនបឋមសិក្សាចំនួន៤ និងភូមិចំនួន ១២០ដែលស្ថិតនៅក្នុងខេត្តចំនួន ៣ នៃប្រទេសកម្ពុជារួមមាន ខេត្តកណ្តាល ខេត្តព្រះវិហារ និងខេត្តតាកែវ ត្រូវបានជ្រើសរើសយកមកធ្វើការសិក្សា។ បន្ទាប់ពីទទួលបានការយល់ព្រមជាលាយលក្ខណ៍អក្សរពីអ្នកចូលរួមរួចហើយ ក្រុមសិក្សា-ស្រាវជ្រាវបានសួរទអ្នកចូលរួមដោយប្រើកំរងសំណួរសំរាប់អ្នកចូលរួម ពីទិន្នន័យដែលពាក់ព័ន្ធនឹងប្រជា-សាស្ត្រ, កត្តាហានិភ័យ ការយល់ដឹងពីជំងឺ និងអាកប្បកិរិយាដែលពាក់ព័ន្ធនឹងជំងឺ។ មេគ្រួសារត្រូវបានសំភាសន៍តាមរយៈកំរងសំណួរសំរាប់មេគ្រួសារ ពីស្ថានភាពសេដ្ឋកិច្ចសង្គមនៃគ្រួសារដូចជា ប្រភេទផ្ទះ សំភារៈក្នុងផ្ទះ បង្គន់អនាម័យ និងសត្វចិញ្ចឹមជាដើម។ បន្ទាប់ពីការសំភាសន៍រួច, អ្នកចូលរួម ទទួលបានប្រអប់ប្លាស្ទិចដែលមានបិទស្លាក (លេខសម្គាល់ឈ្មោះ, ភេទ, អាយុ និងកាលបរិច្ឆេទ) ដើម្បី ប្រមូលសំណាកលាមក។ ក្នុងករណីដែលយើងត្រូវការសំណាកលាមកច្រើនដង ប្រអប់ប្លាស្ទិចថ្មីមួយផ្សេងទៀតត្រូវបានប្រគល់ជូននៅពេលដែលយើងបានប្រមូលសំណាកលាមកទី១ ឬទី២។ សំណាកលាមកត្រូបានវិភាគដោយឧបករណ៍ធ្វើរោគវិនិច្ឆ័យ "Baermann" និង "Koga-agar plate (KAP) culture ដើម្បីវិនិច្ឆ័យរោគដង្កូវព្រូនអង្គីលីស និងឧបករណ៍ធ្វើរោគវិនិច្ឆ័យ "Kato-Katz"សម្រាប់វិនិច្ឆ័យរោគព្រូនពោះវៀនដទៃទៀត។

ការសិក្សាស្រាវជ្រាវ២ បានធ្វើឡើងនៅក្នុងសាលាបឋមសិក្សាចំនួន៤ ដែលស្ថិតនៅក្នុងខេត្តកណ្តាល។ នៅឆ្នាំ២០០៩ ការសិក្សាបែប cross-sectional study ត្រូវបានធ្វើឡើងក្នុងចំណោមសិស្សសាលារៀនបឋមសិក្សាចំនួន ៤៥៨នាក់ ដោយពិនិត្យសំណាកលាមកចំនួនបីក្នុងកុមារម្នាក់ ដើម្បីវាយតម្លៃពី កត្តាហានិភ័យ, ប្រសិទ្ធិភាពនៃឧបករណ៍ធ្វើរោគវិនិច្ឆ័យ និងប្រសិទ្ធភាពនៃការព្យាបាលក្រោយពីបានលេបថ្នាំ ivermectin (១00មីក្រូក្រាម/គីឡូក្រាម/ថ្ងៃ, រយៈពេល២ថ្ងៃ) បាន៣សប្តាហ៍មក។ ការសិក្សាបែបតាមដាន (cohort study) រយៈពេល២ឆ្នាំ ត្រូវបានធ្វើឡើងក្នុងចំណោមសិស្សចំនួន ៣០២នាក់ ពីឆ្នាំ២០០៩ ដល់ ឆ្នាំ២០១១ ដោយពិនិត្យសំណាកលាមកចំនួន២ក្នុងកុមារម្នាក់ ដើម្បីកំណត់ពី ការចំលងមេរោគឡើងវិញ និងកត្តាហានិភ័យនៃជំងឺដង្កូវព្រូនអង្គីលីស។

ការសិក្សាបែប cross-sectional study ទំហំធំចំនួន២ ត្រូវបានធ្វើឡើងក្នុងសហគមន៍ នាឆ្នាំ២០១០ និងឆ្នាំ២០១១ ដើម្បីវាយតម្លៃពី អត្រាប្រេវ៉ាឡង់ និងកត្តាហានិភ័យនៃជំងឺដង្កូវព្រូនអង្គ្លីលីសនៅក្នុងខេត្ត ២ (អ្នកចូលរួមចំនួន ២៣៩៦នាក់ មកពីភូមិចំនួន ៦០ ស្ថិតនៅខេត្តព្រះវិហារ ដោយវិភាគសំណាក លាមកចំនួនពី២ក្នុងម្នាក់ និងអ្នកចូលរួមចំនួន ២៨៦១នាក់ មកពីភូមិចំនួន ៦០ ស្ថិតនៅខេត្តតាកែវ ដោយវិភាគសំណាកលាមកចំនួន១ក្នុងម្នាក់)។ Bayesian kriging ត្រូវបានប្រើដើម្បីទស្សន៍ទាយពី ហានិភ័ក្នុងការកើតជំងឺ នៅភូមិដែលមិនបានអង្កេតស្ថិតនៅខេត្តព្រះវិហារ។ ការសិក្សាលើករណីជំងឺ ដោយដកស្រង់ចេញពីការសិក្សានៅក្នុងខេត្តព្រះវិហារ នាឆ្នាំ២០១០ ត្រូវបានធ្វើឡើង ដើម្បីចងក្រងទុក ជាឯកសារពីលក្ខណៈគ្លីនិករបស់ករណីជំងឺដង្កូវព្រូនអង្គ្លីលីសចំនួន ២១ករណី ដែលមានដង្កូវព្រូនអង្គ្លី លីសច្រើននៅក្នុងសំណាកលាមក ដែលត្រូវបានរកឃើញដោយឧបករណ៍ធ្វើរោគវិនិច្ឆ័យ Baermann។

លទ្ធផល: ការអង្កេតតាមសាលារៀនបែប cross-sectional study ក្នុងចំណោមសិស្សសាលាបឋម សិក្សា ៤៥៨នាក់ នាឆ្នាំ២០០៩ បានរកឃើញថាមានកុមារសិស្សសាលា ២៤,៤% បានកើតជំងឺដង្កូវ ព្រូនអង្គ្លីលីស។ អត្រាប្រេវ៉ាឡង់នៃជំងឺដង្កូវព្រូនអង្គ្លីលីស បានកើនឡើងគួរឱ្យកត់សម្គាល់ (ពី ១៨,៦% ទៅ ២៤,៤%) នៅពេលដែលសំណាកលាមក៣ត្រូវបានពិនិត្យ។ វេទយិតភាពនៃឧបករណ៍ធ្វើរោគ វិនិច្ឆ័យ KAP culture គឺ ៨៨,៤% និង Baermann គឺ ៧៥,០%។ រោគសញ្ញាគ្លីនិកដែលពាក់ពន្ធ័ខ្លាំង ជាមួយនឹងជំងឺដង្កូវព្រូនអង្គ្លីលីសគឺ រមាស់ស្បែក និងរាគរូស។ កុមារដែលបានប្រាប់ថា បានបន្ទោរបង់ នៅក្នុងបង្គន់បានឆ្លងដង្កូវព្រូនអង្គ្លីលីសតិចជាងអ្នកដែលមិនបានប្រើបង្គន់ (OR:0,៤; ៩៥%CI:0,២ -0,៦; P<000១)។ ជំងឺដង្កូវព្រូនអង្គ្លីលីសអាចកាត់បន្ថយបានជិតបីភាគបួន បើកុមារទាំងនោះមាន អនាម័យត្រឹមត្រូវ (PAR:0,៧; ៩៥%CI:0,៥-0,៩)។ ថ្នាំIvermectin (១00មីក្រូក្រាម ក្នុង១គីឡូ ក្រាម ក្នុង១ថ្ងៃ រយៈពេល២ថ្ងៃ)មានប្រសិទ្ធិភាពខ្ពស់ក្នុងការព្យាបាលជំងឺដង្កូវព្រូនអង្គ្លីលីស ដោយវា មានប្រសិទ្ធិភាពដល់ ៩៨,៣% បន្ទាប់ពីការព្យាបាលបាន៣សប្តាហ៍។

អត្រាប្រេវ៉ាឡង់នៃជំងឺដង្កូវព្រូនអង្គ្លីលីសក្នុងចំណោមប្រជាជនទូទៅដែលរស់នៅក្នុងខេត្តព្រះវិហារ គឺ ៤៤,៧% និងអត្រាប្រេវ៉ាឡង់នៃជំងឺដង្កូវព្រូនអង្គ្លីលីសក្នុងខេត្តតាកែវគឺ ២១,0%។ នៅក្នុងខេត្តទាំង២ នេះ បុរសគ្រប់ក្រុមអាយុទាំងអស់បានកើតជំងឺដង្កូវព្រូនអង្គ្លីលីសច្រើនជាងស្ត្រី (P<000១)។ នៅក្នុង ខេត្តព្រះវិហារដែលស្ថិតនៅភាគខាងជើងប្រទេសកម្ពុជា, អត្រាប្រេវ៉ាឡង់នៃជំងឺដង្កូវព្រូនអង្គ្លីលីស បាន កើនឡើងគួរឱ្យកត់សម្គាល់ ដោយចាប់ផ្តើមពី ៣១,៤% នៅក្នុងចំណោមកុមារតិចជាង៦ឆ្នាំ រហូតដល់ កំរិតកំពូល ៥១,២% នៅក្នុងចំណោមអ្នកចូលរួមដែលមានអាយុខ្ពស់ជាង៥០ឆ្នាំ។ អ្នកចូលរួមដែលបាន

ប្រាប់ថាបានបន្ទោរបង់នៅក្នុងបង្គន់ បានឆ្លងមេរោគដង្កូវព្រូនអង្គ្គីលីសតិចជាងអ្នកដែលមិនបានបន្ទោរបង់នៅក្នុងបង្គន់ (OR:0,៥; 95%CI:0,៤-0,៨; P<000១)។ នៅក្នុងខេត្តព្រះវិហារនេះការទស្សន៍ទាយពីជំងឺដង្កូវព្រូនអង្គ្គីលីសតាមរយៈព័ត៌មានពីអវកាសបានបង្ហាញថាអត្រាប្រេវ៉ាឡង់ស្ទើរតែមិនមានការប្រែប្រួលនៅភូមិដែលមិនបានសិក្សា។ ហានិភ័យនៃការឆ្លងជំងឺបានថយចុះ នៅពេលដែលភ្លៀង និងដីមានជាតិកាបោនកើនឡើង ហើយហានិភ័យនៃការឆ្លងជំងឺបានកើនឡើងនៅពេលដែលដីជាដីស្រែ។ នៅក្នុងខេត្តតាកែវដែលស្ថិតនៅភាគខាងត្បូងប្រទេសកម្ពុជា, អត្រាប្រេវ៉ាឡង់នៃជំងឺដង្កូវព្រូនអង្គ្គីលីសមានដល់ទៅ១៤,៥% នៅក្នុងចំណោមកុមារអាយុចាប់ពី៥ឆ្នាំឡើងទៅ និងអត្រាប្រេវ៉ាឡង់ឡើងដល់ ២៨,0% នៅក្នុងចំណោមអ្នកចូលរួមដែលមានអាយុចន្លោះពី៥៦ ទៅ ៦0ឆ្នាំ។ អ្នកចូលរួមដែលបានប្រាប់ថាមានបង្គន់អនាម័យនៅផ្ទះ បានឆ្លងមេរោគដង្កូវព្រូនអង្គ្គីលីសតិចជាងអ្នកដែលមិនមានបង្គន់អនាម័យ (OR:0,៧; ៩៥%CI:0,៤-0,៨;P:0,00៣)។ ការឈឺសាច់ដុំ និងកន្ទាលត្រអាក ជារោគ-សញ្ញាក្លីនិកដែលពាក់ពន្ធ័ខ្លាំងជាមួយនឹងជំងឺដង្កូវព្រូនអង្គ្គីលីស។

ការសិក្សាបែបតាមដាន (cohort study) រយៈពេល២ឆ្នាំក្នុងចំណោមសិស្សសាលា ៣0២នាក់ បានរកឃើញថា អត្រាប្រេវ៉ាឡង់នៃជំងឺដង្កូវព្រូនអង្គ្គីលីសនៅឆ្នាំដំបូង (ឆ្នាំ២00៩)មាន ២៤,២% និងអត្រាប្រេវ៉ាឡង់នៃជំងឺដង្កូវព្រូនអង្គ្គីលីសនៅឆ្នាំតាមដាន (ឆ្នាំ២0១១) មាន ២២,៥%។ ប្រហែលមួយភាគបី (៣១,៥%) នៃកុមារដែលមានជំងឺដង្កូវព្រូនអង្គ្គីលីសនៅឆ្នាំដំបូងចំនួន ៧៣នាក់ បានឆ្លងជំងឺឡើងវិញនៅឆ្នាំតាមដាន។ ប៉ុន្តែ ក្នុងកំឡុងរយៈពេលពីរឆ្នាំមកនេះ ស្ទើរតែ ៧0% នៃកុមារដែលមានជំងឺដង្កូវព្រូនអង្គ្គីលីស និងបានព្យាបាលនៅឆ្នាំដំបូងពុំបានឆ្លងជំងឺឡើងវិញទេ។ នៅឆ្នាំតាមដាន កុមារដែលប្រាប់ថាមានស្បែកជើងនិងបន្ទោរបង់នៅក្នុងបង្គន់ បានឆ្លងជំងឺតិចជាងអ្នកដែលមិនមានស្បែកជើង (OR:0,៣; ៩៥%CI:0,១-0,៥; P:0,0៣១) និងមិនប្រើបង្គន់ (OR:0,៤; ៩៥%CI:0,២-0,៩; P<0,000១)។ គ្មានរោគសញ្ញាក្លីនិកណាមួយពាក់ពន្ធ័នឹងជំងឺដង្កូវព្រូនអង្គ្គីលីសត្រូវបានរកឃើញទេនៅឆ្នាំសិក្សាតាមដាន។

រោគសញ្ញាគ្លីនិកនៃអ្នកជំងឺដង្កូវព្រូនអង្គ្គីលីស ២១នាក់ដែលមានអាំងតង់ស៊ីតេដង្កូវព្រូនច្រើន (ចាប់ពី ២៥0 ដង្កូវព្រូន នៅក្នុងBaermann) នៅខេត្តព្រះវិហារ ត្រូវបានចងក្រងជាឯកសារក្នុងឆ្នាំ២0១0។ អ្នកជំងឺមានអាយុជាមធ្យម ១១ឆ្នាំ (ចន្លោះ:៥ - ៦៧) ២៣,៨% នៃអ្នកជំងឺជាស្ត្រី។អ្នកជំងឺ១១នាក់ (៥២,៤%) មានអាយុតិចជាង១៦ឆ្នាំ។ ក្នុងចំណោមអ្នកជំងឺ២១នាក់នេះ, ២0នាក់ (៩៥,២%) បានប្រាប់ថា ឈឺពោះ, ១៨នាក់ (៨៥,៧%) រាគរូស និង ១៤នាក់ (៦៦,៧%) រមាស់ស្បែក នៅក្នុងអំឡុង

ពេល៦ខែចុងក្រោយនេះ។ មានអ្នកជំងឺ៥នាក់ (២៣,៨%) បានប្រាប់ថាធ្លាប់មានកន្ទាលត្រអាកក្នុងកំឡុង១សប្តាហ៍មុន។ អ្នកជំងឺម្នាក់មានកន្ទាលត្រអាកយ៉ាងធ្ងន់ធ្ងរ។ បន្ទាប់ពីបានលេបថ្នាំ ivermectin (២០០មីក្រូក្រាម/គីឡូក្រាម, លេបតែម្តង) បាន៣សប្តាហ៍មក រោគសញ្ញាដូចជា រាគ, ឈឺពោះ និង កន្ទាលត្រអាក ត្រូវបានបាត់ស្ទើរតែទាំងស្រុង។

សន្និដ្ឋាន: អត្រាប្រេវ៉ាឡង់នៃជំងឺដង្កូវព្រូនអង្គ្លីលីសមានខ្ពស់នៅតាមសហគមន៍ជនបទនៃប្រទេសកម្ពុជាដែលការធ្វើរោគវិនិច្ឆ័យ និងការព្យាបាលនៃជំងឺនេះមិនទាន់មានលក្ខណៈសមស្របនៅឡើយ។ បន្ទាប់ពីបានព្យាបាលដោយថ្នាំ ivermectin បានរយៈពេល២ឆ្នាំ អត្រាចំលងជំងឺដង្កូវព្រូនអង្គ្លីលីសឡើងវិញនៅក្នុងចំណោមសិស្សសាលាមានភាពច្រើនសន្ធឹកសន្ធាប់ ក៏ប៉ុន្តែ ជាងពីរភាគបីនៃកុមារទាំងនោះមិនបានឆ្លងជំងឺដង្កូវព្រូនអង្គ្លីលីសទេ ក្រោយពីបានព្យាបាលបាន ២ឆ្នាំមក។ អ្នកដែលរងផលប៉ះ-ពាល់ខ្លាំងជាងគេគឺ កុមារអាយុមត្តេយ្យសាលា និងកុមារដែលមានអាយុចូលរៀនបឋមសិក្សា។ អនាម័យផ្ទាល់ខ្លួន និងសុខមាលភាព រួមមាន ការពាក់ស្បែកជើង, មានបង្គន់ និងការប្រើប្រាស់បង្គន់គឺសូចណាករណ៍ដ៏សំខាន់ក្នុងការប៉ាន់ប្រមានពីការកើតមានជំងឺដង្កូវព្រូនអង្គ្លីលីស។ រោគសញ្ញាក្រពះពោះវៀន និងសើរស្បែក ជាសញ្ញាក្លីនិកដែលពាក់ពន្ធ័នឹងជំងឺដង្កូវព្រូនអង្គ្លីលីស ហើយរោគសញ្ញាទាំងនេះនឹងបាត់ទៅវិញស្ទើរតែទាំងស្រុងបន្ទាប់ពីបានព្យាបាលដោយថ្នាំ ivermectin។ ដូច្នេះជំងឺដង្កូវព្រូនអង្គ្លីលីសមិនគួរមិនអើពើ ឬមើលរំលងនៅក្នុងប្រទេសកម្ពុជា និងនៅក្នុងប្រទេសត្រូពិកក្រីក្រដ៏ទៃទៀតឡើយ។ ការទទួលបានការធ្វើរោគវិនិច្ឆ័យ និងការព្យាបាលនៃជំងឺដង្កូវព្រូនអង្គ្លីលីសត្រឹមត្រូវ ជាតម្រូវការបន្ទាន់ក្នុងប្រទេសកម្ពុជា។

LIST OF ABBREVIATIONS

AIDS	Acquired Immuno Deficiency Syndrome
CNM	National Center for Parasitology, Entomology and Malaria control, Ministry of Health, Cambodia
DALYs	Disability-Adjusted Life Years
DHS	Demographic Health Survey
EKBB	Ethik Kommission Beider Basel
ELISA	Enzyme-Linked Immuno Sorbent Assay
FECT	Formalin-Ether Concentration Technique
GPAT	Gelatin Particle Agglutination Test
HIV	Human Immunodeficiency Virus
HTLV-1	Human T cell Lymphotropic Virus type 1
IFAT	Indirect immuno-Fluorescence Antibody Techniques
KAP	Koga-Agar Plate
LIPS	Luciferase Immuno Precipitation System
NECHR	National Ethics Committee for Health Research, Cambodia
OR	Odds Ratio
PAR	Population Attributable Risk
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
SAF	Sodium Acetate-Acetic acid-Formalin
SES	Socio-Economic Status
STH	Soil-Transmitted Helminth
Swiss TPH	Swiss Tropical and Public Health Institute, Basel, Switzerland
WHO	World Health Organization
YLDs	Years Lost from Disability

1. INTRODUCTION

This PhD thesis addresses *Strongyloides stercoralis* (threadworm), the most neglected soil-transmitted nematode. Detailed biology, life cycle, epidemiology, risk factors and mode of transmission, classification and definition of syndromes, clinical aspects, diagnostic techniques, and treatment and management of *S. stercoralis* will be shortly described below.

1.1. Biology and life cycle

At least two of 52 species of genus *Strongyloides* are known to infect humans: *S. fuelleborni* and *S. stercoralis*. *S. fuelleborni* is found sporadically in Africa and Papua New Guinea and may cause limited infections in humans. *S. fuelleborni* eggs can be found in stools and although the issue of autoinfection is still not resolved, it probably does not occur in humans with egg-passing infections. The most common and clinically important pathogenic species in humans is *S. stercoralis* [Montes *et al.*, 2010; Olsen *et al.*, 2009; Vadlamudi *et al.*, 2006].

It took nearly half century after the discovery of *S. stercoralis* to elucidate the complete life cycle of the parasite. The life cycle of *S. stercoralis* is more complex than that of most nematodes, with its alternation between free-living and parasitic cycles and its potential for autoinfection and multiplication within the host. *S. stercoralis* is unusual among parasites that infect humans because it can complete an entire cycle of replication within the human host and perpetuate infection for several years or whole life [Foreman *et al.*, 2006; Montes *et al.*, 2010; Siddiqui *et al.*, 2001].

Figure 1 is the picture of *S. stercoralis* larvae found in sediment of Koga agar plate culture. Figure 2 shows the complete life cycle of *S. stercoralis*: free-living and parasitic cycles. In the free-living cycle, the rhabditiform larvae (L1) passed in the stool (parasitic cycle) can either molt twice and become infective filariform larvae (direct development, L3) or molt four times and becomes the free living adult males and females that mate and produce eggs from which rhabditiform larvae hatch. The latter in turn can either develop into a new

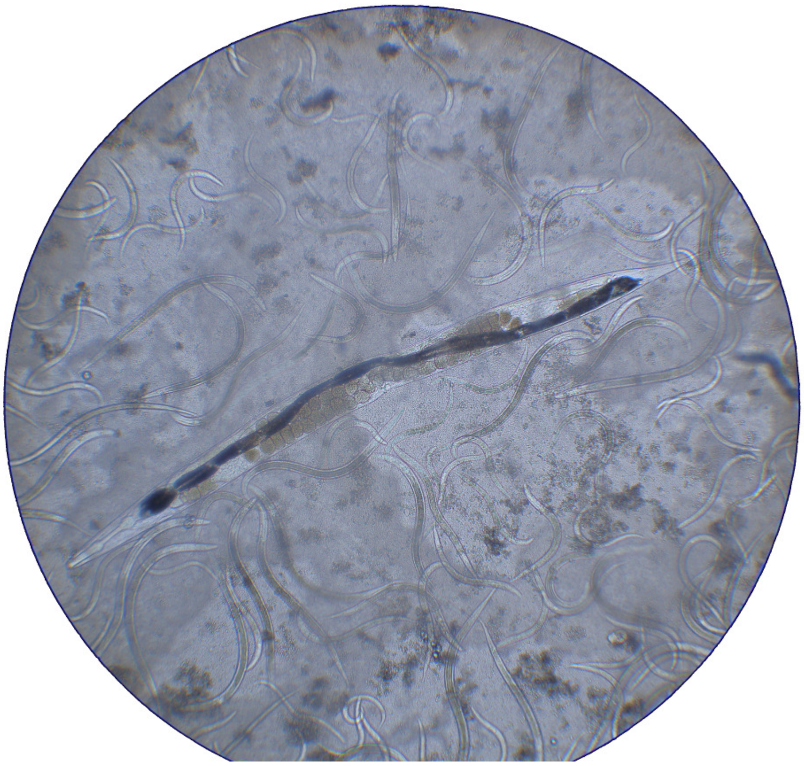

Figure 1: ***S. stercoralis*** **larvae from Koga-agar plate culture**

generation of free-living adults, or into infective filariform larvae. The filariform larvae penetrate the human host skin to initiate the parasitic cycle.

In the parasitic cycle, filariform larvae in contaminated soil penetrate the human skin, and are transported to the lungs where they penetrate the alveolar spaces; they are carried through the bronchial tree to the pharynx, are swallowed and then reach the small intestine. In the small intestine they molt twice and become adult female worms. The females live threaded in the epithelium of the small intestine and by parthenogenesis produce eggs, which yield rhabditiform larvae. The rhabditiform larvae can either be passed in the stool (free-living cycle), or can cause autoinfection.

In autoinfection, the rhabditiform larvae become infective filariform larvae, which can penetrate either the intestinal mucosa (internal autoinfection) or the skin of the perianal area (external autoinfection); in either case, the filariform larvae may follow the previously described route, being carried successively to the lungs, the bronchial tree, the pharynx, and the small intestine where they mature into adults; or they may disseminate widely in the body. To date, occurrence of autoinfection in humans with helminthic infections is recognized only in *S. stercoralis* and *Capillaria philippinensis* infections. In the case of *S. stercoralis*, autoinfection may explain the possibility of persistent infections for many years in persons who have not been in a disease-endemic area and of hyperinfections in immunodepressed individuals (www.dpd.cdc.gov/dpdx.htm).

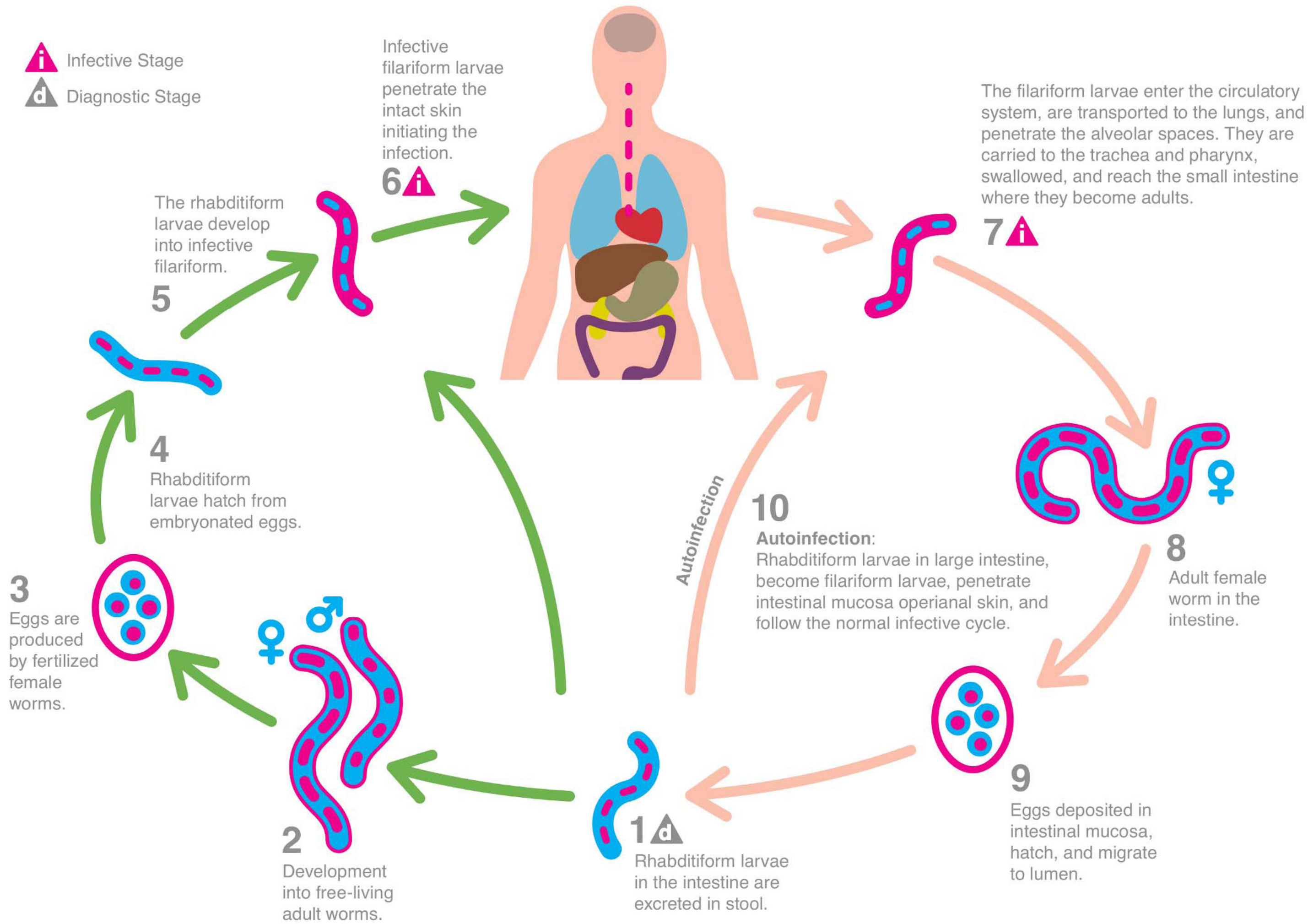

Figure 2: Life cycle of *Strongyloides stercoralis* [Schär *et al.*, 2013b]

1.2. Epidemiology

Infection with *S. stercoralis* was first described in 1876 in the stool of French colonial soldiers working in Vietnam who had severe diarrhea. The disease was known for many years as *Cochin-China Diarrhea* returning from Indochina in 1896. The first strongyloidiasis case was reported by *Fulleborn* in 1926 [Bavay, 1876; Grove, 1996; Normand, 1876; Siddiqui *et al.*, 2001; Vadlamudi *et al.*, 2006].

Many epidemiological aspects of strongyloidiasis are poorly understood or unknown. It is not known in detail where *S. stercoralis* is endemic, which prevalence rates and intensities can typically be expected in different settings and populations, the zoonotic aspects, geographical variations, e.g. in transmission and clinical presentation, host immunity and the risk factors for dissemination, and social, cultural, ecological and environmental aspects [Olsen *et al.*, 2009; Paula *et al.*, 2011].

The worldwide prevalence distribution of *S. stercoralis* is heterogeneous (Figure 3) [Paula *et al.*, 2011; Schär *et al.*, 2013b]. *S. stercoralis* is endemic in areas where sanitary conditions are poor and where the climate is warm and humid [Bannon *et al.*, 1995; Hall *et al.*, 1994]. More than 70 tropical countries are considered as high endemic areas, especially in South-East Asia, Sub-Saharan Africa, West Indies and Latin America. However, the parasite is also prevalent in several subtropical and temperate regions: Australia, Japan, Canada, United States and Europe [Boulware *et al.*, 2007; Foreman *et al.*, 2006; Genta, 1989; Liu *et al.*, 1993; Sampson *et al.*, 1987; Siddiqui *et al.*, 2001; Vadlamudi *et al.*, 2006]. It is believed that about 30 - 100 millions people worldwide are infected with *S. stercoralis* [Bethony *et al.*, 2006]. But the true number of infections and the global burden of strongyloidiasis remain underestimated in many areas of the resource poor countries due to the low sensitivity of the currently available and used diagnostic tools and the paucity of specialized survey. Most available information of strongyloidiasis in tropical resource poor countries originates from Brazil and Thailand. Therefore, strongyloidiasis is arguably considered the most neglected of the neglected tropical diseases [Marcos *et al.*, 2008; Montes *et al.*, 2010; Olsen *et al.*, 2009; Paula *et al.*, 2011].

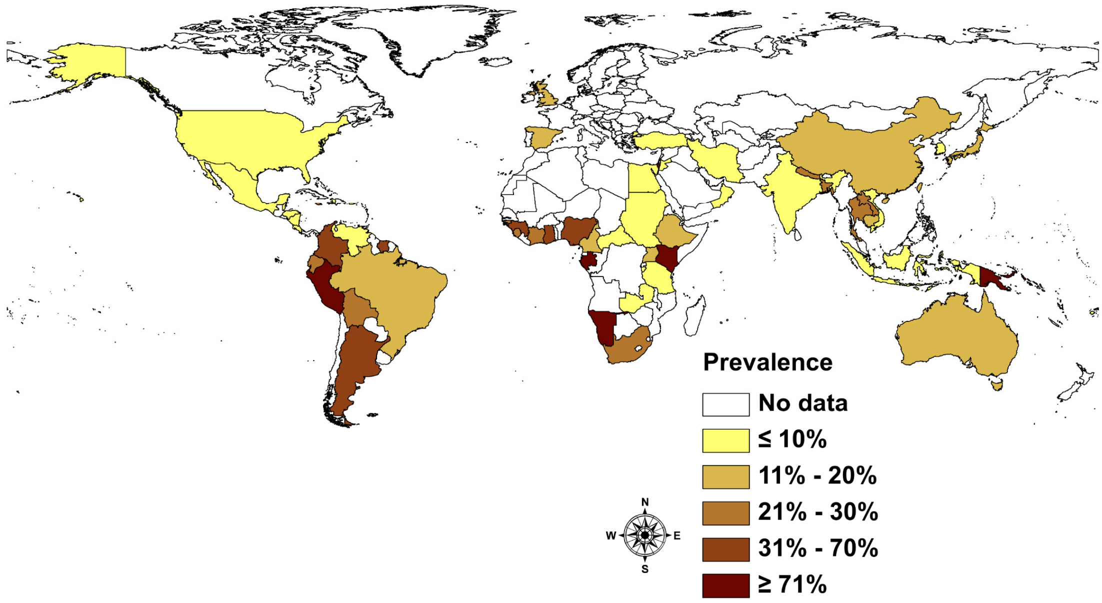

Figure 3: Prevalence of *S. stercoralis* infection by country based community-based studies [Schär *et al.*, 2013b]

1.3. Mode of transmission and risk groups

The transmission of *S. stercoralis* is through skin contact with soil contaminated with infective larvae [Getaneh *et al.*, 2010]. Individuals acquire the infection, usually at a young age through skin penetration and may remain infected into adulthood without further exposure to infected areas [Concha *et al.*, 2005]. The diseases transmission can occur related with occupations that increase contact with soil contaminated with human waste (such as people employed in farms and coal mines) [Vadlamudi *et al.*, 2006; Wagenvoort *et al.*, 1994]. *S. stercoralis* and hookworm infections are the only officially recognized occupational parasitic health hazard for miners in Germany [Olsen *et al.*, 2009]. Swimming or bathing in rivers and consuming non-potable water had not been proven to be a significant source of transmission of *S. stercoralis* [Marcos *et al.*, 2008].

Residents or travelers to Southern, Eastern, and Central Europe, islands of the Caribbean basins, Latin America, sub-Saharan Africa and Southeast Asia, and natives or residents of the Appalachian region in the United States are at risk for acquiring *S. stercoralis* [Concha *et al.*, 2005].

Low socioeconomic status, HIV/AIDS, and alcoholism have been associated with higher prevalence of *S. stercoralis* stool positive. Different prevalence among ethnic groups may simply reflect behavioral socioeconomic factors, but some have suggested that different skin types may be more or less resistant to larval penetration [Schär *et al.*, 2013b; Teixeira *et al.*, 2010; Vadlamudi *et al.*, 2006; Walzer *et al.*, 1982]. The high risk group includes the following: patients with altered cellular immunity, especially those receiving long-term steroid therapy, patients with lymphoma, kidney allograft recipients, travelers to endemic areas, and prisoners and other institutionalized people [Siddiqui *et al.*, 2001].

1.4. Definition of syndromes

Strongyloidiasis is categorized into 3 types: intestinal, hyperinfection and disseminated.

The term "**Intestinal strongyloidiasis**" has been used to define a strongyloidiasis infection with presence of the adult parasite in the intestinal tract. The ability to establish an autoinfection cycle may lead to chronic infection (carrier).

The term "**Hyper-infection**" has been used to denote an increase in parasite burden due to acceleration of autoinfection cycle without an accompanying spread of larvae outside the usual migration pattern. Severe gastrointestinal tract and pulmonary symptoms are commonly seen.

The term "**Disseminated strongyloidiasis**" has been used to describe a form of disease in systemic spread of invasive filariform larvae to sites outside their normal migration pattern with extensive invasion to virtually every organ. While dissemination implies coexisting hyperinfection, hyperinfection can occur without dissemination. However, these two forms are clinically difficult to distinguish [Concha *et al.*, 2005; Fardet *et al.*, 2007].

1.5. Clinical aspects

Little is known about the true clinical relevance of strongyloidiasis, the symptoms associated with different infection patterns, the association between *Strongyloides* and other infectious diseases. Clinical presentation of strongyloidiasis is extremely variable reflecting the complex life cycle of parasite. It also varies greatly from an immune-competent to immune-suppressed individual. Strongyloidiasis can manifest in a wide spectrum of clinical features ranging from asymptomatic infection, disease with mild initial symptoms, disease with chronic symptoms and acute exacerbation with hyper-infection or dissemination of larvae involving respiratory and gastrointestinal systems or multiple organ systems respectively. *Strongyloides* infection is classified into 3 levels: acute; chronic and severe [Cruz *et al.*, 2010; Ly *et al.*, 2003; Montes *et al.*, 2010; Olsen *et al.*, 2009; Vadlamudi *et al.*, 2006].

1.5.1. Acute clinical aspects

Acutely after infestation, most symptomatic patients present within 3 to 4 weeks. The initial symptoms happen soon after the entry of the infective filariform larvae into the human host from its extra-intestinal migration in the host. Though the acute initial manifestations are not well described, the following symptoms are noted in some human infections: serpiginous urticarial rash at the site of entry of the filariform larvae mostly in the legs, cough and tracheal irritation mimicking bronchitis from migration of the larvae through the lungs, abdominal cramping with bloating, watery diarrhea and sometimes constipation due to lodging of the larvae and maturation into adult females in the small intestine of the host. In fact, the most common complaint noted was abdominal bloating. As these initial manifestations are vague and mimic multiple other diseases, they are often misdiagnosed and treated symptomatically with the host still harboring the parasite leading to a chronic state of the disease [Agrawal *et al.*, 2009; Foreman *et al.*, 2006; Vadlamudi *et al.*, 2006].

1.5.2. Chronic clinical aspects

Chronic *S. stercoralis* infection usually results in few or no symptoms which can remain undetected for decades. During the asymptomatic stage, the only clinical finding could be eosinophilia. However, mild symptoms involving gastrointestinal, pulmonary and cutaneous systems can happen in chronic strongyloidiasis. Abdominal pain, the result of intestinal irritation and inflammation, is the most common complaint of gastrointestinal symptoms of chronic infection. Other chronic clinical presentations include nausea, intermittent vomiting, hematemesis, anorexia, diarrhea, constipation, weight loss, gastritis, protein-losing enteropathy, malabsorption and occasional bowel obstruction. Pulmonary symptoms are the next most frequent symptom due to migrating larvae resemble Loerffler's syndrome and typically include nonproductive cough, wheezing, dyspnea, asthma-like symptoms, fever, throat irritation and hemoptysis [Cruz *et al.*, 2010; Liu *et al.*, 2009; Ly *et al.*, 2003; Siddiqui *et al.*, 2001; Sridhara *et al.*, 2008; Vadlamudi *et al.*, 2006]. The main consequence of a chronic infection is the skin involvement, the so-called "**larva currens**", the pathognomonic cutaneous manifestations of strongyloidiasis, which is visualized by urticarial serpiginous eruptions. The serpiginous rash is caused by rapid (approximately 5-15 cm/h) subcutaneous moving of *S. stercoralis* larvae from anal area down the upper thighs. Lesions of larva currens are found on the buttocks, thighs and lower extremities, more so than the head and trunk. The larva currens are usually transient and last for a few hours up to few days, but could recur over weeks, months or years. The larva migration provokes an intensely itchy wheal along its tortuous tract, which is described as linear urticaria, pruritus, edema, inflammation and petechiae. Chronic urticaria has been observed as well-fixed wheals lasting 1 to 2 days and often found on the buttocks and waistline [Amer *et al.*, 1984; Iwamoto *et al.*, 1998].

1.5.3. Severe clinical aspects

In many cases, the development of clinical manifestations is attributed to a decrease in host resistance caused by a debilitating disease, malnutrition or immunosuppressive drugs. Symptomatic strongyloidiasis is then an often fatal infection for immune-compromised hosts, in particular for those receiving immunosuppressive therapy with corticosteroids [Fardet *et al.*, 2007]. In these patients, the immune system is no longer able to keep the larvae emerging from the auto-infective cycle under control, resulting in hyper-infection affecting the proper functioning of the gastrointestinal tract or the dissemination of larvae to multiple organs including the brain. Such massive infections may lead to life-threatening conditions with pneumonia, meningitis, septicaemia and intestinal obstruction. Immuno-suppression with Cyclosporine A is not known to have such devastating effects [Olsen *et al.*, 2009].

1.5.3.1. *Hyper-infection syndromes manifestations*

Although the exact mechanisms involved in the pathogenesis of the hyper-infection syndrome are unknown, it is believed that persons with impaired host immunity are at risk for hyper-infection. In an immune-competent host, the rate of ongoing autoinfection remains controlled, resulting in a low parasite burden. However, with impaired host immunity or delayed gastrointestinal transit time, the normal balance between the larvae being excreted into the stool and the maturation of these forms within the gastro-intestinal tract is altered, which results in increased rates of autoinfection, a higher parasite burden, and hyper-infection syndrome [Foreman *et al.*, 2006]. The hyper-infection syndrome happens from the enormous multiplication and migration of infective larvae especially in an immune-suppressed state. The manifestations of hyper-infection syndrome are divided, based on the system of origin, into intestinal and extra-intestinal disease mainly involving the respiratory tract [Vadlamudi *et al.*, 2006].

The intestinal features include severe cramping abdominal pain, indigestion, and intermittent or persistent watery diarrhea are the most frequent symptoms encountered in patients with severe intestinal strongyloidiasis. Other features include weight loss, nausea, vomiting, steatorrhea, protein-losing enteropathy and adynamic ileus. Small bowel obstruction, occasionally upper and lower gastro-intestinal bleeding, and colitis are features that may mimic Crohn disease or ulcerative colitis [Concha *et al.*, 2005; Foreman *et al.*, 2006; Vadlamudi *et al.*, 2006].

The lung is the most frequent extra-intestinal organ affected in the hyper-infection state with an incidence of 48% to 68%. The extra-intestinal features include mainly asthma-like symptoms such as cough and wheezing, and others such as chronic bronchitis, bronchopneumonia, pneumonia, lung abscess, acute respiratory distress syndrome and pulmonary hemorrhage with diffuse bilateral and lobular infiltrates on the chest X-ray. Pulmonary symptoms due to migrating larvae resemble Loerffler's syndrome. Rare conditions like eosinophilic pleural effusions and eosinophilic granulomatous enterocolitis have also been reported in strongyloidiasis [Concha *et al.*, 2005; Foreman *et al.*, 2006; Vadlamudi *et al.*, 2006].

1.5.3.2. *Disseminated manifestations*

The disseminated form can occur, characterized by the presence of worms in extra-intestinal and extra-pulmonary sites, when larvae load increases, leading to involvement of multiple organs thereby leading to various manifestations along with severe respiratory and gastrointestinal features. Even though, most cases of strongyloidiasis are asymptomatic or present with mild symptoms, fatal disseminated infection with involvement of multiple organ systems other than the respiratory and gastrointestinal systems as in hyper-infection syndrome could also occur especially in patients with immunosuppression from systemic steroids. The mortality from disseminated infection could be as high as 87%. The high mortality rate associated with hyper-infection syndrome and disseminated disease is frequently due to secondary bacterial infections [Olsen *et al.*, 2009; Vadlamudi *et al.*, 2006].

The cutaneous manifestations that could occur from dissemination include widespread petechiae and purpura. Occasionally this may also present as a pruritic, erythematous, morbiliform eruption, or as an intensely itching prurigo. One of the most important and potentially fatal complications that can occur is gram negative bacteremia mainly from pathogens such as *Streptococcus bovis, Escherichia coli, Streptococcus fecalis, Klebsiella pneumoniae, Psedomonas aeruginosa, Alicaligenes faecalis or Enterobacter* sp. as they become blood borne when the larvae penetrate the intestine. Involvement of the central nervous system may lead to headache, altered mental state, seizures and rarely coma. Gram negative bacterial meningitis has also been frequently reported, especially in association immunosuppression. Chronic infection and malnutrition also predispose to systemic strongyloidiasis. The association of diarrhea and abdominal pain, pulmonary infiltrates, or skin lesions with peripheral eosinophilia suggests very strongly the possibility of infection by *S. stercoralis*. Eosinophilia is found in 50% to 80% of infected, frequently asymptomatic patients. In contrast, a low eosinophil count is common in patients with severe strongyloidiasis and is considered a poor prognostic marker [Concha *et al.*, 2005; Vadlamudi *et al.*, 2006].

1.5.4. Other clinical aspects

Multiple case reports indicate a potential increase in the frequency of fatal hyper-infection or disseminated infection with corticosteroid therapy in patients with asymptomatic or mild strongyloidiasis [Fardet *et al.*, 2007; Vadlamudi *et al.*, 2006]. *S. Stercoralis* hyper-infection has been described in AIDS patients as part of the immune reconstitution syndrome after starting highly active antiretroviral therapy. HIV-infected pregnant women, *S. stercoralis* infection was associated with a higher risk of low birth weight, which is the single most important factor for high infant mortality. In contrast, human T cell lymphotropic virus type 1

(HTLV-1) infection is associated with a higher *S. stercoralis* prevalence as well as with an increased incidence of hyper-infection. It is apparent that strongyloidiasis is not an important opportunistic infection associated with AIDS; the infection should still be searched for and promptly treated in HIV-infected patients who have a history of residence in and/or travel to endemic areas. On the other hand, strongyloidiasis appears to be relevant opportunistic infection in patients infected with HTLV-1 [Fardet *et al.*, 2007; Olsen *et al.*, 2009; Siddiqui *et al.*, 2001].

1.6. Diagnostic techniques

Today, there is no gold standard for diagnosing *S. stercoralis*. The diagnosis is often delayed or overlooked as patients present with non-specific gastro-intestinal complaints [Agrawal *et al.*, 2009]. Patients with chronic strongyloidiasis usually have a low parasite load and irregular larval output making it exceedingly difficult to diagnose [Siddiqui *et al.*, 2001]. A number of diagnostic methods have been used to detect *S. stercoralis*, including coprological, serological and molecular methods. However, all of these techniques have problems with sensitivity, specificity, availability, sophistications or need qualified technicians in endemic developing countries [Montes *et al.*, 2010; Olsen *et al.*, 2009; Requena-Mendez *et al.*, 2013].

1.6.1. Coprological techniques

Several coprological diagnostic techniques have been used to detect parasites larvae in fecal samples including formalin-ether concentration techniques (FECT) [Sato *et al.*, 1995]; modified Baermann's method [Garcia *et al.*, 2001], Harada-Mori filter paper culture [Harada *et al.*, 1955] and stool culture on a blood agar plate [Koga *et al.*, 1991]. Stool can be examined for presence of rhabditiform larvae in direct fecal smears or using FECT. Stool examination has poor sensitivity with a single stool sample being positive in only 25 to 50% of cases of gastrointestinal strongyloidiasis [Basile *et al.*, 2010; Siddiqui *et al.*, 2001]. Multiple repeated stool samples are needed to improve sensitivity which could be increased up to 60%, if five or more stool samples are examined [Cruz *et al.*, 2010].

A modified FECT resulted in higher recovery rates of *S. stercoralis* larvae and presumably an improved diagnostic efficiency, but dead individual larvae are more difficult to discern at low magnification [Anamnart *et al.*, 2010; Siddiqui *et al.*, 2001]. However, the chemical component used for this method has been considered hazardous by the US Environmental Protection Agency and many state environmental agencies and it might also not be suitable in limited resource settings [Requena-Mendez *et al.*, 2013].

The Baermann method is cheap and simple technique, based on the ability of *S. stercoralis* to enter a free-living cycle of development. In the Baermann procedure, stool is placed on mesh screen and a coarse fabric in a funnel that is filled with warm water and connected to a clamped-tubing. After two hours larvae crawl out of the fecal suspension and migrate into the warm water, from where they can be collected by centrifugation [Garcia *et al.*, 2001]. However, this technique is labor intensive and it is not usually available in parasitological laboratory. Furthermore, the big challenge of Baermann test is that it requires a huge amount and fresh stool samples [Requena-Mendez *et al.*, 2013; Siddiqui *et al.*, 2001].

In the Harada-Mori technique, filter paper containing fresh fecal material is placed in a test tube with water that continuously soaks the filter paper by capillary action. Incubation at 30 ^{o}C provides conditions suitable for the development of larvae, which can migrate to either side of the filter paper [Harada *et al.*, 1955]. The method is less sensitive than Baermann or Koga-agar culture but much more sensitive than single stool-smears. However, it is not frequently used as a standard procedure in clinical parasitology laboratories [Requena-Mendez *et al.*, 2013; Siddiqui *et al.*, 2001].

In the Koga agar culture method, developed in early the 1990s, stool is placed on a nutrient agar plate, incubated in a humid chamber at 28 °C for at least 48 hours (2 days) and evaluated for visible tracks created as larvae carry bacteria over the agar [Koga *et al.*, 1991]. Motile *S. stercoralis* larvae can also be seen with the aid of a dissecting microscope. Although the agar culture method has a higher sensitivity (96%) than direct fecal smears or the Baermann test, it is more time consuming, laborious and expensive [Requena-Mendez *et al.*, 2013; Siddiqui *et al.*, 2001].

In chronic infection, the sensitivity of these diagnostic methods might not be satisfactory. Thus they require multiple stool samples to achieve adequate sensitivity [Montes *et al.*, 2010; Requena-Mendez *et al.*, 2013].

1.6.2. Serological and molecular techniques

Because it is imperative to examine multiple stool samples to make a correct diagnosis, it is important to note that failure to detect larvae in a stool examination does not necessarily indicate the unequivocal absence of the infection [Siddiqui *et al.*, 2001]. Hence, there is a great need for a highly sensitive and efficient serodiagnostic test for *S. stercoralis* that has the potential to be used even in multiple helminth infections. Several immunodiagnostic assays have been tested including enzyme-linked immunosorbent assay (ELISA) [Sato *et al.*, 1985], skin testing with larval extracts [Sato *et al.*, 1986], indirect immunofluorescence antibody test (IFAT) of fixed larvae [Boscolo *et al.*, 2007], gelatin particle agglutination test (GPAT) [Sato *et al.*, 1991] and luciferase immunoprecipitation system (LIPS) [Ramanathan *et al.*, 2008a]. These assays show variable sensitivity and specificity depending on antigen preparation and immunoglobulin isotypes used, and on the population tested. In specific target populations, such as immigrants to non-endemic regions, serology has proven to be a valuable tool for screening and for the evaluation of therapies; however, serology seems to lack sufficient sensitivity to diagnose recently acquired infections. Although serology seems to have good negative predictive value if used in endemic areas, specificity appears to be more problematic as pronounced cross-reactivity is observed with other helminths, especially filarial infection [Olsen *et al.*, 2009].

A serologic test may be positive because of a resolved or unresolved previous *Strongyloides* infection [Marcos *et al.*, 2008]. Thus, the copro-antigen [Sykes *et al.*, 2011], Western blot [Silva *et al.*, 2003], polymerase chain reaction (PCR) [Moghaddassani *et al.*, 2011], real-time PCR [Schär *et al.*, 2013a; Verweij *et al.*, 2009] should follow a positive serologic test. Although appropriate facilities for PCR are difficult to maintain in endemic countries with scarce resources, real-time PCR has a promising role in industrialized countries for detection of *S. stercoralis* and post-treatment analysis [Verweij *et al.*, 2009].

1.6.3. Other diagnostic techniques

Biopsy via endoscopy or bronchoscopy is effective method for *Strongyloides* diagnosis [Cruz *et al.*, 2010]. It is an invasive procedures and recommended only in patients suspected of having an overwhelming infection, i.e., as patients with hyper-infection syndrome with dissemination may present with severe gastrointestinal complaints (gastrointestinal bleeding or ulcers) and/or significant respiratory complaints [Montes *et al.*, 2010].

Intra-dermal skin test, immediate hypersensitivity reaction in skin to different somatic excretory/secretory antigens, has been used to diagnose the strongyloidiasis [Neva *et al.*, 2001]. However, the test is not realistic option for routine diagnosis of strongyloidiasis due to the low sensitivity, particularly in HTLV-1 subject, and cross-reactivity with other nematode infections [Requena-Mendez *et al.*, 2013].

1.7. Management and treatment

The infection with *S. stercoralis* is very large problem in endemic areas and severe with high morbidity and mortality in population in communities. Limited guidelines exist for the treatment and management of *S. stercoralis* infection in immune-compromised patients [Marcos *et al.*, 2008]. Treatment of *Strongyloides* infection can be challenged. Strongyloidiasis is a difficult infection to treat because, for many helminth infections, a treatment is considered sufficient if worm burden is below the level at which clinical disease develops [Siddiqui *et al.*, 2001]. All patients with strongyloidiasis, regardless of the severity of symptoms, have to be treated to prevent long-term complications [Montes *et al.*, 2010].

The treatment options include Ivermectin and Benzimidazoles. Ivermectin, an antibiotic used initially in veterinary medicine to treat nematode infections, became available for human use in the late 1980s. The World Health Organization lists ivermectin as the drug of choice for the treatment of hyperinfection syndrome and disseminated *S stercoralis* [Foreman *et al.*, 2006]. Ivermectin is highly effective when given orally (200microgram/kg/day for 1-2 days) for complicated intestinal strongyloidiasis [Sridhara *et al.*, 2008]. Eradication rates two years after treatment with ivermectin are as high as 97% [Zaha *et al.*, 2000]. Its reported adverse effects are similar to those reported for the benzimidazoles, but they generally occur with less frequency and severity [Foreman *et al.*, 2006]. Tiabendazole at a dose of 25 mg/kg orally twice a day for three days is an alternative for complicated infections. Albendazole at a dose of 10mg/kg/day can be used as an alternative if nothing else is available as it has a lower efficacy (38-45%) [Montes *et al.*, 2010].

2. GOAL AND OBJECTIVES

2. 1. Goal

The investigations included in this PhD thesis aimed to understand the importance of S. *stercoralis* infection in Cambodia. The overarching goal was to understand the epidemiology and clinical aspects of *S. stercoralis* infection in Cambodia.

2. 2. Objectives

The PhD thesis pursued the following 4 main objectives:

1. To assess risk factors, diagnostic methods and ivermectin treatment efficacy of *S. stercoralis* infection in schoolchildren in semi-rural Cambodia.

2. To assess *S. stercoralis* infection and risk factors in two socioeconomic and ecological distinctly different settings: Preah Vihear in Northern and Takeo province in Southern Cambodia.

3. To determine re-infection of *S. stercoralis* in schoolchildren in semi-rural Cambodian villages.

4. To document clinical features before and after ivermectin treatment in *S. stercoralis* patients of rural Cambodian communities with high infection intensity.

3. Materials and methods

Detailed material and method section of each study performed in the framework of this PhD including study areas, subjects, and field and laboratory procedures etc. are provided in respective chapters. This chapter provides overview on ethical considerations, research approach and study design, and study sites used in this PhD.

Figure 4: Field and Laboratory activities

3.1. Ethical considerations

Prior to the implementation of each research study of this PhD, the study protocols were submitted and received clearances from Ethics Committees from Cambodia (National Ethics Committee for Health Research "NECHR", Ministry of Health) and from Basel, Switzerland (Ethikkommission beider Basel "EKBB", www.ekbb.ch). Moreover, the permission for field work was obtained from all relevant local institutions and authorities such as CNM, Provincial Health Departments and local health and administrative authorities of the respective provinces (village chiefs, headmasters, school teachers and so on).

Written informed consent in Khmer language, which explained the purpose and the detail procedures of the research, was obtained from all participants and patients. Participants aged under or equal to 18 years were first asked their assent and then a written informed consent was obtained from their parents or legal guardian or appropriate literate substitutes. In case participants were not able to read Khmer, we asked the village chief or relatives acting as witnesses for their participation in the research. Specifically, we obtained a written informed consent from a patient for publication of the image (Chapter 8).

All participants infected with *S. stercoralis* were treated with ivermectin [WHO, 2002]. All parasitic infections were treated according to the guidelines of the National Helminth Control Program of Cambodia [CNM, 2004].

3.2. Research approach and study design

This chapter will briefly describe the research approaches and study designs of the PhD studies. Figure 5 shows an overview of research approach and study populations of the thesis project. The activities done in this PhD works were separated in four main parts: (1) assessment of diagnostic methods, risk factors and treatment efficacy, (2) assessment of large-scale community-based infection prevalence and risk factors, (3) determining of re-infection rate, and (4) documenting of clinical features of strongyloidiasis.

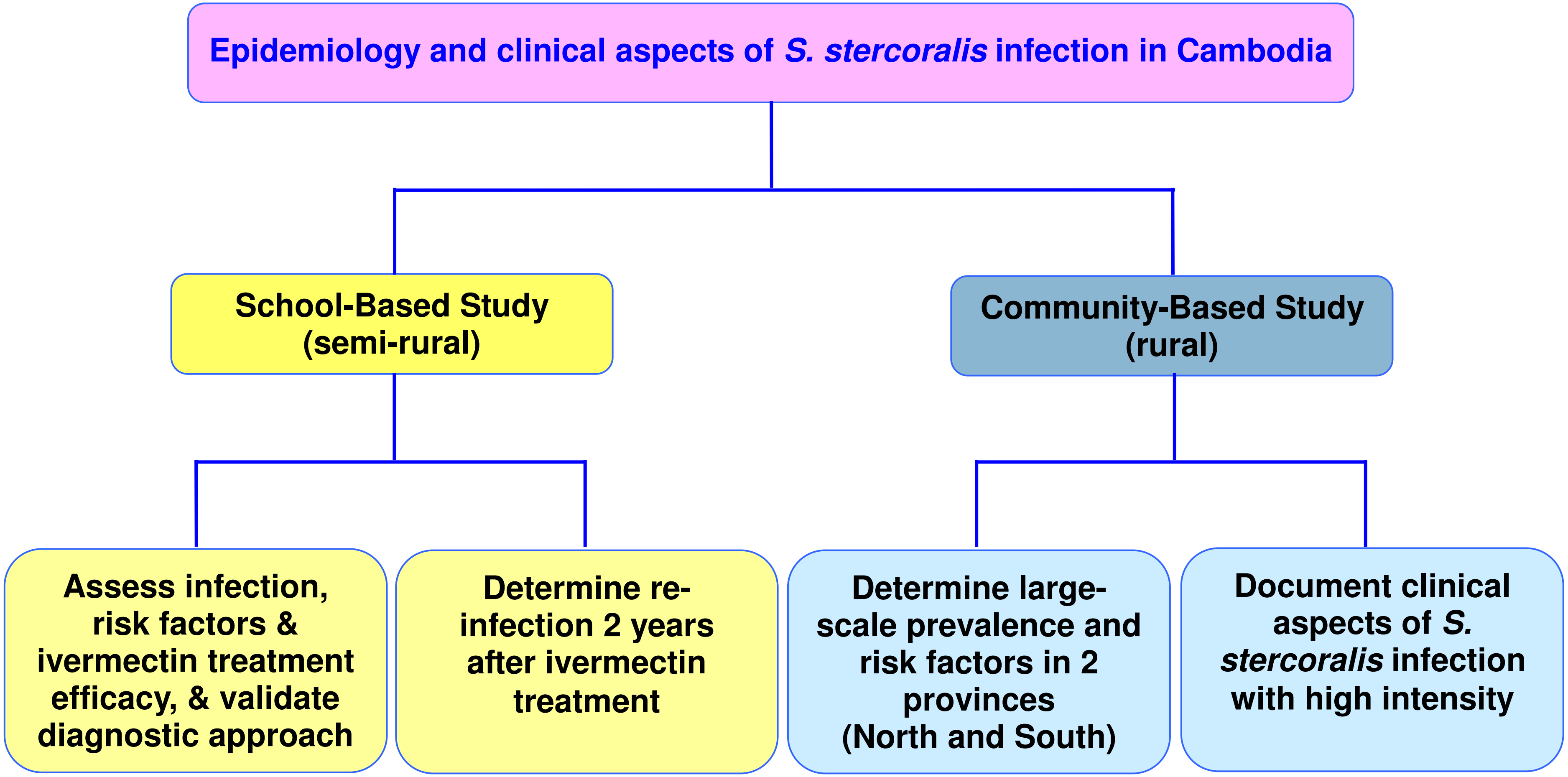

Figure 5: Overview of research approach

3.2.1. Assess diagnostic methods, risk factors and treatment efficacy

During the dry season in 2009, we conducted a cross-sectional study among schoolchildren living in four villages of Saang district, Kandal province. The villages were selected because hookworm infections were previously reported (used as a proxy for likely *S. stercoralis* transmission), and the villages were accessible by car. Parent or legal guardians of children and children were interviewed, using pre-tested household and child questionnaires, at home and school, respectively. At school, each child was asked to provide three stool samples over five days. Stool specimen was examined by KAP culture [Koga *et al.*, 1991] and Baermann technique [Garcia *et al.*, 2001] for the detection of *S. stercoralis* larvae. All children infected with *S. stercoralis* were treated with ivermectin 100µg/kg/day for two days (total 200 µg/kg BW) [Igual-Adell *et al.*, 2004], under direct observation of a medical doctor. At 21-23 days after treatment, *S. stercoralis* infected children were asked to provide another three stool samples (over five days), which were then examined with the same procedure as at baseline (KAP culture and Baermann technique).

3.2.2. Assess infection and risk factors in large scale community studies

Two cross-sectional studies focused on assessing infection prevalence and risk factors of *S. stercoralis* were carried out in Preah Vihear province (2010) and Takeo province (2011), socioeconomic and ecologically distinctly different settings. Fifteen households were randomly selected from the list of all the households of the selected villages (60 villages per province). All household members older or equal to one year of age were eligible. Pre-tested household and individual questionnaires were administrated the head of household and participant, respectively. Stool samples were examined by KAP culture [Koga *et al.*, 1991] and Baermann technique [Garcia *et al.*, 2001] for the detection of *S. stercoralis* larvae. Furthermore, we used Bayesian logistic models to explore the village-level correlation of *S. stercoralis* infection risk. Bayesian kriging was employed to predict infection risk at non-surveyed locations. The spatial prediction analysis was performed in Preah Vihear province in 2010 only.

3.2.3. Determine re-infection rate

A two-year cohort study (2009: baseline; 2011: follow-up) among schoolchildren living in four villages of Saang district, Kandal province, was conducted to determine the re-infection rate with *S. stercoralis*. We used KAP culture [Koga *et al.*, 1991] and Baermann technique [Garcia *et al.*, 2001] to detect *S. stercoralis* larvae in the stool sample in both stages (baseline and follow-up). At baseline, the schoolchildren were asked to submit two stool samples for diagnosing *S. stercoralis* infection. All infected participants were treated with ivermectin 100µg/kg/day for two days [Igual-Adell *et al.*, 2004] and re-examined their stool samples three weeks after treatment. At follow-up, infection status in all schoolchildren was re-assessed with the same laboratory procedures as at baseline (number of stool sample and diagnostic method used).

3.2.4. Document clinical features

In early 2010, we documented clinical features of 21 strongyloidiasis cases found in the community-based survey in Rovieng district, Preah Vihear province. Two stool samples were obtained on two consecutive days from each patient and examined with Baermann [Garcia *et al.*, 2001] and KAP culture [Koga *et al.*, 1991] techniques for the presence of *S. stercoralis* larvae. Patients with more than 250 larvae in one of the Baermann examinations were considered to have a high intensity infection were revisited and a detailed clinical assessment was performed. Patients were then treated with a single oral dose of ivermectin, 200µg/kg body weight. All patients were observed for one hour following treatment for the occurrence of adverse effects. Three weeks later, all patients were visited again and the laboratory and clinical assessment were repeated.

3.3. Study sites

The fieldwork of this PhD thesis was conducted in 3 provinces of Cambodia, including Kandal (Central-south), Preah Vihear (North) and Takeo (South) provinces (Figure 6).

Two school-based surveys were carried out among schoolchildren during the dry season in March-June 2009 and in May-June 2011 in four villages (Ang, Roka, Koh Khel and Damrey Chhlang villages) of Saang district, Kandal province, south of Phnom Penh, Capital of Cambodia. Kandal (11.41° N latitude and 104.71° E longitude) is one of the most populated provinces of Cambodia with a total population of 1'265'085 inhabitants. It is situated in Central-South of Cambodia. This plain wet province has a surface of 3'568 square kilometers and shares the border to the North with Kampong Chhnang and Kampong Cham provinces, to the East with Prey Veng province, to the West with Kampong Speu and Takeo provinces and to the South with Vietnam. The province's average temperature is about 27°C with minimum temperature of 16°C and maximum temperature of 36°C. Rice subsistence farming is the main economic activity in the province. Pigs, poultry and cattle are the most common domestic animals [NIS, 2008; NIS *et al.*, 2011]. There was one study on prevalence of intestinal parasitic infection available in Kandal province. It was a cross-sectional study among 3'574 participants living in three villages, along Bassak River, of Prek Russey commune, Takmao city. It reported a *S. stercoralis* infection rate of 14.6%. Additionally, the infections prevalence of hookworm, *Ascaris lumbricoides, Trichuris trichiura and Enterobius vermicularis* were 21.8%, 15.4%, 0.8% and 0.4%, respectively [Koga-Kita, 2004].

From February to June 2010, a community-based study was conducted among general population living in 60 randomly selected villages of Preah Vihear province, northern Cambodia. Preah Vihear (13.45° N latitude and 105.16° E longitude) is one of the least populated provinces (170'852 inhabitants) of Cambodia. It borders to Thailand and Laos in the North, Stung Treng province in the East, Oddar Meanchey and Siem Reap provinces in the West and Kampong Thom province in the South. The province's surface is 13'788 square kilometers. Much of the province is hilly forested and extremely remote, with no proper major existing roads. It has an average temperature of 27°C with minimum temperature of 16°C and maximum temperature of 35°C. Farming and agriculture are the main sources of income in the province [NIS, 2008; NIS *et al.*, 2011]. There was no information on the prevalence of STHs in Preah Vihear province.

From January to April 2011 a community-based survey was undertaken among the general population living in 60 randomly selected villages of Takeo province (10.98° N latitude and 104.78° E longitude). The typical plain wet province is located in the south of Cambodia, total area of 3'563 square kilometers, bordering in the South to Vietnam, in the North and East with Kandal, and in the West with Kampong Speu and Kampot provinces. The total population of Takeo province is 843'931 inhabitants. Takeo's temperature is about 27°C in average with minimum temperature of 16°C and maximum temperature of 36°C. Province's economy consists basically of agricultural farming, fishery, rice and fruit cropping [NIS, 2008; NIS *et al.*, 2011]. There was no information on prevalence of *S. stercoralis* in Takeo province. However, a study on intestinal parasitic infections conducted by *Yong et al* found a prevalence of hookworm in Ang Svay Chek village, Prey Kabas district between 1.9% and 16.0% [Yong *et al.*, 2012].

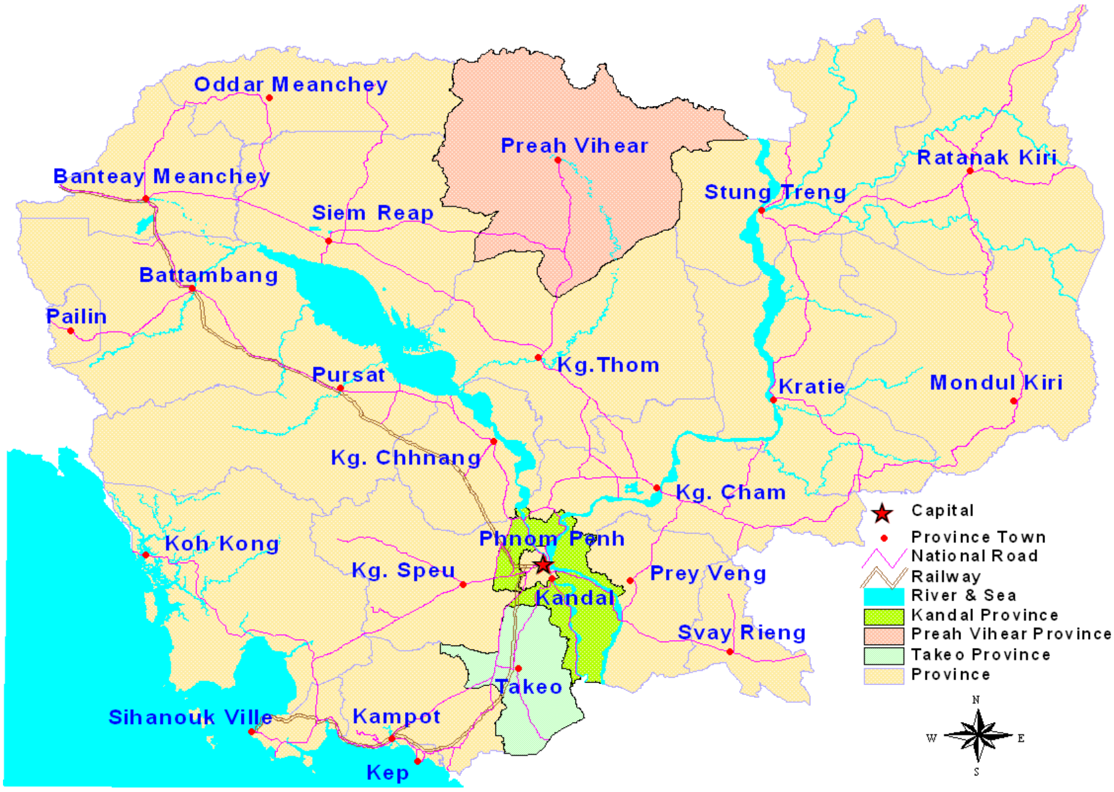

Figure 6: Map of Cambodia and study provinces

4. DIAGNOSIS, TREATMENT AND RISK FACTORS OF *STRONGYLOIDES STERCORALIS* IN SCHOOLCHILDREN IN CAMBODIA

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4.1. Abstract

Worldwide, an estimated 30 to 100 million people are infected with *Strongyloides stercoralis*, a soil-transmitted helminth. Information on the parasite is scarce in most settings. In semi-rural Cambodia, we determined infection rates and risk factors; compared two diagnostic methods (Koga agar plate [KAP] culture and Baermann technique) for detecting *S. stercoralis* infections, using a multiple stool examination approach; and assessed efficacy of ivermectin treatment.

We performed a cross-sectional study in 458 children from four primary schools in semi-rural villages in Kandal province, using three diagnostic procedures (Kato-Katz, KAP culture and Baermann technique) on three stool samples. Infected children were treated with ivermectin (100µg/kg/day for two days) and re-examined three weeks after treatment. Hookworm, *S. stercoralis*, *Trichuris trichiura*, and small trematode eggs were most prevalent, with 24.4% of children being infected with *S. stercoralis*. The sensitivity of KAP culture and Baermann technique was 88.4% and 75.0%, respectively and their negative predictive values were 96.4% and 92.5%, respectively. The cumulative prevalence of *S. stercoralis* increased from 18.6% to 24.4%, after analyzing three stool samples, which was close to the modeled 'true' prevalence of 24.8%. Children who reported defecating in latrines were significantly less infected with *S. stercoralis* than those who did not use latrines ($p<0.001$). Itchy skin and diarrhea were significantly associated with *S. stercoralis* infection. The cure rate of ivermectin was 98.3%.

S. stercoralis infection is highly prevalent among semi-rural Cambodian schoolchildren. The sensitivity of KAP culture is higher than that of the Baermann technique. In the absence of a ''gold standard'', analysis of multiple stool samples by different diagnostic methods is required to achieve a satisfactory level of sensitivity. Almost three-quarters of the infections could have been avoided by proper sanitation. Ivermectin is highly efficacious against *S. stercoralis* but prohibitive costs render the drug inaccessible to most Cambodians.

Key words: *Strongyloides stercoralis*, Diagnostic Methods, Schoolchildren, Ivermectin, Cambodia

Authors Summary

The difficulty of diagnosing *Strongyloides stercoralis* infections is the reason why up to date, accurate information on its geographic distribution in endemic regions and the total global burden is lacking. We conducted a cross-sectional study among 458 schoolchildren, with the purpose of comparing two methods for diagnosing S. *stercoralis* infection (Koga agar plate 'KAP' culture and Baermann technique) on three stool samples from each individual and to assess the efficacy of ivermectin three weeks after treatment. About one quarter of the schoolchildren examined were infected with *S. stercoralis*. The sensitivity of KAP culture and Baermann technique was 88.4% and 75.0%, respectively. The prevalence of *S. stercoralis* infection increased considerably (from 18.6% to 24.4%) when three stool samples were examined. Almost three-quarters of the infections could have been avoided by proper sanitation. Ivermectin was highly efficacious against *S. stercoralis* infection, with a cure rate of 98.3%. In the absence of a ''gold standard'', it is necessary to examine multiple stool samples using different diagnostic techniques in order to reach a ''true'' prevalence.

4.2. Introduction

The threadworm *Strongyloides stercoralis* affects about 30-100 million people worldwide [Bethony *et al.*, 2006; Olsen *et al.*, 2009]. *S. stercoralis* is the only soil-transmitted helminth (STH) with the ability for auto-infection, and thus may lead to systemic infections with high parasite densities, particularly in immune-compromised hosts [Basile *et al.*, 2010; Marcos *et al.*, 2008; Vadlamudi *et al.*, 2006]. Disseminated strongyloidiasis may lead to severe complications with substantial mortality [Liu *et al.*, 2009; Marcos *et al.*, 2008].

Strongyloidiasis is endemic in areas where sanitary conditions are poor and where the climate is warm and humid [Hall *et al.*, 1994], such as Central and South America, sub-Saharan Africa, and South and Southeast Asia [Concha *et al.*, 2005; Grove, 1994; Liu *et al.*, 1993; Siddiqui *et al.*, 2001]. However, little information is available on *S. stercoralis* prevalence in most of these settings. Today, most studies conducted in resource poor countries originate from Brazil and Thailand [Ines Ede *et al.*, 2011; Nontasut *et al.*, 2005; Paula *et al.*, 2011; Suputtamongkol *et al.*, 2011]. The sensitivity of widely used diagnostic procedures, such as direct fecal smear, Baermann technique and Koga agar plate (KAP) culture is not satisfactory when used on a single stool specimen [Koga-Kita, 2004; Longfils *et al.*, 2005; Sayasone *et al.*, 2009; Sithithaworn *et al.*, 2006].

The clinical manifestations of strongyloidiasis vary greatly in different situations, depending on infection intensities and the immune status of the individual. More than 50% of the infections may remain asymptomatic [Cruz *et al.*, 2010; Fardet *et al.*, 2007; Foreman *et al.*, 2006]. Gastrointestinal symptoms, including diarrhea and abdominal pain, are the most common symptoms [Grove, 1996; Lim *et al.*, 2004]. The most commonly described dermatologic aspects of chronic strongyloidiasis are itching and rash (urticaria) [Ly *et al.*, 2003]. The recommended treatment for strongyloidiasis is ivermectin [WHO, 2002].

The detection of *S. stercoralis* larvae in the stool is proof of an infection [Siddiqui *et al.*, 2001]. Several diagnostic methods such as direct fecal smear, Baermann concentration, formalin-ethyl acetate concentration (FECT), Harada-Mori filter paper culture, or nutrient agar plate culture [Garcia *et al.*, 2001; Gutierrez, 2000; Koga *et al.*, 1991; Leventhal *et al.*, 1989] have been used to identify larvae in stool samples. The exact sensitivity of these different diagnostic approaches is still debated [de Kaminsky, 1993; Knopp *et al.*, 2008; Marchi Blatt *et al.*, 2003; Steinmann *et al.*, 2007].

In Cambodia, information on *S. stercoralis* infection is scarce. Earlier studies indicate prevalence rates of up to 20% in schoolchildren. However, they were assessed using a diagnostic approach with low sensitivity [Chhakda *et al.*, 2006; Koga-Kita, 2004; Longfils *et al.*, 2005].

In semi-rural Cambodia, we determined infection rates and risk factors, compared two methods (KAP culture and Baermann technique) for diagnosing *S. stercoralis* infection using a multiple stool examination approach, and assessed efficacy of ivermectin treatment. We performed a cross-sectional study on *S. stercoralis* infection in four primary schools in semi-urban villages in Kandal province, examining three stool samples per child.

4.3. Materials and methods

Ethical Considerations

The study was approved by the National Ethics Committee for Health Research (NECHR; number 033, dated 20 March 2009), Ministry of Health, Cambodia and by the Ethics Committees of the Cantons of Basel-Stadt and Baselland (EKBB; number 21/09, dated 29 January 2009), Switzerland. All relevant authorities (village chiefs, school teachers and headmasters) were informed about the purpose and procedures of the study. Written informed consent was obtained from the parent or the legal guardian of the child or appropriate literate substitutes, prior to study onset.

All diagnosed infections were treated according to Cambodian standard treatment guidelines [CNM, 2004]. All children infected with *S. stercoralis* were treated with ivermectin 100µg/kg/day for two days [Igual-Adell *et al.*, 2004].

Study Setting and Population

The study was carried out in four semi-rural villages (Ang, Roka, Koh Khel and Damrey Chhlang villages), located in the Saang District (11.22° N and 105.01° E longitude), Kandal province, 45 kilometers south of Phnom Penh. Rice subsistence farming is the main economic activity in the villages. Pigs, poultry and cattle are the most common domestic animals. The villages were selected because hookworm infections were previously reported (used as a proxy for likely *S. stercoralis* transmission), and the villages were accessible by car to ensure rapid transfer of stool samples to the Parasitological Laboratory of the National Center for Parasitology, Entomology and Malaria Control (CNM) in Phnom Penh.

A school-based survey was conducted during the dry season from March to June 2009 among the schoolchildren of the four semi-rural villages mentioned above.

Field Procedures

First, parents or legal guardian of the children were interviewed at home, using a pre-tested household questionnaire, to obtain the demographic data (age, sex, education level, profession), personal risk-perception (knowledge about helminth infections, health seeking behavior), living conditions (type of house, sanitation infrastructure, domestic animals) and personal hygiene.

Second, at school, a pre-tested child questionnaire was administered to the schoolchildren to obtain demographic data (age, sex, school grade), personal risk-perception (knowledge about helminth infection) and behavior data (wearing shoes, food consumption and personal hygiene) from the child.

After the interview, each child received a pre-labeled plastic container (ID code, name, sex, age and date) for stool sample collection. Each morning, after collecting the filled container, another empty pre-labeled one was provided for the following day. This procedure was repeated until three stool samples were obtained per child or over a period of five days.

Within 90 minutes after collection, the stool samples arrived at the laboratory at ambient temperature. Upon arrival, experienced laboratory technicians from the Parasitological Laboratory of CNM immediately examined the specimens, as explained below.

Laboratory Procedures

Stool samples were first subjected to a KAP culture, then a Kato-Katz thick smear examination and finally a Baermann technique was performed.

First, KAP culture [Koga *et al.*, 1991] was used for identifying *S. stercoralis* and possibly hookworm larvae. For this purpose, agar plates were prepared once per week and stored at 4°C in humid conditions. A hazelnut-sized stool sample was placed in the middle of the plate and the closed Petri dish was incubated in a humid chamber for 48 hours at 28°C. Afterwards, the plates were rinsed with sodium acetate-acetic acid-formalin (SAF) solution. The eluent was centrifuged and the sediment microscopically examined for the presence of *S. stercoralis* and hookworm larvae. The two species were distinguished by the characteristic morphology of the larvae (i.e., size of buccal cavity, presence of genital primordium (L_1), presence of forked tail-end (L_3)).

Then, a single Kato-Katz thick smear [Katz *et al.*, 1972] was prepared using the WHO standard template and examined under a light microscope for the presence of helminth eggs.

Finally, the Baermann technique [Garcia *et al.*, 2001] was performed. A walnut-sized stool sample was placed on gauze inserted into a glass funnel, and covered with water. The apparatus was exposed for two hours to artificial light directed from below. After centrifuging of the collected liquid, the sediment was examined under a microscope for presence of *S. stercoralis* larvae. If insufficient stool was submitted Baermann technique was dropped first.

For quality control, the technicians were specifically trained for three days on morphological criteria distinguishing hookworm and *S. stercoralis* larvae. During the whole study period, beside the permanent and rigorous supervision by a qualified microscopist from the Swiss Tropical and Public Health Institute (Swiss TPH), Basel, Switzerland, any unclear diagnosis was immediately discussed and solved with the qualified microscopist and study supervisor. Additionally, ten percent of the slides were re-examined by the same qualified technician from Swiss TPH. Slides yielding discrepant results were re-read by involved reader. A definitive infection(s) was found to be such by consensus.

Follow-Up after Treatment of S. stercoralis Patients

S. stercoralis infected children were treated with ivermectin over two days (100µg/kg/day). Stromectol 3 mg (commercial name of ivermectin), was manufactured on November 2008 by Merck Sharp & Dohme BV in the Netherlands. The drug was registered under no. 3523885; expiry date November 2011 (Manufactured batch: NK03350; Packed batch: NK18050). At 21-23 days after treatment, the infected children were asked to provide another three stool samples (over five days), which were then examined with the same procedure as at baseline (KAP culture, Kato-Katz and Baermann technique). Ivermectin treatment was provided under direct observation of a medical doctor. Adverse events occurring within three hours after treatment were recorded. Parents or legal guardian of the child were asked to report any adverse event occurring within a week after treatment to a medical doctor by telephone.

Statistical Analyses

Questionnaire and laboratory data were double-entered in EpiData version 3.1 (EpiData Association; Odense, Denmark) and validated. Statistical analyses were performed with STATA version 10.1 (StataCorp.; College Station, TX, USA). Only schoolchildren with a complete record (three stool samples examined with all three methods and complete questionnaire information) were retained for analyses.

Prevalence, sensitivity (i.e., proportion of true positives identified as positive) and negative predictive values (i.e., proportion of un-infected children among negative results) of the different *S. stercoralis* diagnostic methods employed were assessed. A mathematical model developed by Marti and Koella (Marti *et al.* (1993] was used to estimate species-specific 'true' prevalence rates. This model employs the number of positive test results among stool samples submitted by the same person, to estimate the sensitivity of the diagnostic method and to calculate the number of stool samples needed for the test to be below a given percentage of false negative results. The procedure has been employed before to estimate the 'true' infection rates of soil-transmitted helminths, including *S. stercoralis* [Bogoch *et al.*, 2006; Knopp *et al.*, 2008; Steinmann *et al.*, 2007]. All *S. stercoralis* positive children, regardless of the number of stool samples provided, were followed up after treatment.

Univariate logistic regression was used to associate infection status with demographic variables, hygienic status, and knowledge of the child's guardian and the recent medical history of the child. Population attributable fraction was calculated for significantly associated risks. *P-values* under or equal to 0.05 were considered as significant.

4.4. Results

Study Sample and Compliance

In total, 500 children from four primary schools were enrolled (Figure 7), of which 461 (92.2%) submitted three stool samples over five days. The analysis focused on 458 (91.6%) schoolchildren with complete data records, i.e. three stool samples examined with all diagnostic tests. The participants were between 6 and 19 years old (median age 11 years); 227 (49.6%) were girls. There was no age difference between genders (p=0.06).

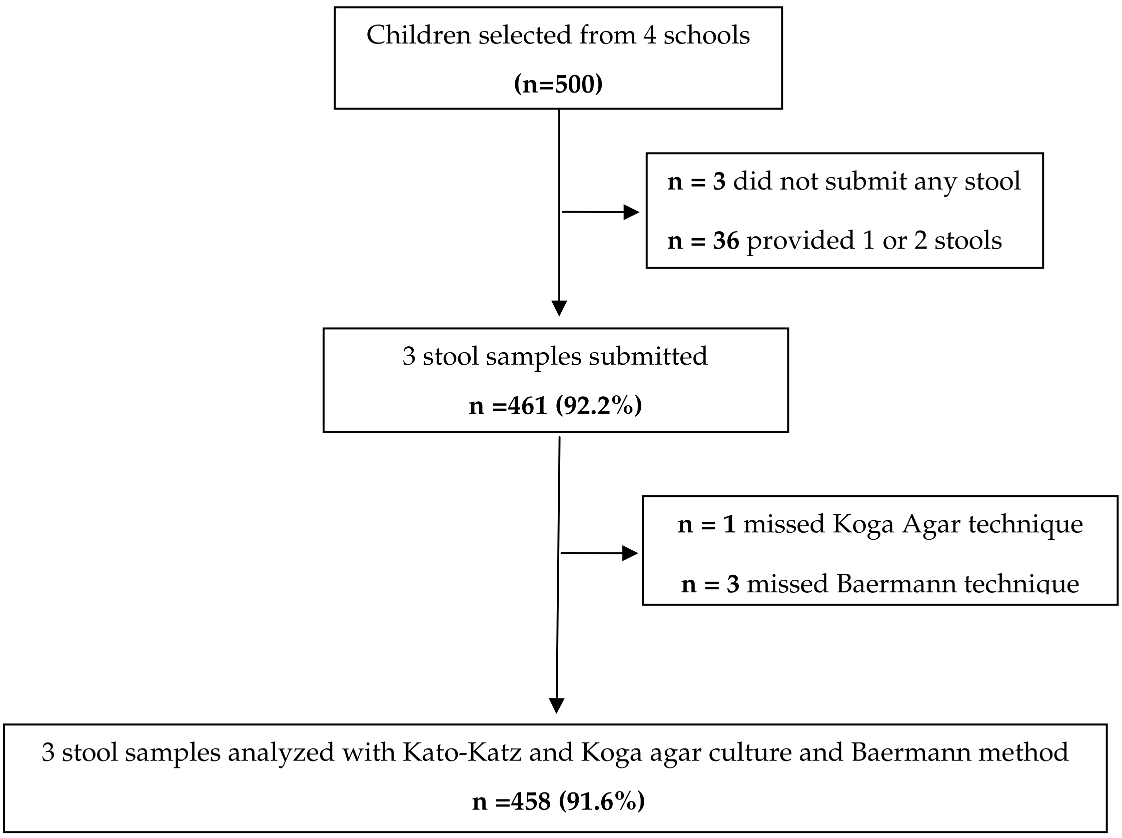

Figure 7: Flow chart detailing the study and compliance for stool examination, 2009

Parasitological Findings

The observed and estimated prevalence of eight different intestinal parasite species found from different stool samples and diagnostic methods are detailed in Figure 8. In total, half of the schoolchildren (49.3%) were infected with hookworm, and one quarter of them (24.4%) were diagnosed with *S. stercoralis* infection. *T. trichiura* was found in 17.3%, while 7.9% harbored small trematode eggs. Both, *A. lumbricoides* and *E. vermicularis*, were observed in 2.0% of participants, whereas *H. nana* and *Taenia* sp. were found in 3.7% and 0.4% of children, respectively.

Performance of the Diagnostic Methods, Sampling Efforts and Prevalence Prediction

In total, 112 *S. stercoralis* infected schoolchildren (24.5%) were diagnosed by either KAP culture and/or Baermann technique out of 458 study participants who submitted three stool samples (Table 1). KAP culture and Baermann technique detected 99 and 84 *S. stercoralis* infections, respectively. The combination of the two tests was considered to be the "diagnostic gold standard". The prevalence of *S. stercoralis* as estimated by KAP culture or Baermann technique alone was 21.6 % and 18.3%, respectively. The sensitivity of KAP culture was 88.4%, whereas the sensitivity of Baermann technique was 75.0%. The negative predictive value of KAP culture and Baermann technique was 96.4% and 92.5%, respectively.

The effect of the sampling effort for multiple stool samples examination on infection prevalence and on the sensitivity of the different diagnostic methods are presented in Table 2 for *S. stercoralis* and in Table 3 for hookworms. The number of *S. stercoralis* and hookworm infections detected by either method increased considerably by analyzing three stool samples. For *S. stercoralis* the prevalence rose from 15.9% to 21.6% and from 12.0% to 18.3%, as detected by KAP culture and Baermann method, respectively. The combined results of both methods showed an increase from 18.6% to 24.4% when three stool samples were examined instead of one.

The 'true' *S. stercoralis* prevalence was estimated at 24.8% (SD = 4.1%) when the two methods were combined. The probability of correctly diagnosing *S. stercoralis* infected children by examining only a single stool sample was similar (70.0%) for both methods. The sensitivity and negative predictive value of all methods combined for three stool examinations was above 97% and 99%, respectively.

The predicted prevalence of hookworms was 51.4% (SD = 5.0%) when three tool samples were analyzed by a combination of all three methods (KAP culture, Kato-Katz and Baermann technique). The predicted prevalence for other intestinal parasites is shown in Figure 8.

Table 1: Koga agar plate culture and Baermann method for the diagnosis of *S. stercoralis* in schoolchildren, 2009

		Combined Methods (KAP culture and Baermann)		Total
		Positive	Negative	
KAP culture	Positive	99	0	99
	Negative	13	346	359
	Total	112	346	458
Baermann method	Positive	84	0	84
	Negative	28	346	374
	Total	112	346	458

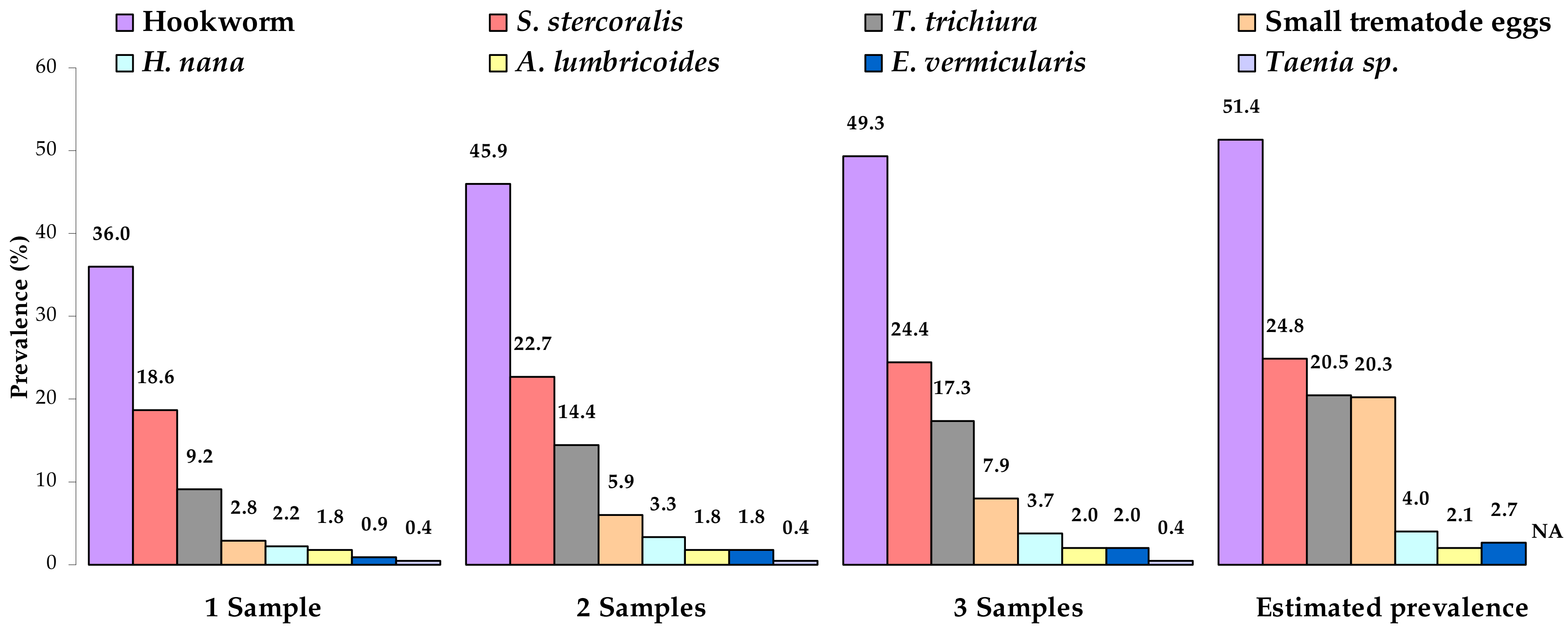

Figure 8: **Observed cumulative and estimated prevalence of intestinal helminth infections among 458 schoolchildren in Cambodia, 2009**

Table 2: ***S. stercoralis*** **larvae in Koga agar plate culture and Baermann method of three stool samples in schoolchildren, 2009**

	KAP culture		Baermann method		Combined 2 methods*	
	Number	%	Number	%	Number	%
Cumulative result after examination of						
1 stool sample	73	15.9	55	12	85	18.6
2 stool samples	90	19.6	78	15	104	22.7
3 stool samples	99	21.6	84	18.3	112	24.4
Estimated prevalence** (SD)		22.1 (3.9)		18.9 (3.7)		24.8 (4.1)
Sensitivity (3 samples)		97.7		97.3		98.6
Sensitivity of an individual test (SD)		71.3 (5.7)		69.9 (6.4)		76.0 (5.0)
Negative predictive value		99.3		99.4		99.6

*Koga agar plate culture and Baermann method; ** 'Estimated' prevalence according to a model developed by Marti and Koella [Marti *et al.*, 1993]; SD: Standard Deviation

Table 3: Hookworm in Kato-Katz, Koga agar plate culture and Baermann method in three stool samples in schoolchildren, 2009

	Kato-Katz Method		KAP culture		Baermann method		Combined 3 methods*	
	Number	%	Number	%	Number	%	Number	%
Cumulative result after examination of								
1 stool sample	115	25.1	79	17.3	55	12.0	165	36.0
2 stool samples	152	33.2	122	26.6	79	17.3	210	45.9
3 stool samples	169	36.9	142	31.0	99	21.6	226	49.3
Estimated prevalence** (SD)		39.0 (4.8)		36.7 (5.8)		30.2 (6.8)		51.4 (5.0)
Sensitivity (3 samples)		94.7		82.7		71.5		96.0
Sensitivity of an individual test (SD)		62.1 (4.9)		44.2 (6.2)		34.2 (7.7)		65.9 (4.1)
Negative predictive value		96.6		90.6		89.0		96.0

* Kato-Katz and Koga agar plate culture and Baermann method; ** 'Estimated' prevalence according to a model developed by Marti and Koella [Marti *et al.*, 1993]; SD: Standard Deviation

Efficacy of Ivermectin

Among all those children who submitted at least one stool sample (n= 497) during baseline examinations, 117 schoolchildren were diagnosed with a *S. stercoralis* infection. They were treated with ivermectin (100µg/kg/day for 2 days). Three weeks after treatment, all children submitted two stool samples and 106 (90.6%) provided an additional third stool sample for examination. In two children, an infection with *S. stercoralis* was diagnosed (In one case, it was diagnosed in the first stool sample and in the other case, in the second stool sample. Both children were retreated with ivermectin.). No infection was detected in the third stool specimen. The cure rate of ivermectin was 98.3%. None of the children experienced an adverse event within three hours after treatment and none reported adverse events within a week after treatment.

The cure rates of treatments on the other intestinal helminth infections are presented in Table 4. Overall, 97 (82.9%) cases were co-infected with hookworm. Three weeks after mebendazole treatment (500mg single dose), 65 (55.5%) participants were found hookworm positive in the follow-up examination. The cure rate of mebendazole was 33.0%.

Table 4: Cure rates of helminth infections among 117 *Strongyloides stercoralis* cases treated according to the Cambodian national guidelines, 2009

Infection	Drug	Before treatment n (%)	After treatment n (%)	Cure rate
Strongyloides stercoralis	Ivermectin	117 (100.0)	2 (1.7)	98.3%
Hookworm	Mebendazole	97 (82.9)	65 (55.5)	33.0%
Ascaris lumbricoides	Mebendazole	2 (1.7)	0 (0.0)	100.0%
Trichuris trichiura	Mebendazole	38 (32.5)	1 (0.9)	97.4%
Enterobius vermicularis	Mebendazole	3 (2.6)	0 (0.0)	100.0%
Taenia spp.	Praziquantel	2 (1.7)	0 (0.0)	100.0%
Hymenolepis nana	Praziquantel	2 (1.7)	0 (0.0)	100.0%
Small trematode eggs	Praziquantel	18 (15.4)	1 (0.9)	94.4%

Predictors of Strongyloides stercoralis Infection

Of 112 *S. stercoralis* cases, 108 (96.4%) were diagnosed in schoolchildren under 16 years, 42.0% were girls. As shown in Table 5, gender and age were not statistically different between infected and non-infected children. However, reported personal hygiene characteristics were significantly associated with *S. stercoralis* infection. The children who reported having shoes, and defecating in a toilet were half as likely to be infected with *S. stercoralis* than those who did not report shoes and latrine use (p<0.001). Itchy skin and diarrhea symptoms in the previous two weeks were reported more frequently among *S. stercoralis* cases.

Population attributable risk analysis showed that the number of strongyloidiasis cases would be reduced by 72% and 40% if all children had a toilet at home and used it for defecation, respectively. Bivariate analysis of population attributable risk showed that when the children in this population had toilet at home and defecated in it, strongyloidiasis cases could be reduced by 74%.

Table 5: Risk factors for *Strongyloides stercoralis* infection in 458 schoolchildren from Cambodia, 2009

	S. stercoralis Negative (N=346) n (%)	*S. stercoralis* Positive (N=112) n (%)	Relative Risk (95% CI)	Population Attributable Risk (95% CI)
Demographic characteristics of children				
Gender (female)	180 (52.0)	47 (42.0)	1.3 (0.9 - 1.8)	
Age group				
6-10 years old	173 (50.0)	52 (46.4)	Reference	
11-15 years old	170 (49.1)	56 (50.0)	1.0 (0.7 - 1.4)	
>15 years old	3 (0.9)	4 (3.6)	2.4 (1.2 - 4.9)	
Hygiene behavior of children				
Defecates usually in toilet (yes)	160 (46.2)	26 (23.2)	2.2 (1.5 - 3.3)	0.4 (0.2 - 0.5)
Child washed hands after defecation, last time (yes)	237 (68.5)	47 (42.0)	2.2 (1.6 - 3.1)	0.3 (0.1 - 0.4)
Child washed hand before eating, last time (yes)	172 (49.7)	42 (37.5)	1.4 (1.0 - 2.0)	0.2 (0.01 - 0.3)
Child has shoes (yes)	306 (88.4)	77 (68.7)	2.3 (1.7 - 3.1)	0.1 (0.1 - 0.2)
Toilet at home (yes)	125 (36.1)	9 (8.0)	4.7 (2.4 - 9.0)	0.7 (0.5 - 0.8)
Recent medical history of children (last 2 weeks)				
Itchy skin (yes)	18 (5.2)	13 (11.6)	0.5 (0.3 - 0.8)	
Lost weight (yes)	23 (6.7)	7 (6.2)	1 (0.5 - 2.0)	
Nausea (yes)	26 (7.5)	7 (6.2)	1.1 (0.6 - 2.3)	
Vomiting (yes)	64 (18.5)	26 (23.2)	0.8 (0.5 - 1.2)	
Diarrhea (yes)	61 (17.6)	32 (28.6)	0.6 (0.4 - 0.9)	
Cold or cough (yes)	164 (47.4)	53 (47.3)	1 (0.7 - 1.4)	
Seen worm in stool (yes)	30 (8.7)	16 (14.3)	0.6 (0.4 - 1.0)	
Abdominal pain (yes)	226 (65.3)	67 (59.8)	1.2 (0.8 - 1.6)	
Knowledge of child's guardian				
Guardian reported child has been treated for Worm (yes)	216 (62.4)	45 (40.2)	1.9 (1.4 - 2.7)	0.3 (0.1 - 0.4)
Guardian knows about Worm/Worm Infection (yes)	258 (45.6)	25 (22.3)	2.3 (1.5 - 3.4)	0.4 (0.2 - 0.6)

4.5. Discussion

An in-depth parasitological investigation of *S. stercoralis* in Cambodia, including the performance of different diagnostic methods and the efficacy of treatment has not been carried out before. Our study confirms the validity of the KAP culture and Baermann method for detecting *S. stercoralis* larvae with high sensitivity and the high efficacy of oral ivermectin treatment (100µg/kg/day for two days) in curing *S. stercoralis* infection. A cumulative prevalence of 24.4% was found among 458 schoolchildren in four semi-rural villages in Kandal province, south of Phnom Penh, by applying two methods on three stool samples collected over five days. This prevalence is substantially higher than those stated in three previous reports from Cambodia [Chhakda *et al.*, 2006; Koga-Kita, 2004; Longfils *et al.*, 2005], and in reports from neighboring Laos and Thailand[Nontasut *et al.*, 2005; Sayasone *et al.*, 2009]. This is most likely due to the fact that we used a much more rigorous diagnostic approach (number of stool samples, multiple diagnostic methods) than did the other studies, where it was common to examine a single stool sample with a single method. Nevertheless, a prevalence (20.2%) similar to the one found in our study was observed in 2006, among school-aged children living in villages bordering Tonlé Sap Lake, northern Cambodia [Chhakda *et al.*, 2006] and using only the Baermann technique to analyze a single stool sample. This observation indicates that in villages close to Tonlé Sap Lake the true prevalence was actually considerably higher. It further underlines the reason why *S. stercoralis* is so often underdiagnosed.

The prevalence observed in our study area is particularly high when compared to other studies that used a similar diagnostic approach. In Zanzibar, Stefanie Knopp and colleagues diagnosed *S. stercoralis* in 10.8% of schoolchildren [Knopp *et al.*, 2008] and in China, Peter Steinmann and colleagues found a prevalence of 11.7% in the general population [Steinmann *et al.*, 2008; Steinmann *et al.*, 2007].

In the scientific literature, no agreement seems to exist regarding the respective sensitivity of the KAP culture and Baermann method [de Kaminsky, 1993; Glinz *et al.*, 2010; Knopp *et al.*, 2008; Steinmann *et al.*, 2007; Stothard *et al.*, 2008]. In our study, the sensitivity of the KAP culture was higher than that of the Baermann method, which was also reported in the studies of de Kaminsky [de Kaminsky, 1993] and Glinz [Glinz *et al.*, 2010]. In contrast, in recent studies conducted in China [Steinmann *et al.*, 2007], Zanzibar [Knopp *et al.*, 2008] and Uganda [Stothard *et al.*, 2008], the Baermann method detected up to more than an additional 20% of *S. stercoralis* larvae in terms of the observed cumulative prevalence. KAP culture requires expertise in distinguishing hookworm from *S. stercoralis* larvae, and it is not always easy to perform it in rural settings of developing countries. The Baermann method is less time consuming, but it needs a considerably larger quantity of stool, which might lead to compliance problems if an additional sample is required due to insufficient volume. Hence, neither of the methods is sufficiently valid on its own and ideally, the two should be combined whenever possible. Today, 30 -100 million people are estimated to be infected with *S. stercoralis* [Bethony *et al.*, 2006]. Given the low sensitivity of stool examination techniques most commonly used, this figure is likely to be an underestimation of the true burden of infection.

Since a true "gold standard" is not available, the results of analyzing one stool sample with a single test may not be sufficient to reach an acceptable estimate of the "true" prevalence of *S. stercoralis.* To overcome this problem, Siddiqui and Beck [Siddiqui *et al.*, 2001] proposed to analyze a single stool sample by multiple diagnostic methods simultaneously. In our study, multiple stool samples were examined using multiple diagnostic methods, which considerably increased the observed prevalence of *S. stercoralis,* consistent with observations in previous studies [Knopp *et al.*, 2008; Steinmann *et al.*, 2008; Steinmann *et al.*, 2007]. Using this approach, the final prevalence observed was close to the "true prevalence" as estimated in a mathematical model [Marti *et al.*, 1993]. Collecting a large quantity of multiple stool samples on consecutive days from children, who are at high risk of *S. stercoralis*, is always a challenge. The pointed diagnostic methods including molecular (PCR in stool sample) [Moghaddassani *et al.*, 2011] and serological (copro antigen) methods [Sykes *et al.*, 2011], which need a small amount of feces specimen, might be alternative option for prevalence studies of *S. stercoralis*. These techniques, however, require further validation and might need further development before they can be recommended for wider use. However, their cost and sophistication might hamper their introduction in resource poor countries where *S. stercoralis* is most prevalent.

Ivermectin, the drug of choice for treatment of strongyloidiasis, was highly efficacious and shows few side-effects at a single dose of 200µg/kg or 100µg/kg/day for two days. Our results confirm observations of previous studies [Bisoffi *et al.*, 2011; Datry *et al.*, 1994; Igual-Adell *et al.*, 2004; Marti *et al.*, 1996; Naquira *et al.*, 1989; Nontasut *et al.*, 2005; Shikiya *et al.*, 1992; Suputtamongkol *et al.*, 2011]. However, to demonstrate full eradication of a *S. stercoralis* infection is difficult as it could have dropped temporarily below the detection level and increase thereafter. Therefore, short follow-up periods might overestimate the complete cure. However, in populations exposed to on-going *S. stercoralis* transmission a longer follow-up period bears the risk of a re-infection. Therefore, ideal efficacy assessments for drugs against *S. stercoralis* should be conducted in non-exposed populations.

Ivermectin is not included in the list of essential drugs of the Ministry of Health of Cambodia. Although there are at least two big pharmacies in Phnom Penh where the drug is sold, the extremely high price (USD 10 per tablet) excludes its wide-scale use.

One third of cases were clear of hookworm after three weeks of single dose mebendazole treatment. Our observed low cure rate of single dose mebendazole is coinciding with the recent control trial study among school-aged children in Lao PDR [Soukhathammavong *et al.*, 2012]. Our study did not determine the efficacy of ivermectin against other STH infections.

Nevertheless, ivermectin has shown low efficacy against hookworm and *T. trichiura* infections, except *A. lumbricoides* [Marti *et al.*, 1996].

It is not surprising that the age of participants was not associated with *S. stercoralis* infection. The literature explains that individuals can acquire the infection, usually at a young age, which persists until the time of diagnosis in adulthood, thus without further exposure to infected areas [Concha *et al.*, 2005]. Nevertheless, we observed that the personal hygiene of children was a significant predictor for a *S. stercoralis* infection. *S. stercoralis* is mainly transmitted through skin penetration by the infective larvae from contaminated soil [Montes *et al.*, 2010]. The transmission of strongyloidiasis could be interrupted by improving basic personal hygiene, such as defecating in a toilet and wearing shoes when in contact with soil. Our study found almost three-quarter of strongyloidiasis cases in the population could be prevented if personal hygiene (possession and use of latrine) was improved.

In conclusion, *S. stercoralis* is highly prevalent in rural Cambodian schoolchildren. Almost two-thirds of the infections could be avoided by proper sanitation. In the absence of a “gold standard”, the examination of multiple stool samples with different diagnostic methods is required in order to reach a reliable estimate of the prevalence. An adequate therapeutic regimen in the treatment of chronic uncomplicated strongyloidiasis is ivermectin at a dose of 100μg/kg/day for two days. The availability and cost of ivermectin are critical issues in Cambodia.

Conflict of interests

The authors declare no conflict of interests

Author Contributions

Conceived and designed the experiments: VK FS HM SD SS SM PO. Performed the experiments: VK FS. Analyzed the data: VK PO. Wrote the paper: VK PO. Coordination of field work in Cambodia: SM SD.

4.6. Acknowledgments

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5. HIGH PREVALENCE AND SPATIAL DISTRIBUTION OF *STRONGYLOIDES STERCORALIS* IN RURAL CAMBODIA

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5.1. Abstract

The threadworm, *Strongyloides stercoralis*, endemic in tropical and temperate climates, is a neglected tropical disease. Its diagnosis requires specific methods and accurate information on its geographic distribution and global burden are lacking. We predicted prevalence, using Bayesian geostatistical modeling, and determined risk factors in northern Cambodia.

From February to June 2010, we performed a cross-sectional study among 2,396 participants from 60 villages in Preah Vihear Province, northern Cambodia. Two stool specimens per participant were examined using Koga agar plate culture and the Baermann method for detecting *S. stercoralis* infection. Environmental data was linked to parasitological and questionnaire data by location. Bayesian mixed logistic models were used to explore the spatial correlation of *S. stercoralis* infection risk. Bayesian Kriging was employed to predict risk at non-surveyed locations.

Of the 2,396 participants, 44.7% were infected with *S. stercoralis*. Of 1,071 strongyloidiasis cases, 339 (31.6%) were among schoolchildren and 425 (39.7%) were found in individuals under 16 years. The incidence of *S. stercoralis* infection statistically increased with age. Infection among male participants was significantly higher than among females (OR: 1.7; 95% CI: 1.4 – 2.0; P<0.001). Participants who defecated in latrines were infected significantly less than those who did not (OR: 0.6; 95% CI: 0.4 - 0.8; P=0.001). Strongyloidiasis cases would be reduced by 39% if all participants defecated in latrines. Incidence of S. stercoralis infections did not show a strong tendency toward spatial clustering in this province. The risk of infection significantly decreased with increasing rainfall and soil organic carbon content, and increased in areas with rice fields.

Prevalence of *S. stercoralis* in rural Cambodia is very high and school-aged children and adults over 45 years were the most at risk for infection. Lack of access to adequate treatment for chronic uncomplicated strongyloidiasis is an

urgent issue in Cambodia. We would expect to see similar prevalence rates elsewhere in Southeast Asia and other tropical resource poor countries.

Authors Summary

Data on the prevalence and distribution of *Strongyloides stercoralis* (threadworm) is scarce in many resource-poor countries. We carried out a cross-sectional study during the dry season among 2,396 rural Cambodians of all ages. We used a rigorous diagnostic approach, involving two stool samples per person and two examination techniques, namely, Koga agar plate culture and the Baermann method. We predicted the spatial distribution of *S. stercoralis* using Bayesian Kriging analysis. Almost half of the participants (44.7%) were infected with *S. stercoralis*. Of the *S. stercoralis* cases, 39.7% involved participants under 16 years old. *S. stercoralis* infection prevalence was significantly higher in males than in females. Participants younger than 10 years old had a lower risk of infection than did older participants. Furthermore, our study showed that toilet use could prevent threadworm infections by 39%. Infection prevalence in the province was negatively associated with rainfall and soil organic content and positively associated with land covered by rice fields. We conclude that access to adequate treatment for *S. stercoralis* must be addressed in Cambodia. Infection prevalence is likely to be similar in other countries of the region and the developing world.

5.2. Introduction

Strongyloides stercoralis, a soil-transmitted nematode, is a neglected tropical helminthiasis [Olsen *et al.*, 2009; Schär *et al.*, 2013b] and endemic in tropical, subtropical and temperate settings where sanitary and hygiene conditions are poor [Foreman *et al.*, 2006; Marcos *et al.*, 2008]. However, the worldwide prevalence of *S. stercoralis* is heterogeneously distributed [Schär *et al.*, 2013b] and the current estimation of infection remains underestimated due to the use of inadequate diagnostic method [Bisoffi *et al.*, 2013].The available information about *S. stercoralis* infection in developing countries mostly comes from studies in Brazil and Thailand [Schär *et al.*, 2013b].

The gastrointestinal symptoms of the disease include diarrhea and abdominal pain, while dermatological symptoms include itching, rash (urticaria) and migrating larvae in the skin (larva currens) [Khieu *et al.*, 2013b; Koczka *et al.*, 2012; Ly *et al.*, 2003; Vadlamudi *et al.*, 2006]. However, more than 50% of all infections remain asymptomatic [Foreman *et al.*, 2006]. Due to its particular ability for autoinfection, *S. stercoralis* is the only soil-transmitted helminth (STH) that can lead to systemic infection with high parasite densities and severe to potentially fatal complications, especially in immunosuppressed hosts [Basile *et al.*, 2010; Marcos *et al.*, 2008; Vadlamudi *et al.*, 2006]. Ivermectin is recommended as the most effective treatment [WHO, 2002].

The presence of *S. stercoralis* larvae in stool specimens is proof of infection [Siddiqui *et al.*, 2001]. Koga-agar plate (KAP) culture [Koga *et al.*, 1991] and the Baermann method [Garcia *et al.*, 2001] are specific diagnostic methods for strongyloidiasis. However, their sensitivity is not satisfactory when testing a single stool sample in cases of chronic, uncomplicated strongyloidiasis [Khieu *et al.*, 2013a; Marchi Blatt *et al.*, 2003; Requena-Mendez *et al.*, 2013; Schär *et al.*, 2014a].

In Cambodia, data from several cross-sectional studies in community and hospital settings revealed *S. stercoralis* prevalences between 2.6 % and 31.5%.

However, in all but three studies, a diagnostic approach with low sensitivity was used on a single stool sample [Chhakda *et al.*, 2006; Koga-Kita, 2004; Longfils *et al.*, 2005; Moore *et al.*, 2012b]. Three recent studies used a combined diagnostic approach (KAP culture and Baermann technique) on two [Khieu *et al.*, 2013b; Schär *et al.*, 2014b] and three stool samples [Khieu *et al.*, 2013a].

We aimed to determine the prevalence, risk factors and spatial distribution of *S. stercoralis* infection in Preah Vihear province. We conducted a cross-sectional study of *S. stercoralis* infection, using KAP culture and the Baermann method on two stool samples from each participant in 60 villages of Preah Vihear province, northern Cambodia.

5.3. Materials and methods

Ethical considerations

The research was approved by the Ethics Committee of the Cantons of Basel-Stadt and Baselland (EKBB, #16/10, dated 1 February 2010), Switzerland, and by the National Ethics Committee for Health Research, Ministry of Health, Cambodia (NECHR, #004, dated 5 February 2010). Written informed consent was obtained from each participant prior to the start of the study. For participants between the ages of 1 and 18 years, written informed consent was obtained from the parents, legal guardian or appropriate literate substitute. All participants were informed of the study's purpose and procedures prior to enrolment.

All participants infected with *S. stercoralis* were treated with a single oral dose of ivermectin (200µg/kg BW) [Marti *et al.*, 1996]. All other parasitic infections were treated according to the guidelines of the National Helminth Control Program of Cambodia [CNM, 2004].

Study setting and population

The study was conducted in 60 rural villages of Preah Vihear province, Northern Cambodia (Figure 9). The villages were randomly selected from a list of all villages in six of the seven districts in Preah Vihear province (total number of villages: 184). The district of Chhaeb was not included as most villages in this district are difficult to access by car, which was necessary to ensure the rapid transfer (three hours by car) of stool samples to one of the two temporary laboratories established in the health centers of Kulen and Rovieng districts.

A cross-sectional study was carried out from February to June 2010 among all the population living in 60 villages. Fifteen households were randomly selected from the list of all households in the selected villages. All household members one year of age and older were eligible for inclusion in the study and all household members present on the day of the survey were enrolled.

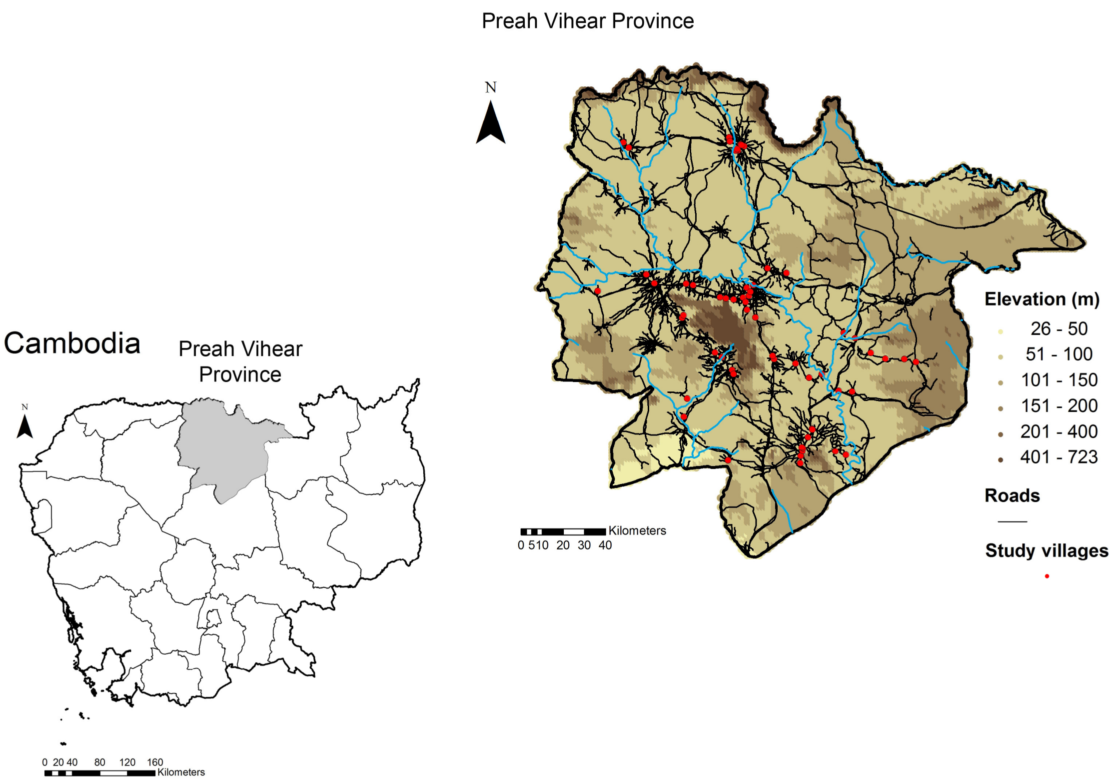


Figure 9: Map of the study villages in Preah Vihear province, Cambodia, 2010

Field and laboratory procedures

After obtaining written informed consent from participants, an individual questionnaire was administered to obtain demographic information (age, gender, educational level and profession), personal risk-perception (knowledge about worm infections), and behavioral data (personal hygiene practices, wearing shoes, and latrine use). The head of household was interviewed, based on a household questionnaire, about socioeconomic indicators such as house type, household assets, latrine and livestock. All questionnaires were pre-tested. After the interview, each participant was given a pre-labeled plastic container (ID code, name, sex, age and date) for stool sample collection. The next morning, the filled stool container was collected and a second empty, pre-labeled one was handed out for a second stool sample of the following day.

Stool samples were transported at ambient temperature and arrived at the laboratory within three hours of collection. Laboratory technicians from the National Center for Parasitology, Entomology and Malaria Control (CNM), Phnom Penh, processed the stool specimens in one of two laboratories established in Kulen and Rovieng health centers, respectively. First, a single Kato-Katz thick smear [Katz *et al.*, 1972] was prepared using the WHO standard template and examined under a light microscope to detect helminth eggs. Eggs were counted and recorded for each helminth species separately. Second, KAP culture [Koga *et al.*, 1991] was used to detect *S. stercoralis* larvae. A hazelnut-sized stool sample was placed in the middle of the agar plate and the closed Petri dish was incubated in a humid chamber for 48 hours at 28°C. Afterwards, the plates were visually examined for the presence of larval tracks. The plates were then rinsed with sodium acetate-acetic acid-formalin (SAF) solution. The eluent was centrifuged and the sediment was examined under a microscope for the presence of larvae. Based on morphology, larvae were identified (i.e., size of buccal cavity, presence of genital primordium (L1), presence of forked tail-end (L3)) as either *S. stercoralis* or hookworm larvae. Finally, the Baermann technique [Garcia *et al.*, 2001] was performed to detect *S. stercoralis* larvae. A walnut-sized stool sample was placed on gauze inserted into a glass funnel and covered with water. The apparatus was exposed for two hours to artificial light directed from below. After centrifuging the collected liquid, the sediment was examined under a microscope for the presence of *S. stercoralis* larvae.

Quality control

For quality control, the technicians were specifically trained on the morphological criteria for distinguishing hookworm and *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.

Environmental data collection

Day and night land surface temperature (LST), enhanced vegetation index (EVI) and land use/land cover (LULC) 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 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 at a spatial resolution of 9 x 9 km, including bulk density, soil organic carbon content and pH, was extracted from the International Soil Reference and Information Center's (ISRIC) World Inventory Soil Emission Potentials (WISE), version 1.0 (http://www.isric.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.

Statistical analyses

Questionnaire and laboratory data

Questionnaire and laboratory data collected from each participant were entered twice and validated in EpiData version 3.1 (EpiData Association; Odense, Denmark). Statistical analyses were performed with STATA version 12.1 (StataCorp.; College Station, TX, USA). Only participants with complete records (two stool samples examined with all diagnostic methods and completed questionnaires) were included in the final analyses. Smoothed age prevalence was used to present the infection prevalence distribution by participant's age. *P-values* less than 0.05 were considered to indicate a significant association.

Data on household assets and livestock were used to build the socioeconomic status (SES), employing principle component analysis (PCA). SES status was defined according to one of three wealth tiers, from poor to least poor [Vyas *et al.*, 2006].

Generalized Estimating Equations (GEE) were used to assess the association between infection status and demographic variables, hygienic status, knowledge, recent medical history of participants and environmental factors. Variables with odds ratios below 0.80 and above 1.25 in the bivariate models were selected for inclusion in the multivariate GEE model. Population attributable fraction was calculated for significantly associated risks.

Environmental data

ArcGIS version 10.0 (ESRI; Redlands, CA, USA) was used for environmental data processing, geo-referencing and map making. Environmental data was linked to parasitological and questionnaire data according to location. Data management and bivariate regressions were performed in STATA version 12.1. Bayesian multivariate models were fitted using WinBUGS version 1.4.3 (Imperial College & Medical Research Council; London, UK). Spatial analysis was performed using mixed logistic regression models. The association between infection risk and environmental covariates was assessed at a 15% significance level, as determined by the likelihood ratio test (LRT), with mixed bivariate logistic regressions accounting for village-level correlation with an exchangeable random effect. To explore the village-level correlation of *S. stercoralis* infection risk, Bayesian mixed logistic models were run in absence of covariates. Bayesian models with or without environmental covariates were run alternately with a spatial and a non-spatial exchangeable random effect. 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. Non-informative prior distributions were chosen for all other parameters. Markov chain Monte Carlo (MCMC) simulation was used to estimate model parameters [Gelfand *et al.*, 1990]. Convergence was assessed by examining the ergodic averages of selected parameters. For all models, a burn-in of 5,000 was followed by 50,000 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]. DIC was retrieved after 10,000 additional iterations.

S. stercoralis infection risk was predicted at non-surveyed locations using Bayesian Kriging [Diggle *et al.*, 1998]. For model validation, 48 randomly selected villages were used for fitting and the 12 remaining were used as test locations. Models were run with either a spatial random effect, using the WinBUGS "spatial.unipred" function, or with an exchangeable random effect [Lunn *et al.*, 2000]. Predictive ability was assessed using the probability coverage of the shortest Bayesian credible interval, the Mean Squared Error and a χ^2 test analogue [Gosoniu *et al.*, 2006; Riedel *et al.*, 2010]. Based on environmental factors only, predictions were made at 16,532 pixels of a 1x1 km resolution, using an exchangeable random effect.

5.4. Results

Compliance and study population

Overall, 3,560 individuals from 616 households (average household size: 5; range: 1 – 12) were enrolled, of which 2,748 (77.2%) participants submitted two stool samples. The final analysis included 2,396 (67.3%) participants with complete data records, i.e., two stool specimens examined with all diagnostic tests and all questionnaires completed.

The median age of the participants was 20 years, with a range from 1 to 85 years. One thousand three hundred and fifty-five (56.5%) participants were females. Half of the participants (48.5%) were farmers and 33.0% were pupils. The majority of participants (58.3%) had attended primary school; one third (32.2%) had not received primary education.

Parasitological findings

Seven intestinal parasite species were found in the stool samples. Hookworm and *S. stercoralis* were most common, with a prevalence of 46.7% and 44.7%, respectively. *Taenia* sp. was found in 0.4% of participants, while *Hymenolepis nana* and *Enterobius vermicularis* were observed in 0.2% and 0.1% of participants, respectively. Both *Ascaris lumbricoides* and *Trichuris trichiura* were observed in 0.3% of participants. Of the 1,071 *S. stercoralis* cases, 642 (59.9%) were co-infected with hookworm.

Performance of the diagnostic methods

Table 6 summarizes the results of KAP culture and Baermann tests for the 1,071 *S. stercoralis* cases (44.7%) detected. KAP culture and the Baermann technique detected 877 and 823 cases, respectively. The total of all positive cases diagnosed by any of the two methods was considered the "diagnostic gold standard". The sensitivity of the KAP culture was 81.9%, and that of the Baermann technique, 76.8%. The negative predictive values were 87.2% and 84.2%, while the positive predictive values were 81.8% and 76.8% for KAP culture and Baermann technique, respectively.

Table 6: Koga agar plate culture and Baermann methods for the detection of *S. stercoralis* in 2396 participants, Preah Vihear province, Cambodia, 2010

		Combined Methods (KAP culture and Baermann)		Total
		Positive	Negative	
KAP culture	Positive	877	0	877
	Negative	194	1325	1519
	Total	1071	1325	2396
Baermann method	Positive	823	0	823
	Negative	248	1325	1573
	Total	1071	1325	2396

Characteristics of Strongyloides stercoralis cases

Of 1,071 *S. stercoralis* cases, half were females (50.1%), half were farmers (51.1%), and 425 (39.7%) cases were diagnosed in individuals under 16 years. The majority (57.0%) attended primary school, while one third (33.6%) reported no schooling. Figure 10 shows the smoothed age prevalence stratified by gender. The prevalence of *S. stercoralis* increased rapidly with age, particularly in the first eight years of life, where after it leveled off in females but continued to rise slowly in males. Prevalence rose from 31.4% in children, aged five, to 51.2% in participants older than 50. In all age groups, prevalence was higher in males than in females.

The multivariate GEE found that gender was significantly associated with *S. stercoralis* infection (mOR: 1.7; 95% CI: 1.4 – 2.0; *P*<0.001). Compared to children under six years old, all age groups had a higher risk for infection. Participants who reported having been treated for worms were less frequently infected with *S. stercoralis* than those who did not report taking anthelminthic drugs (mOR: 0.7; 95% CI: 0.6 – 0.8; *P*<0.001). In addition, participants who usually defecated in latrines were significantly less infected with *S. stercoralis* than those who did not use latrines (mOR: 0.6; 95% CI: 0.4 – 0.8; *P*=0.001). No additional predictor of *S. stercoralis* infection relating to personal disease perception and hygiene was found in the multiple regression analysis. Looking at environmental factors, risk significantly decreased with increasing rainfall (mOR: 0.8; 95% CI: 0.7 - 0.9; *P*= 0.004) and soil organic carbon content (mOR: 0.6; 95% CI: 0.5 - 0.9; *P*=0.003). The land cover class corresponding to croplands was associated with an increased risk for infection (mOR: 1.7, 95% CI: 1.2 - 2.4; *P*=0.004) (Table 7).

During the two weeks preceding examinations for *S. stercoralis*, 50.5% of participants reported an episode of diarrhea, 12.7% had experienced nausea and 59.1% complained about abdominal pain. However, none of these clinical symptoms was significantly associated with *S. stercoralis* infection.

Population attributable risk analysis found that the number of strongyloidiasis cases would be reduced by 39% if all participants used a latrine for defecation.

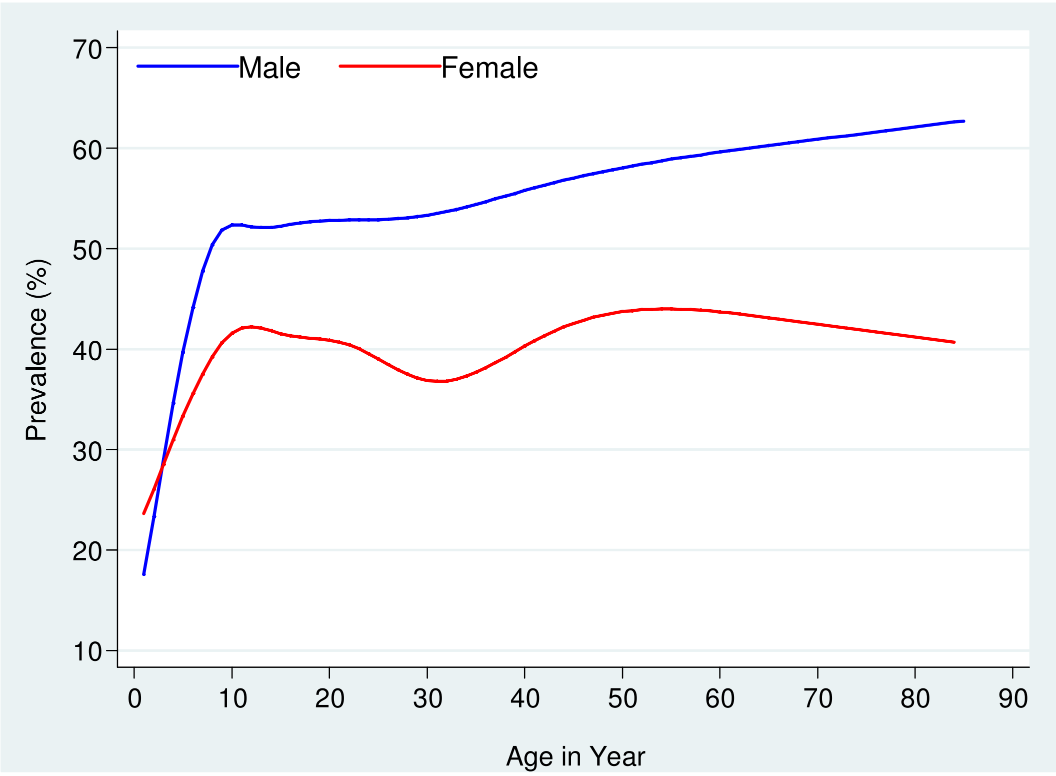


Figure 10: Smoothed age prevalence of *S. stercoralis* infection by sex among 2396 participants in Preah Vihear province, Cambodia, 2010

Table 7: Risk factors for *S. stercoralis* infection in the multivariate GEE among 2396 participants, Preah Vihear province, Cambodia, 2010

	Non-*S. stercoralis* (N=1325) n (%)	*S. stercoralis* (N=1071) n (%)	mOR (95% CI)	p-Value
Gender (male compared to women)	507 (38.37)	534 (49.9)	1.7 (1.4 – 2.0)	<0.001
Age group				
1 - 5 years				
6 - 15 years				
16 - 30 years	129 (9.7)	59 (5.5)	Reference	
31 - 45 years	453 (34.2)	366 (34.2)	2.3 (1.4 - 3.6)	<0.001
> 45 years	352 (26.6)	287 (26.8)	1.8 (1.2 – 3.0)	0.01
Profession	220 (16.6)	174 (16.2)	1.7 (1.0 - 2.8)	0.04
Farmer/Rice-Grower	171 (12.9)	185 (17.3)	2.2 (1.4 - 3.7)	0.001
Pupil				
Others	615 (46.4)	547 (51.1)	Reference	
Has been treated for worms (yes)	451 (34.0)	339 (31.6)	0.8 (0.6 - 1.2)	0.275
Knows about worms/infection with worms (yes)	259 (19.6)	185 (17.3)	1.0 (0.7 - 1.3)	0.859
Usually defecated in toilet (yes)	450 (33.9)	279 (26.0)	0.7 (0.6 - 0.8)	<0.001
Had shoes (yes)	240 (18.1)	234 (21.8)	1.3 (1.1 - 1.7)	0.017
Rainfall (year mean)	195 (14.7)	81 (7.5)	0.6 (0.4 - 0.8)	0.001
Soil organic carbon content (10-20g/kg)	1203 (90.8)	999 (93.3)	1.1 (0.8 - 1.6)	0.657
Land cover	-	-	0.9 (0.8 – 1.1)	0.359
Savanna and shrubland	-	-	0.8 (0.7 - 0.9)	0.004
Forest	773 (58.3)	485 (45.3)	0.6 (0.5 - 0.9)	0.003
Grassland				
Cropland and crop-natural vegetation mosaic	441 (33.3)	283 (26.4)	Reference	

mOR: Multiple Odds Ratio ; 95% CI: 95% Confidence Interval

Spatial analysis of Strongyloides stercoralis infection risk

The spatial model run without covariates indicated very little spatial correlation of infection risk, as indicated by the 1 km range. The small residual (unexplained) within village variance (σ) also indicated a weak clustering tendency of *S. stercoralis* infection risk. Parameters of these models are presented in Table 8. After introducing LST night, rainfall, soil carbon content and land cover, the model with an exchangeable random effect fitted the data slightly better, as indicated by the lower DIC. Environmental covariates explained 45% of the village-level variability and the range dropped under a kilometer after covariates were introduced in the model.

Table 8: Model parameters in absence or presence of covariates for the spatial models and their non-spatial counterparts

Model parameters	No covariates		Environmental covariates	
	Non-spatial (95% CI)	Spatial (95% CI)	Non-spatial (95% CI)	Spatial (95% CI)
DIC	3136.4	3135.6	3130.6	3130.8
σ	0.4 (0.2 - 0.7)	0.4 (0.2 - 0.7)	0.2 (0.1 - 0.4)	0.2 (0.1 - 0.4)
ρ	n.a.	317.8 (23.6 - 1558.0)	n.a.	812.5 (98.8 - 1594.0)
Range (km)	n.a.	1.1 0.2 - 14.0)	n.a.	0.4 (0.2 - 3.2)

CI, credible interval; DIC, deviance information criterion (a measure of model fit; a lower DIC indicates a better fit); σ 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%

Prediction of Strongyloides stercoralis infection risk and model validation

Mixed bivariate logistic regressions revealed no association at 15% significance level between *S. stercoralis* infection risk and any yearly summary measure of altitude, LST day, EVI, soil pH or bulk density. LST night (P=0.072), yearly means of rainfall estimates (P<0.0001), soil organic carbon content (P=0.002) and land cover (P=0.107) were associated with infection risk and were used to predict *S. stercoralis* infection risk throughout Preah Vihear province. Apart from LST night, all covariates remained significant in the multivariate model and ORs were similar to those obtained in the multivariate GEE for the risk factor analysis (data not shown). Maps of the covariates used predict infection in Preah Vihear province are presented in Figure 11.

Model validation revealed that both models were able to correctly predict prevalence for 100% of the test locations, within a 95% credible interval. However the non-spatial model, i.e. with an exchangeable random effect, had slightly better predictive ability (MSE: 0.0226 and 0.0229, χ^2: 13.22 and 13.59 for the non-spatial and spatial models, respectively). Therefore, the non-spatial model was used to predict *S. stercoralis* infection risk in Preah Vihear province, Cambodia. Figure 12 displays the *S. stercoralis* predicted median prevalence in Preah Vihear province (Figure 12A), together with the uncertainty of the estimates (Figure 12B) as expressed by the error coefficient (the ratio between the predicted median and its standard deviation). The lower (2.5%) and upper (97.5%) credible intervals of the predicted *S. stercoralis* prevalence are presented in Figure (12C) and (12D), respectively. Results were consistent with observed prevalence at surveyed locations.

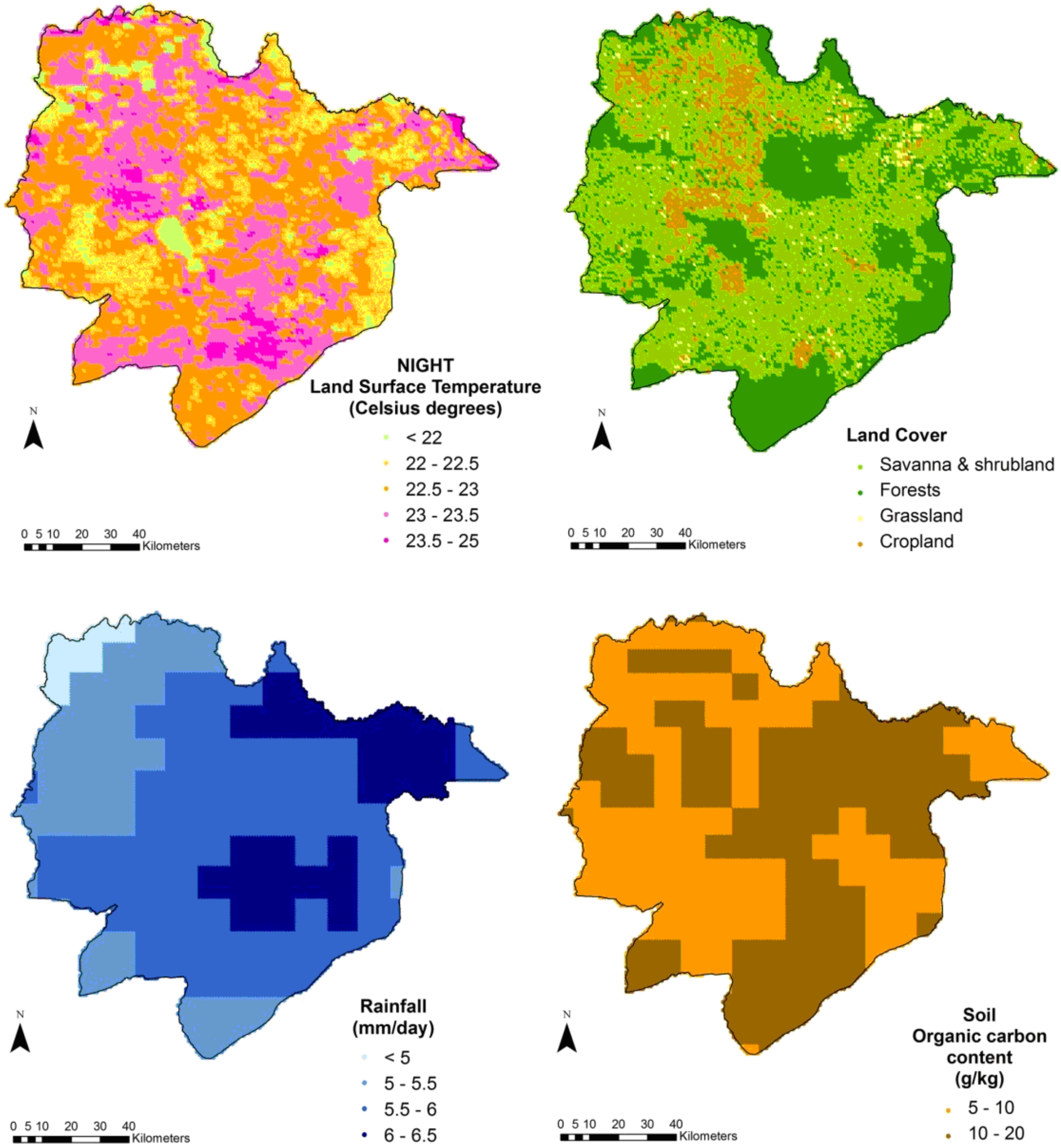
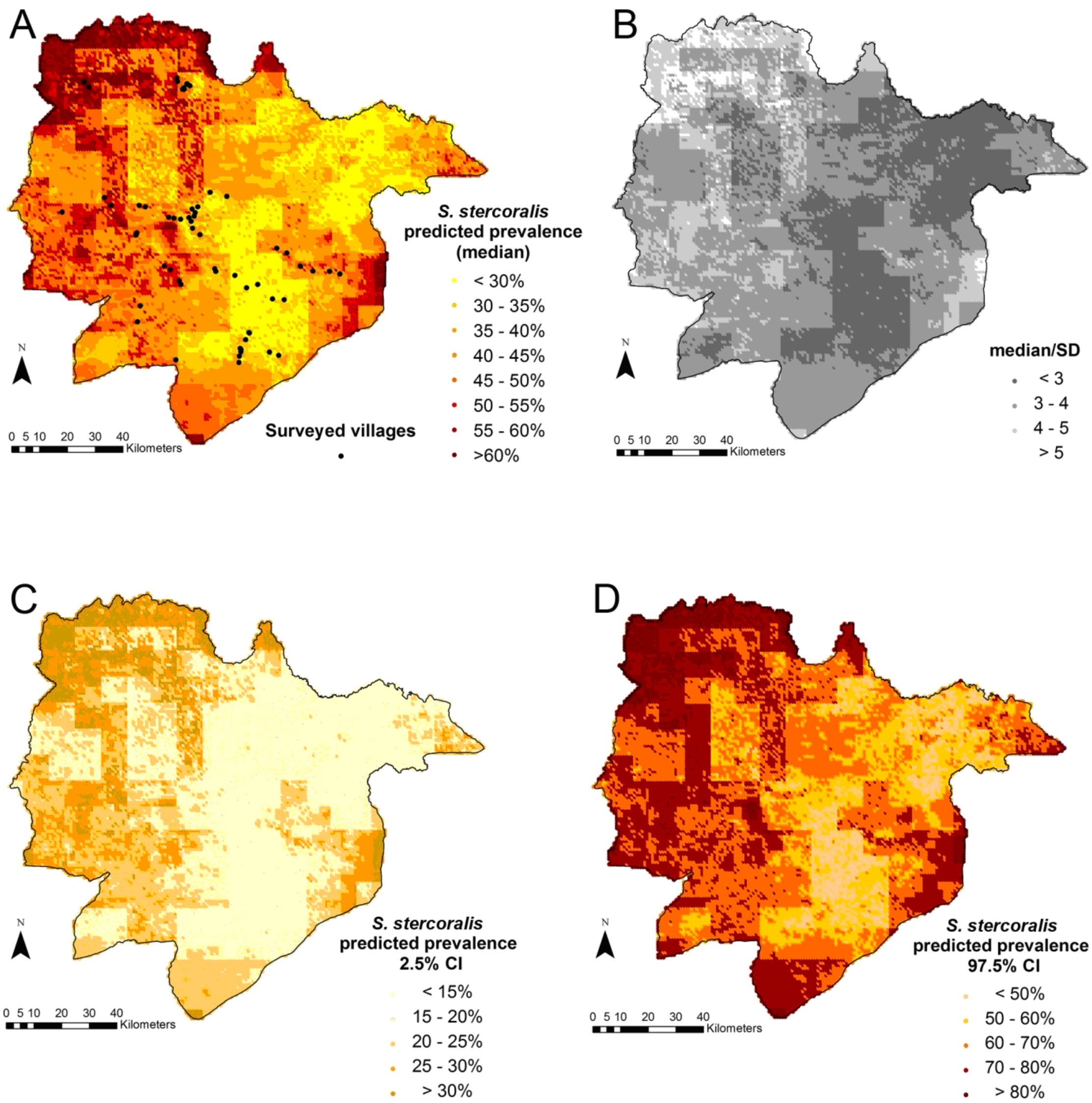


Figure 11: Distribution of environmental factors used to predict *S. stercoralis* prevalence in Preah Vihear province, Cambodia



The error coefficient is the ratio of the predicted median over its standard deviation; a higher value indicates a higher precision. The lower and upper estimates correspond to the 2.5% and 97.5% borders of the Bayesian credible interval, respectively.

Figure 12: Predicted *S. stercoralis* median prevalence (A), error coefficient (B); lower (C), and higher (D) estimates of *S. stercoralis* predicted prevalence in Preah Vihear Province, Cambodia

5.5. Discussion

Many epidemiological aspects of *S. stercoralis* infection are poorly understood [Paula *et al.*, 2011]. The available information on the prevalence of *S. stercoralis* comes from studies on other STHs, where diagnostic methods with low-sensitivity for *S. stercoralis* and only a single stool sample were mostly used [Khieu *et al.*, 2013a; Olsen *et al.*, 2009; Schär *et al.*, 2013b]. To reach an acceptable estimate of the ''true'' prevalence of *S. stercoralis*, Siddiqui and Beck [Siddiqui *et al.*, 2001], and Khieu et al. [Khieu *et al.*, 2013a] proposed analyzing multiple stool samples with multiple diagnostic techniques simultaneously.

In our study of *S. stercoralis* among a rural population living in 60 villages in northern Cambodia, we examined two stool samples using two diagnostic techniques (KAP culture and Baermann method) specifically targeting *S. stercoralis* and found that 44.7% of the participants were infected. Children under the age of six accounted for 5.5% of the infections, while prevalence increased with age.

Almost every second individual in our study population was infected with *S. stercoralis*. To our knowledge, this is one of the highest prevalence ever reported, compared to other studies in highly endemic areas like Cambodia [Khieu *et al.*, 2014; Khieu *et al.*, 2013a; Schär *et al.*, 2013b], Laos [Sayasone *et al.*, 2009], Thailand [Nontasut *et al.*, 2005], Brazil [Paula *et al.*, 2011] and China [Wang *et al.*, 2013], or in other countries. The main reason for such high prevalence is likely to be due to the more rigorous diagnostic approach employed in our study (number of stool specimen, multiple diagnostic methods), compared to the other studies, where a single method to examine a single fecal sample was used. Yet, the prevalence we observed is also substantially higher than that of other studies using the similar diagnostic approaches. Two recent studies in Kandal and Takeo provinces in Cambodia reported that about a quarter (24.4%) of schoolchildren and 21.0% of the general population were infected, respectively [Khieu *et al.*, 2014; Khieu *et al.*, 2013a]; while Steinmann et al., and Knopp et al. found a prevalence rate of 11.7% in a village in Yunnan, China and of 10.8% among schoolchildren in Zanzibar, respectively [Knopp *et al.*, 2008; Steinmann *et al.*, 2007]. Hence, other factors such socioeconomic and sanitary conditions are likely to contribute to the differences observed.

In the absence of a gold standard for diagnosing *S. stercoralis*, KAP culture [Koga *et al.*, 1991] and the Baermann method [Garcia *et al.*, 2001] are widely used for detecting the parasite microscopically. Our study found that KAP culture was more sensitive than the Baermann method, which is consistent with reports from Cambodia [Khieu *et al.*, 2014; Khieu *et al.*, 2013a], rural Côte d'Ivoire [Glinz *et al.*, 2010], Brazil [Ines Ede *et al.*, 2011] and Honduras [de Kaminsky, 1993]. However, the opposite was observed in studies in south-central Côte d'Ivoire [Becker *et al.*, 2011], Zanzibar [Knopp *et al.*, 2008], China [Steinmann *et al.*, 2007] and Uganda [Stothard *et al.*, 2008]. This seems to indicate that neither method is superior. As either technique will fail to identify a certain number of infections, the combined use of both methods is recommended for optimal sensitivity.

We found that about one third of children under six (59 of 188 children) were already infected with *S. stercoralis*. This hints at a high contamination of the environment, such that children easily become infected when playing on the ground around the house or barefoot in the village. The fact that prevalence steadily increases with age can be explained by the fact that once infected at a young age, an infection can persist in an untreated individual for their entire life [Concha *et al.*, 2005; Prendki *et al.*, 2011]. Personal hygiene (not using a toilet for defecating) as a significant predictor of *S. stercoralis* infection was also observed in a study in south-central Cambodia [Khieu *et al.*, 2013a]. This connection is obvious: with proper disposal of the feces, contamination of the surrounding area with infective larvae decreases. We calculated that 39.0% of *S. stercoralis* cases in the study area could be prevented if everyone were to defecate in a toilet. The cycle of *S. stercoralis* transmission could thus be interrupted by improving personal hygiene and sanitation. Strongyloidiasis is almost non-existent in countries where sanitation and human waste disposal have improved [WHO, 2013].

S. stercoralis infections were ubiquitous the study setting and exhibited a weak tendency to spatial clustering in the Preah Vihear province, as indicated by the low location-specific variance parameter. A low clustering tendency was also observed for hookworm, in the Region of Man, Côte d'Ivoire and Ghana [Raso *et al.*, 2006; Soares Magalhaes *et al.*, 2011]. However, the lack of spatial correlation in this analysis is likely due to the study's small scale. This does not preclude *S. stercoralis* infection risk from spatially clustering at country or regional level, since environmental factors delimit suitable ecological zones for parasites at larger scales [Brooker, 2007].

Still, even at this provincial scale, we found significant associations with rainfall, soil organic carbon content and croplands both in the predictive model and after adjusting for demographic and behavioral factors. Our risk predictions yielded two broad risk zones: a lower risk zone in the East of the province and a higher risk zone in the West, characterized by lower rainfall and soil organic carbon content and a higher proportion of zones occupied by cropland. Since there was no indication of spatial correlation, risk prediction was carried out using an exchangeable random effect and relied on the predictors only. While a negative association between rainfall and infection risk was also identified in Thailand, a laboratory study found that *S. stercoralis* development was impaired by submersion of stools in water [Anamnart *et al.*, 2013]. Hypothetically, the decreased risk of *S. stercoralis* infection in the East of the Province where rainfall was higher, might relate to more extensive or long lasting flooding that could negatively affect *S. stercoralis* transmission. Another possibility might be that higher rainfall in the East reduces parasite survival rates, as parasites are washed away by run-off water down steeper slopes. We found that lower soil carbon content was associated with increased risk of infection (in the West). A full profile of soil type information was unavailable for this setting and soil organic carbon content depends on a complex interplay of environmental and soil features, so interpretation is limited. But, in general, soil organic content tends to decrease with increased forest destruction, burning of savannas and land use for agriculture [Buringh, 1984]. Hence, the association of increased risk of infection with lower soil carbon contents in our setting might relate to human activities such as slash-and-burn practices that destroy forests to create agricultural lands. Moreover, risk of infection was found to increase in croplands, a MODIS land cover category that specifically corresponds to soils that are alternately bare and cultivated. In our setting, these are rice fields [Friedl *et al.*, 2002]. Half (51.7%) of the study villages are surrounded by rice fields and 54.9% of participants infected with *S. stercoralis* live in such environments. Risk might be increased further by regular soil contamination by defecation around the fields and exposure during agricultural activities. Indeed, open defecation was the usual habit for 88.5% of participants. Finally, the small cluster size (1 km) of infection risk suggests that *S. stercoralis* transmission occurs within villages rather than between them and may relate to the location of defecation sites within and close to the villages.

We conclude that *S. stercoralis* infection is highly prevalent in rural communities of Cambodia. School-aged children and adults over 45 years were the most at risk for infection. Almost 40% of infections could be avoided by proper personal hygiene. Access to adequate treatment for chronic uncomplicated strongyloidiasis is low. Given its potential to produce potentially fatal disseminated infections, further epidemiological data on this parasite in other endemic areas are urgently needed.

Competing Interests

The authors have declared that no competing interests exist.

Author Contributions

VK, FS, SM and PO conceived and designed the study; VK and FS performed the fieldwork under the supervision of SM and PO; AF downloaded and managed environmental data; AF performed spatial analysis with PV; HM coordinated the laboratory activities; SD and SM coordinated the field work in Cambodia; VK and JH analyzed data and interpreted results together with PO; VK wrote the manuscript with PO; PO supervised the first author in all aspect of the study; all authors have read and approved the final version of manuscript.

5.6. Acknowledgments

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6. PREVALENCE AND RISK FACTORS OF *STRONGYLOIDES STERCORALIS* IN TAKEO PROVINCE, CAMBODIA

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6.1. Abstract

The threadworm *Strongyloides stercoralis*, the most neglected helminth, affects an estimated 30-100 million people worldwide. Information on *S. stercoralis* infection is scarce in tropical and sub-tropical resource poor countries, including Cambodia. We determined *S. stercoralis* infection prevalence and risk factors for infection in the general population in Southern Cambodia.

A cross-sectional study was carried out between January and April 2011 among 2,861 participants living in 60 villages of Takeo province, using Koga-agar plate culture, the Baermann technique on a single stool sample.

Eight intestinal helminth species were diagnosed. Hookworm (31.4%) and *S. stercoralis* (21.0%) occurred most frequently. Prevalence of *S. stercoralis* infection increased with age. In all age groups a higher prevalence was found among males than among females (OR: 1.7; 95% CI: 1.4 – 2.0; *P* <0.001). Participants who had a latrine at home were significantly less frequently infected with *S. stercoralis* than those who did not (OR: 0.7; 95% CI: 0.4 – 0.8; *P*: 0.003). Muscle pain (OR: 1.3; 95% CI: 1.0 – 1.6; *P*: 0.028) and urticaria (OR: 1.4; 95% CI: 1.1 – 1.8; *P*: 0.001) were significantly associated with *S. stercoralis* infection.

S. stercoralis is highly prevalent among the general Cambodian population and should no longer be neglected. Access to adequate diagnosis and treatment is urgently needed.

Keywords: *Strongyloides stercoralis*, prevalence, risk factors, clinical manifestation, Cambodia

6.2. Introduction

Strongyloides stercoralis, a soil-transmitted nematode, is arguably the most neglected tropical disease [Olsen *et al.*, 2009], yet an estimated 30 – 100 million people are infected worldwide [Bethony *et al.*, 2006]. Despite its frequency, epidemiological data on prevalence and geographical variations are largely lacking [Paula *et al.*, 2011]. The prevalence of *S. stercoralis* is often underestimated, as most diagnostic methods used have a low sensitivity for *S. stercoralis* [Khieu *et al.*, 2013a; Requena-Mendez *et al.*, 2013; Schär *et al.*, 2013b]. Furthermore, in resource poor countries, environmental conditions and poor hygiene behaviour favour transmission. For Cambodia, only a few reports are available, giving prevalences ranging from 2.6% to 24.4% [Chhakda *et al.*, 2006; Khieu *et al.*, 2013a; Koga-Kita, 2004; Longfils *et al.*, 2005; Moore *et al.*, 2012a].

To date, there is no universally agreed upon gold standard for diagnosing *S. stercoralis*. Molecular and serological methods have been reported as promising diagnostic tools, yet these techniques require further validation or further development [Schär *et al.*, 2013a]. Therefore, definitive diagnosis relies on the detection of larvae in stool specimens [Requena-Mendez *et al.*, 2013]. The Baermann technique [Garcia *et al.*, 2001] and Koga-agar plate (KAP) culture [Koga *et al.*, 1991] are the coprological diagnostic methods most often employed. The sensitivity and specificity of either technique alone is not satisfactory [Glinz *et al.*, 2010; Ines Ede *et al.*, 2011; Khieu *et al.*, 2013a; Steinmann *et al.*, 2007]. Thus, to increase the likelihood of detecting *S. stercoralis*, combined use of these diagnostic tests has been suggested [Khieu *et al.*, 2013a].

S. stercoralis infection is acquired by infective filariform larvae (L_3), originating from contaminated soil [Getaneh *et al.*, 2010], directly penetrating the skin. The clinical presentation of strongyloidiasis is extremely variable, ranging from asymptomatic patients to patients with gastrointestinal symptoms (e.g., abdominal pain, diarrhoea) and urticaria [Khieu *et al.*, 2013b], to disseminated infections with mortality rates as high as 87% [Vadlamudi *et al.*, 2006].

The present cross-sectional study aimed to determine the prevalence, risk factors and associated clinical manifestations of *S. stercoralis* infection in a random population sample living throughout 60 villages of Takeo province, southern Cambodia.

6.3. Materials and methods

Ethical considerations

The study was approved by the National Ethics Committee for Health Research, Ministry of Health of Cambodia (NECHR # 185, dated 20 December 2010) and by the Ethics Committee of the Cantons of Basel-Stadt and Baselland, Switzerland (EKBB #14/11, 13 January 2011). All participants were informed in Khmer language about the purpose and procedures of the study. Written informed consent was obtained from all participants prior to enrolment. For participants aged 1 to 18 years, consent was obtained from the parents, legal guardian or appropriate literate substitutes.

All participants infected with *S. stercoralis* were treated with a single oral dose of ivermectin (200µg/kg body weight) [Marti *et al.*, 1996]. All other parasitic infections were treated according to the guidelines of the National Helminth Control Program of Cambodia [CNM, 2004].

Study design, area and population

A cross-sectional study was carried out from January to April, 2011, among the general population living throughout 60 villages of Takeo province, southern Cambodia (Figure 13). The villages were randomly selected from a list of all villages in the 10 districts of Takeo province (total number of villages: 1,118).

Takeo province, with its expansive floodplains, is located in the South of Cambodia, about 80 kilometres from the capital, Phnom Penh, along the border with Vietnam. Takeo has 843,931 inhabitants, with a density of 237 people per square kilometre. The economy is based on agriculture, namely fisheries, rice and fruit cropping. Pigs, dogs, cats, poultry and cattle are the most common domestic animals [NIS, 2008]. No information on the prevalence of *S. stercoralis* is available for Takeo province. However, the prevalences of intestinal helminth infections have recently been reported in Ang Svay Chek villages, Prey Kabas district, and were 13.2% for hookworm and 47.5% for *Opisthorchis viverrini and* minute intestinal flukes togehter [Yong *et al.*, 2012].

Fifteen households were randomly selected from each village. All household members, 12 months or older, were eligible and informed about the study. All household members present on the day of the survey were enrolled.

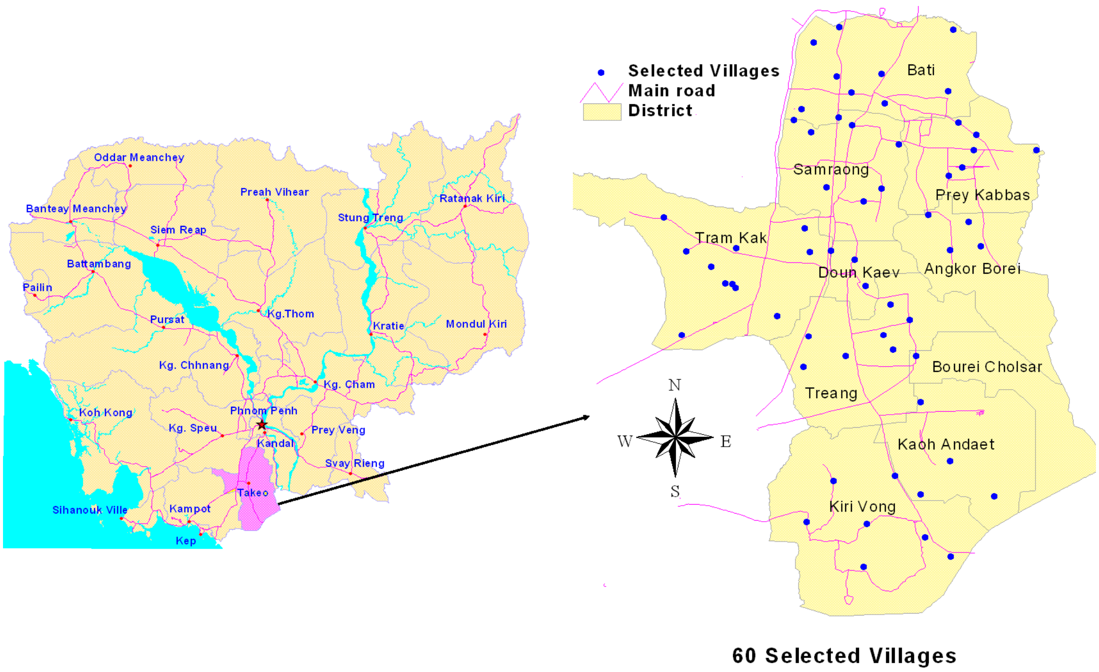

Figure 13: Map of selected villages in Takeo province, Cambodia, 2011

Field procedures

A pre-tested household questionnaire was administered to the head of household to obtain information on socioeconomic indicators such as house type (concrete, wood or leaves), household assets (sewing machine, television, radio, motorcycle or car), livestock (cattle, pigs or poultry) and availability of a latrine at home. Participants were interviewed based on a pre-tested individual questionnaire in order to collect information about demographics (age, gender, educational level and profession), personal risk-perception (knowledge about worm infections) personal hygiene practices (washing hands after defecating or before eating), wearing shoes (at home/work) and use of latrines for defecation. All questionnaires were developed in English and translated into Khmer. A different person translated the questionnaires back into English to ensure correct content. The interviews were conducted in Khmer language. After the interview, each participant received a pre-labelled plastic container (ID code, name, sex, age and date) for collecting a stool sample the next morning. Within two hours of collection, the stool specimens were sent (at ambient temperature) to the laboratory in Takeo town, where they were immediately processed.

Laboratory procedures

First, two Kato-Katz thick smears were prepared, using the World Health Organization's (WHO's) standard template [Katz *et al.*, 1972]. After a clearance time of 30 minutes, the smears were examined under a light microscope for the presence of helminth eggs. These were counted and recorded for each helminth species separately. Second, the KAP culture was used to identify *S. stercoralis* and hookworm larvae [Koga *et al.*, 1991]. A hazelnut-sized stool sample was placed in the middle of the agar plate and the closed Petri dish was incubated in a humid chamber for 48 hours at 28°C. Then, the plate was rinsed with sodium acetate-acetic acid-formalin (SAF) solution. The eluent was centrifuged and the sediment microscopically examined for the presence of *S. stercoralis* and hookworm larvae. Finally, the

Baermann technique was performed [Garcia *et al.*, 2001]. A walnut-sized stool sample was placed on gauze inserted into a glass funnel, and covered with tap water. The apparatus was exposed for two hours to artificial light directed from below. After centrifugation of the collected liquid, the sediment was examined under a microscope for the presence of *S. stercoralis* larvae.

For quality control, the technicians were specifically trained on the morphological criteria for distinguishing hookworm and *S. stercoralis* larvae. During the entire study period, a qualified microscopist from the Swiss Tropical and Public Health Institute, Basel, Switzerland, provided continuous and rigorous supervision. Further, any unclear diagnosis was immediately discussed with a qualified microscopist and the study supervisor.

Data management and statistical analyses

Questionnaire and laboratory data collected from each participant was double-entered and validated in EpiData version 3.1 (EpiData Association; Odense, Denmark). Statistical analyses were performed with STATA version 12.1 (StataCorp.; College Station, TX, USA). Only participants with complete records (stool sample examined with all diagnostic methods and completed questionnaires) were included in the final analysis. A 'smoothed' age prevalence curve was used to present the prevalence distribution of the mean age of each participant.

Principle component analysis (PCA) was applied to variables pertaining to ownership of various household assets, and was used to build the socioeconomic status (SES) profile. Socioeconomic status was categorized by one of three wealth levels: least poor, less poor and poor, as previously described in detail [Vyas *et al.*, 2006].

Generalized Estimating Equations (GEE) were used to determine the association between infection status and demographic variables, hygienic status, knowledge and the recent medical history of participants. Variables with an odd ratio below 0.80 and above 1.25 in the bivariate models were selected for inclusion in the multivariate GEE model.

6.4. Results

Study population and compliance

In total, 3,568 individuals from 900 households (median household size: 5 members, range: 1 – 12) were enrolled, of which 3,154 (88.4%) submitted a stool sample. The final analysis included 2,861 (80.2%) participants with complete data records, i.e., enough stool for all three diagnostic tests (two Kato-Katz, KAP culture and Baermann method) and all questionnaire data.

Of the 2,861 participants with complete data records, 1,560 (54.5%) were female. The median age of all participants was 26 years, with a range from 1 to 90 years and 57.2% aged 30 years or under. One third or 31.7% were schoolchildren; 40.5% were farmers; three-quarters or 74.7% had attended school, with 50.5% and 24.2% attending primary and secondary school or higher, respectively.

Parasitological findings and performance of diagnostic methods

Figure 14 shows the eight intestinal helminth species found among the participants. Hookworm and *S. stercoralis* were most frequent, with 31.4% and 21.0%, respectively. *Ascaris lumbricoides*, *Trichuris trichiura*, eggs of minor intestinal flukes (MIF), *Hymenolepis nana*, *Enterobius vermicularis* and *Taenia* sp. were detected, but with prevalences of less than 1%. Of 601 *S. stercoralis* cases, 46.9% were co-infected with hookworms.

Detailed information on the diagnostic performance of the Baermann technique and KAP culture is shown in Table 9. When combining the two methods as a "diagnostic gold standard", the sensitivity of KAP culture and Baermann technique was 78.5% and 67.1%, respectively, with negative predictive values of 94.6% and 91.9%.

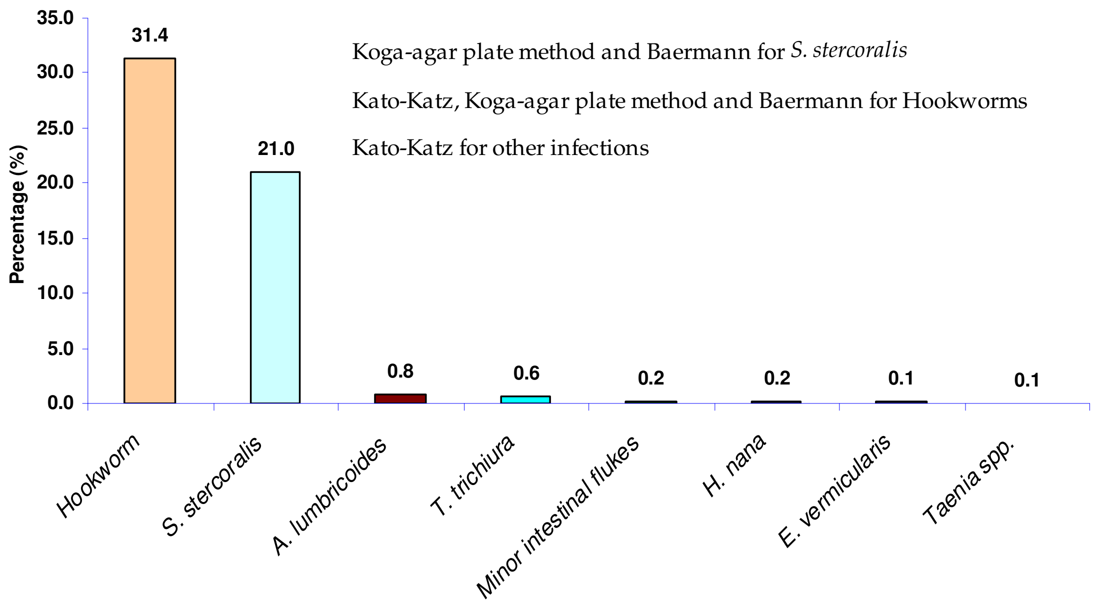

Figure 14: Distribution of intestinal parasitic infections among 2861 participants in Takeo province, Cambodia, 2011

Table 9: Yield of Koga-agar culture and Baermann for the detection of *S. stercoralis* in 2861 participants, Takeo province, Cambodia

		Combined Methods (KAP culture and Baermann)		Total
		Positive	**Negative**	
KAP culture	**Positive**	472	0	472
	Negative	129	2260	2389
	Total	601	2260	2861
Baermann method	**Positive**	403	0	403
	Negative	198	2260	2458
	Total	601	2260	2861

Risk factors for Strongyloides stercoralis infection

A total of 16 risk factor variables were tested to identify the univariate association between *S. stercoralis* infection and socio-demographic status (6 variables), personal disease perception (4 variables) and hygiene data (6 variables). Table 10 contains 10 variables that were included in the multivariable analysis. The most important risk factors associated with *S. stercoralis* infection were gender and possession of a latrine at home. Males had a significantly higher risk of *S. stercoralis* infection than females (OR: 1.7; 95% CI: 1.4 – 2.0; *P*<0.001), while participants with a latrine at home were less frequently infected than those who did not (OR: 0.7; 95% CI: 0.4 – 0.8; *P:* 0.003). The prevalence of *S. stercoralis* increased with age for both sexes (Figure 15), starting from 14.5 % in children five years and under to a peak of 28.0% in individuals aged 56 to 60 years. In all age groups, males displayed a higher infection rate than females. Age, profession, education level and SES were not statistically different between non-infected and infected individuals.

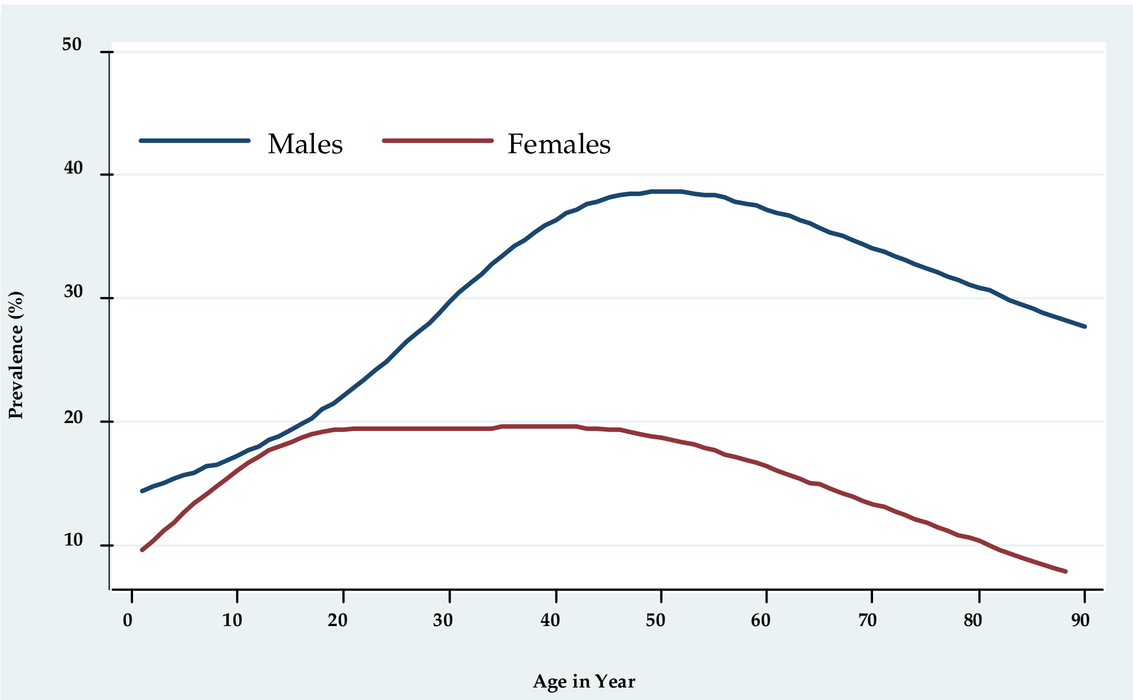

Figure 15: Smoothed age prevalence of *S. stercoralis* infection by sex among 2861 participants in Takeo province, Cambodia, 2011

Characteristics of Strongyloides stercoralis cases

Of the 601 *S. stercoralis* cases, 332 (55.2%) were male and 308 (51.3%) were farmers. Almost one-third (28.0%) of the cases were children under 16 years, while 256 (42.6%) were between the ages of 16 and 45 years. A quarter (25.6%) of the cases occurred among schoolchildren (Table 10).

Table 10: Risk factors for *S. stercoralis* infection among 2861 participants, Takeo province, Cambodia, 2011

Multiple model		Non-*S. stercoralis* (N=2260) n (%)	*S. stercoralis* (N=601) n (%)	OR (95% CI)	p-Value
Demographic information					
Gender (male)		969 (42.9)	332 (55.2)	1.7 (1.4 – 2.0)	<0.001
Age group					
	1 - 15 years	868 (38.4)	168 (28.0)	Reference	
	16 - 30 years	471 (20.8)	129 (21.5)	1.2 (0.8 - 1.7)	0.378
	31 - 45 years	386 (17.1)	127 (21.1)	1.3 (0.9 - 1.9)	0.229
	> 46 years	535 (23.7)	177 (29.4)	1.3 (0.9 - 2.0)	0.145
Profession					
	Farmer/Rice-Grower	851 (37.7)	308 (51.3)	Reference	
	Schoolchildren	752 (33.3)	154 (25.6)	0.7 (0.5 - 1.1)	0.097
	Others	657 (29.1)	139 (23.1)	0.7 (0.5 - 0.9)	0.006
Socio-economic status					
	Poor	631 (27.9)	195 (32.4)	Reference	
	Less poor	745 (32.9)	185 (30.8)	0.8 (0.6 - 1.0)	0.092
	Least poor	884 (39.1)	221 (36.8)	0.9 (0.7 - 1.2)	0.523
Personal Disease Perception					
Have been treated for worms (yes)		1249 (55.3)	269 (44.8)	0.8 (0.7 - 1.0)	0.055
Know about worms/infection with worms (yes)		933 (41.3)	277 (46.1)	1.0 (0.8 - 1.2)	0.890
Personal Hygiene					
Toilet at home (yes)		949 (42.0)	192 (31.9)	0.7 (0.4 - 0.8)	0.003
Usually defecated in toilet (yes)		941 (41.6)	209 (34.8)	1.2 (0.8 - 1.7)	0.351
Had shoes (yes)		1982 (87.7)	542 (90.2)	1.1 (0.7 - 1.9)	0.697
Wore shoes when go to defecate/toilet (yes)		1846 (82.7)	514 (85.5)	1.1 (0.7 - 1.6)	0.786

OR: Odds Ratio; 95% CI: 95% Confident Interval

The medical problems reported during the two weeks prior to diagnoses are shown in Table 11. Muscle pain (OR: 1.3; 95% CI: 1.0 - 1.6; *P:* 0.028) and cutaneous rash (urticaria) (OR: 1.4; 95% CI: 1.1 - 1.8; *P:* 0.001) were significantly more frequent among those with *S. stercoralis* infection. No other reported clinical symptom was associated with *S. stercoralis* infection.

Table 11: Clinical symptoms for *S. stercoralis* cases in 2861 participants, Takeo province, Cambodia, 2011

Symptoms in the past 2 weeks	Non-*S. stercoralis* (N=2260) n (%)	*S. stercoralis* (N=601) n (%)	OR (95% CI)	p-Value
Anorexic/loss of appetite (yes)	410 (18.1)	115 (19.1)	1.0 (0.8 - 1.3)	0.785
Sensation of tiredness (yes)	532 (23.5)	154 (25.6)	1.1 (0.9- 1.3)	0.492
Nausea (yes)	307 (13.6)	80 (13.3)	1.0 (0.8 - 1.3)	0.925
Vomiting (yes)	286 (12.7)	63 (10.5)	0.8 (0.6 - 1.1)	0.180
Diarrhoea (yes)	753 (33.3)	204 (33.9)	1.0 (0.9 - 1.3)	0.677
Bloody diarrhoea (yes)	83 (3.7)	22 (3.7)	1.0 (0.6 - 1.7)	0.852
Greasy diarrhoea (yes)	177 (7.8)	50 (8.3)	1.0 (0.7 - 1.4)	0.855
Constipation (yes)	305 (13.5)	63 (10.5)	0.8 (0.6 - 1.0)	0.088
Itching (yes)	822 (36.4)	225 (37.4)	1.1 (0.9 - 1.3)	0.334
Seen worm in stool (yes)	215 (9.5)	45 (7.5)	0.8 (0.6 - 1.1)	0.278
Cough (yes)	1308 (57.9)	351 (58.4)	1.1 (0.9 - 1.3)	0.552
Coughed out a worm (yes)	11 (0.5)	2 (0.3)	0.7 (0.2 - 3.0)	0.625
Cutaneous rash "urticaria" (yes)	397 (17.6)	138 (23.0)	1.4 (1.1 - 1.8)	0.001
Abdominal pain (yes)	1121 (49.6)	278 (46.3)	0.9 (0.8 - 1.1)	0.300
Muscle pain (yes)	360 (15.9)	118 (19.6)	1.3 (1.0 - 1.6)	0.028
Lost weight (yes)	310 (13.7)	90 (15.0)	1.1 (0.8 - 1.4)	0.578

OR: Odd Ratio; 95% CI: 95% Confident Interval

6.5. Discussion

The worldwide distribution of *S. stercoralis* infection varies greatly from country to country and even within the same country, depending on ecological and socioeconomic conditions [Schär *et al.*, 2013b]. *S. stercoralis* infection is commonly overlooked, especially in endemic resource poor countries, as the most suitable diagnostic methods for *S. stercoralis* (KAP culture and Baermann technique) are not used in most epidemiological studies of soil-transmitted helminths [Olsen *et al.*, 2009; Schär *et al.*, 2013b]. Despite the high endemicity of *S. stercoralis* in Southeast Asia, specific information is often lacking. Thailand is the only country in the region with a considerable amount of information available on *S. stercoralis* prevalence [Schär *et al.*, 2013b]. Our community-based study of *S. stercoralis* infection among 2,861 participants from 60 villages in a southern province of Cambodia, employing KAP culture and the Baermann method, found a prevalence of 21.0%.

To our knowledge, this is the first community-based report in Cambodia of the importance of *S. stercoralis* infection in a large province-wide setting, using a rigorous diagnostic technique (KAP culture and the Baermann technique). Recent surveys on *S. stercoralis* infection in Cambodia studied mainly the diagnosis, treatment and risk factors of infection among schoolchildren [Khieu *et al.*, 2013a], the clinical aspects of high intensity infection [Khieu *et al.*, 2013b] and the molecular diagnostic approach (PCR) [Schär *et al.*, 2013a]. Our report highlights the high frequency of the parasite in the general population, and points to key risk factors, which need to be addressed in large-scale control programmes (country-wide).

Our observed prevalence is particularly high compared to those of two previous studies that used the Baermann technique on samples from school-aged children (12.0%) and from the general population in Cambodia (14.6%) [Koga-Kita, 2004; Longfils *et al.*, 2005]. One possible explanation is that the present study used a combined diagnostic method (KAP culture and Baermann technique) to diagnose *S. stercoralis*, while others used a single test. However, a similar prevalence rate (20.2%) was observed in a 2006 study that had only used the Baermann technique to analyse a single stool sample from school-aged children living in villages bordering Tonlé Sap Lake, central-northern Cambodia [Chhakda *et al.*, 2006]. Moreover, a recent study among schoolchildren in Kandal province, central-southern Cambodia, which used the same laboratory approach on multiple stool samples, reported a prevalence of 24.4% [Khieu *et al.*, 2013a], which is consistent with our findings when using the same diagnostic methods on a single stool sample. This observation suggests that the overall prevalence of *S. stercoralis* in our study population would actually have been higher if several stool samples had been examined.

The prevalence of *S. stercoralis* in our study setting is higher than those shown in other studies among the general population in Southeast Asian countries: Lao PDR, Thailand and China. In Lao PDR, the study conducted by Sayasone *et al.* in 2009, using a formalin ethyl-acetate concentration technique on a single stool sample, found that 10.3% of participants were infected with *S. stercoralis*, but this diagnostic approach is not very sensitive [Sayasone *et al.*, 2009]. In Thailand, Nontasut *et al.* used KAP culture to analyse a single stool sample and reported a prevalence of 15.9% [Nontasut *et al.*, 2005]. In China, Steinmann *et al.* examined three stool samples by KAP culture and Baermann method and found a prevalence of 11.7% [Steinmann *et al.*, 2007].

In our study, the observed prevalence of *S. stercoralis* was significantly higher in males than in females, which coincides with previous reports from Thailand [Nontasut *et al.*, 2005] and China [Steinmann *et al.*, 2007]. In addition, males had a higher infection rate than females in all age groups. This is likely due to agricultural practices and activities. Most men are rice farmers and work in muddy rice fields without footwear. In contrast, Cambodian females usually work as housewives and wear shoes when walking around the household or village. The prevalence of *S. stercoralis* also increased with age, starting from 14.5% in children 5 years and under, to a peak prevalence of 28.0% in participants aged 56 to 60, decreasing slightly thereafter. This suggests that infection first occurs in the village or household where young children play, usually without shoes. After contracting the parasite at a young age, the infection may remain for decades in the host if the infection is not treated [Concha *et al.*, 2005; Montes *et al.*, 2010; Prendki *et al.*, 2011], which would account for the increased prevalence associated with age.

The clinical manifestations of strongyloidiasis vary greatly between immune-competent and immune-suppressed individuals. Gastrointestinal (nausea and diarrhea) and cutaneous (itchiness and urticaria) symptoms are frequently described. However, more than 50% of strongyloidiasis cases are asymptomatic [Khieu *et al.*, 2013b; Koczka *et al.*, 2012; Ly *et al.*, 2003; Vadlamudi *et al.*, 2006]. In our study, we observed that muscle pain and urticaria reported during the preceding two weeks were associated with *S. stercoralis* infection; a finding that coincides with recent reports from the Northern province of Cambodia [Khieu *et al.*, 2013b]. The clinical features of strongyloidiasis and the association between strongyloidiasis and other infectious diseases are not well understood [Ly *et al.*, 2003; Vadlamudi *et al.*, 2006]. With the limitations of our study, it was not possible to differentiate underlying infections that might mimic strongyloidiasis. Therefore, an in-depth assessment, on the clinical symptoms of *S. stercoralis* infection is required.

The sensitivity of KAP culture and the Baermann technique has been reviewed in several studies, with contradictory results. Our study found the sensitivity of KAP culture to be higher than that of the Baermann method, contrary to previous reports from south-central Côte d'Ivoire [Becker *et al.*, 2011], Zanzibar [Knopp *et al.*, 2008], Uganda [Stothard *et al.*, 2008] and China [Steinmann *et al.*, 2007]. However, our findings are consistent with those reported in studies conducted in Cambodia [Khieu *et al.*, 2013a], Brazil [Ines Ede *et al.*, 2011], rural Côte d'Ivoire [Glinz *et al.*, 2010] and Honduras [de

Kaminsky, 1993].

Our study examined only a single stool sample per participant, thus the prevalence of *S. stercoralis* in our study setting was likely under-estimated, since the excretion of *S. stercoralis* larvae in stool specimens varies considerably from day-to-day [Knopp *et al.*, 2008; Schär *et al.*, 2014a]. A recent study showed that the output of larvae in faecal specimens ranged from 0.003 larvae per gram to 151.2 larvae per gram, as observed over seven consecutive days [Schär *et al.*, 2014a]. However, even when multiple stool samples are available, no single diagnostic test, KAP culture or Baermann method, can detect all *S. stercoralis* infection [Khieu *et al.*, 2013a; Knopp *et al.*, 2008]. Therefore, the combined use of both diagnostic methods (KAP culture and Baermann technique) on several stool specimens will increase sensitivity and currently represents the best diagnostic approach for this infection.

S. stercoralis infection is highly prevalent among the general population in Cambodia and should be given more attention due to its potential for disseminating infection. Access to adequate diagnosis and treatment is urgently required in Cambodia.

Competing interests

The authors declared no conflicts of interest.

Author Contributions

VK, FS, SM and PO conceived and designed the study. VK collected field data, FS and PJB analysed the stool specimens. HM coordinated the laboratory activities. MCC and SM coordinated the field work in Cambodia. VK analysed the data and wrote the manuscript together with PO. PO supervised the first author in all aspect of the study. All authors have read and approved the final version of manuscript.

6.6. Acknowledgments

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7. *STRONGYLOIDES STERCORALIS* INFECTION AND RE-INFECTION IN A COHORT OF CHILDREN IN CAMBODIA

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7.1. Abstract

Information on *Strongyloides stercoralis* re-infection after ivermectin treatment is scarce in *S. stercoralis* endemic countries. In semi-rural Cambodia, we determined *S. stercoralis* infection and re-infection rates among schoolchildren, two years after ivermectin treatment (2x100µg/kg PO, 24 hours apart). The study was conducted among 484 children from four primary schools in semi-rural villages in Kandal province from 2009 to 2011, using Koga agar plate culture and the Baermann method on two stool samples per child. Complete data were available for 302 participants. We observed infections in 24.2% and 22.5% of the children at baseline and at follow-up, respectively. At baseline, 73 children were treated for *S. stercoralis* infection. At follow-up, one-third of those treated for *S. stercoralis* infection had been reinfected, while 19.6% of the 229 healthy children (at baseline) had been newly infected with *S. stercoralis*. Possession of shoes and defecation in toilet were associated with *S. stercoralis* infection at follow-up. Infection and re-infection rates of *S. stercoralis* among schoolchildren are considerably high. However, 68.5% of infected children remained free of infection for at least two years. A large-scale cohort study is required to understand age-specific infection and re-infection dynamics in endemic countries.

Key words: *Strongyloides stercoralis*, infection, re-infection, schoolchildren, Cambodia, risk factors

7.2. Introduction

Strongyloides stercoralis, a soil-transmitted nematode, infects an estimated 30–100 million people worldwide [Bethony *et al.*, 2006]. *S. stercoralis* infection is endemic in the tropical and humid areas of Central and South America, Sub-Saharan Africa and South and Southeast Asia [Agrawal *et al.*, 2009; Concha *et al.*, 2005; Siddiqui *et al.*, 2001], and in temperate climates such as Japan, Australia, Spain, Italy, Romania and the United States [Ramanathan *et al.*, 2008b; Valerio *et al.*, 2013]. Despite its negative impact on public health, *S. stercoralis* is still one of the most neglected infections among the so-called neglected tropical diseases [Buonfrate *et al.*, 2012].

The global prevalence of *S. stercoralis* infection is heterogeneously distributed, depending on the ecological and socioeconomic conditions in which the population live [Schär *et al.*, 2013b]. In many resource poor countries, *S. stercoralis* infection is generally underreported. One underlying reason for underreporting is that most available information on *S. stercoralis* originates from studies on other soil-transmitted helminths (STHs), such as *Ascaris lumbricoides*, hookworms and *Trichuris trichiura*, that use the Kato-Katz method, which only has a very low sensitivity for *S. stercoralis* infection [Olsen *et al.*, 2009; Schär *et al.*, 2013b]. Koga-agar plate (KAP) culture and the Baermann technique are considered to be the most appropriate diagnostic methods [Khieu *et al.*, 2013a], however, they are hardly used, as *S. stercoralis* is rarely included in such studies. As a result, information on incidence rates of *S. stercoralis* infection is entirely missing [Schär *et al.*, 2013b].

Ivermectin is the medicine of choice for treating *S. stercoralis* infection [WHO, 2006]. Even though the efficacy of ivermectin treatment is high [Bisoffi *et al.*, 2011; Khieu *et al.*, 2013a; Marti *et al.*, 1996; Suputtamongkol *et al.*, 2011], no guideline exists for large-scale chemotherapy against strongyloidiasis [WHO, 2006]. A major reason for this is the lack of information about re-infection after successful treatment [Schär *et al.*, 2013b].

Using a sensitive diagnostic approach on multiple stool samples and multiple examination techniques, we assessed *S. stercoralis* infection and re-infection rates among schoolchildren in four primary schools in semi-rural villages in Kandal province, central-southern Cambodia. Specifically, we assessed *S. stercoralis* infection at baseline and two years after infected children had been treated with ivermectin.

7.3. Materials and methods

Ethical considerations

The study was approved by the Ethics Committee of the Cantons of Basel-Stadt and Baselland, Switzerland (EKBB; number 21/09, dated 29 January 2009 and number 159/11, dated 19 May 2011) and by the National Ethics Committee for Health Research, Ministry of Health, Cambodia (NECHR; number 033, dated 20 March 2009 and number 30 dated 11 April 2011). Written informed consent was obtained from the children's parents, legal guardian or appropriate literate substitute for both the baseline (2009) and follow-up (2011) surveys. All relevant authorities (village chiefs, school teachers and headmasters) were informed of the study's purpose and procedures.

All children infected with *S. stercoralis* were treated with ivermectin under the direct supervision of a medical doctor [WHO, 2006]. All other diagnosed parasitic infections were treated according to the guidelines of the National Helminth Control Program of Cambodia [CNM, 2004].

Study setting

The study was carried out in four rural villages (Ang, Roka, Koh Khel and Damrey Chhlang villages), located in the Saang District (11.22° N and 105.01° E longitude), Kandal province, 45 kilometres south of Phnom Penh. These villages were selected because they were readily accessible by the project car, assuring rapid transfer of stool samples to the Parasitological Laboratory of the National Center for Parasitology, Entomology and Malaria Control (CNM) in Phnom Penh (up to 90 minutes travel time by car).

Study design

A school-based survey was conducted during the dry season between March and June 2009 (baseline) and again between May and June 2011 (follow-up). In the 2009 baseline survey, KAP culture and the Baermann method were performed on two stool samples per participant, to detect *S. stercoralis* infection. All *S. stercoralis* cases were followed-up three weeks after ivermectin treatment (2x100µg/kg PO, 24 hours apart) [Igual-Adell *et al.*, 2004] to ensure that *S. stercoralis* larvae had been cleared. For the 2011 follow-up survey, all schoolchildren who had participated in the baseline survey were re-enrolled. They were examined using the same laboratory procedures as at baseline (two stool samples per child examined by KAP culture and the Baermann method).

Field and laboratory procedures

During home visits, after obtaining written informed consent from the children's parents or legal guardian, a pre-tested household questionnaire was administered to the head of household to obtain socioeconomic data, such as ownership of household assets. All schoolchildren present on the day of the school visit were interviewed using a pre-tested children's questionnaire, to obtain demographic data, personal risk-perception (knowledge about worm infection) and behavioural data (wearing shoes, personal hygiene practices). After the interview, each participant received a pre-labelled plastic container (ID code, name, sex, age and date) for stool sample collection and instructions on how to collect the faeces. The next morning, after collecting the filled stool container, another empty pre-labelled container was given to the child. Every participant was asked to provide two stool samples.

Within two hours of receipt, the stool samples were sent to the laboratory at ambient temperature (25-30 °C). As soon as the specimen arrived in the laboratory, trained laboratory technicians from the Parasitological Laboratory of CNM examined the samples using KAP culture: a hazelnut-sized stool was placed on a nutrient agar plate and the closed Petri dish was incubated in a humid chamber at 28°C for 48 hours and evaluated for visible tracks created by larvae as they carried bacteria over the agar [Koga *et al.*, 1991]. The Baermann technique was then performed by placing faecal specimens on a mesh screen in a funnel filled with warm water and connected to clamped-tubing. After two hours any larvae present had crawled out of the stool suspension and migrated into the warm water, from where they were collected by centrifugation [Garcia *et al.*, 2001].

Follow-up study

From May to June 2011, two years after the baseline survey, the school-children were individually re-assessed. The same field and laboratory procedures were performed as at baseline (i.e., written informed consent of the parents or legal guardian was obtained, two stool samples per child were collected and analysed by KAP culture and Baermann technique for detecting *S. stercoralis* larvae). In addition, questionnaires were used to obtain information about risk factors.

Data management and statistical analyses

Questionnaire and laboratory data collected from each individual for both surveys were entered twice and validated by EpiData version 3.1 (EpiData Association; Odense, Denmark). Statistical analyses were performed with STATA version 12.1 (StataCorp.; College Station, TX, USA). Only participants with completed records in 2009 and 2011 (completed questionnaires and two stool samples examined with all diagnostic tests) were included in the final analyses.

Univariate logistic regression was used to associate infection status with the participants' demographic variables, hygienic practices, knowledge and recent medical history. *P-values* less than 0.05 indicated a significant association. Re-infection was defined as treated *S. stercoralis* cases in 2009 that were *S. stercoralis* positive in 2011.

7.4. Results

Study population and compliance

In total, 484 schoolchildren participated in the study in 2009, of whom 409 (84.5%) were present at the follow-up visit in 2011 (Figure 16). The final analysis focused on 302 (62.6%) participants with complete data records (completed questionnaire and two stool samples analysed with all diagnostic methods) in 2011. Children were between 8 and 18 years old (median age 12 years). The ratio of males to females was close to 1.

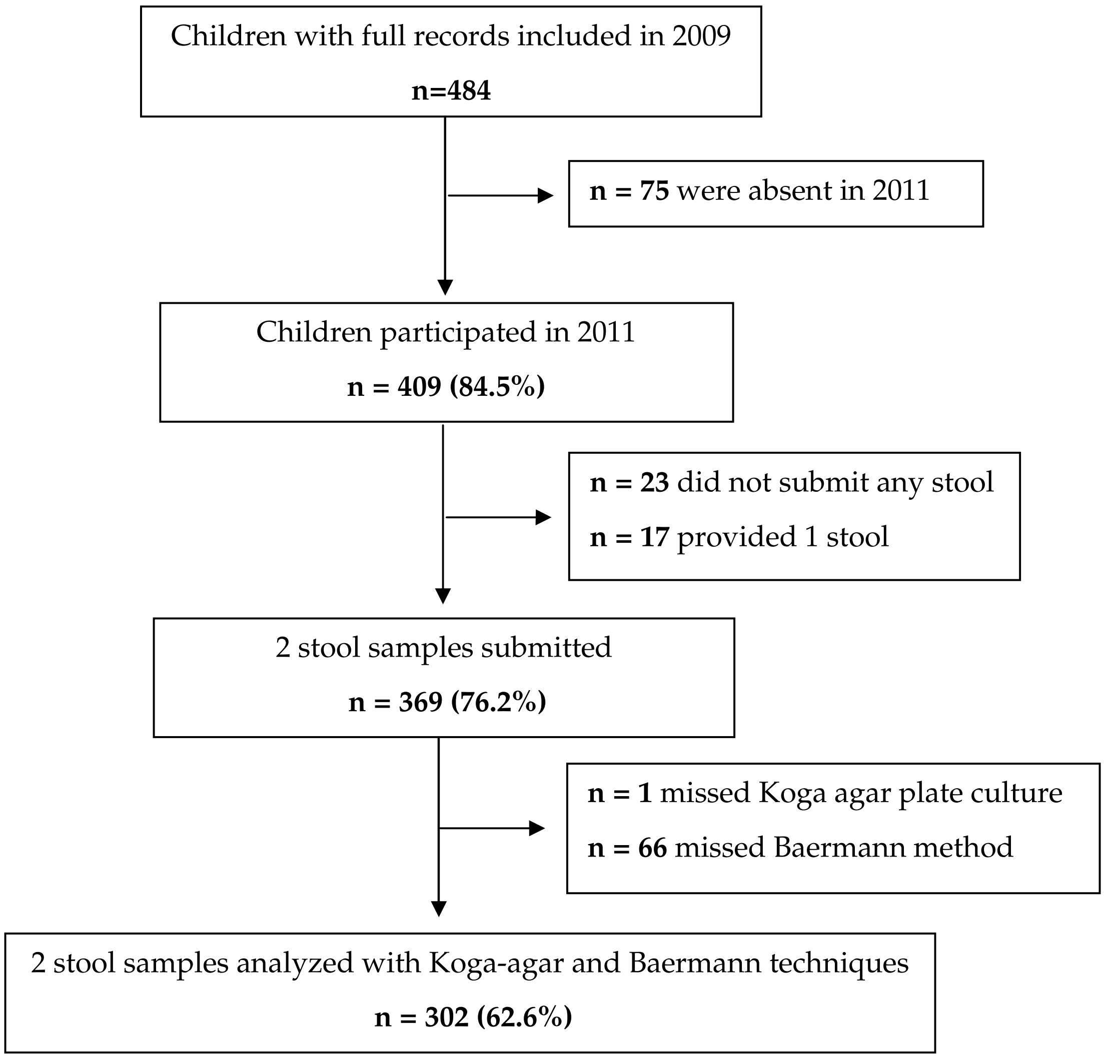

Figure 16: Flow chart detailing the compliance of participants in Cambodia, 2011

The characteristics of participants obtained during the baseline survey (both compliant and non-compliant study participants) are given in Table 12. Participant characteristics were very similar between the two groups. However, non-compliant children were, in general, slightly older and had better personal hygiene practices than the children retained in the cohort.

Table 12: Characteristics of compliant and non-compliant study participants

Characteristic	Present in 2011 (N=302) % (n)	Absent in 2011 (N=182) % (n)
Infection		
Strongyloides stercoralis (yes)	24.2 (73)	18.7 (34)
Hookworms (yes)	45.7 (138)	44.5 (81)
Demography		
Gender (female)	49.3 (149)	48.3 (88)
Median age in year (min - max)	10 (6 - 16)	12 (6 - 19)
Personal hygiene practices		
Child has ever taken Anthelmintic drug (yes)	58.3 (176)	65.4 (119)
Child usually defecates in toilet (yes)	35.7 (108)	48.3 (88)
Child has washed hand after last defecating (yes)	60.6 (183)	64.3 (117)
Child has shoes (yes)	79.8 (241)	90.1 (164)
Child wears shoes when go to defecate/toilet (yes)	36.4 (110)	29.7 (54)
Children's parent or guardian		
Toilet at home (yes)	26.8 (81)	31.3 (57)
Guardian knows about worm/worm infection (yes)	39.4 (119)	43.4 (79)

Infection and re-infection

Figure 17 shows the *S. stercoralis* cases diagnosed at baseline and at follow-up. Of the 302 children, 24.2% and 22.5% tested positively for *S. stercoralis* infection at baseline and at follow-up; respectively. Of the 73 children diagnosed and treated in 2009, 23 (31.5%) were re-infected in 2011. Of the 229 infection free children at baseline, 45 (19.6%) were infected with *S. stercoralis* at follow-up. Two of the 73 positive cases diagnosed at baseline were not *S. stercoralis* infection-free three weeks after ivermectin treatment.

The distribution of *S. stercoralis* infection and re-infection rate varied among the study villages (Figure 18). Compared to the prevalence at baseline, the follow-up prevalence decreased in Roka and Ang villages, and increased in Damrey Chlong and Koh Khel villages. The re-infection rate of *S. stercoralis* in Damrey Chlong and Ang villages was 24.0% and 42.0%, respectively. However, the different prevalence and re-infection rates in the study villages was not statistically significant.

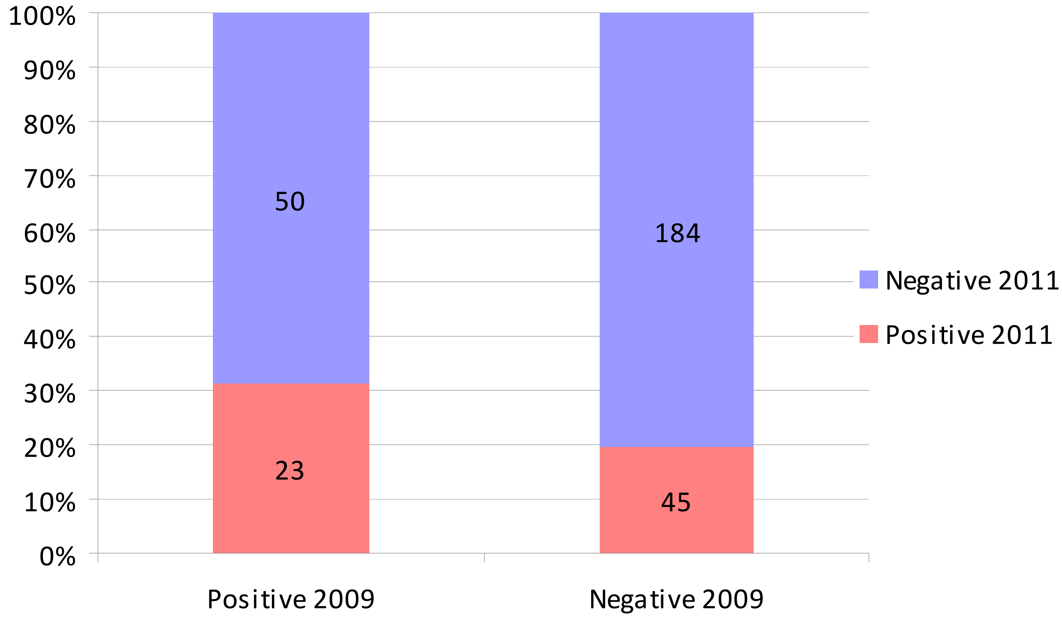

Figure 17: ***Strongyloides stercoralis*** **cases diagnosed among 302 children in 2009 and 2011, Cambodia**

Risk of Strongyloides stercoralis infection in 2011

Of the 68 *S. stercoralis* cases diagnosed in 2011, 50 (73.5%) were in children between the ages of 8 and 13 years and 70.6% were boys (Table 13). There were significantly more boys infected with *S. stercoralis* than girls (*P*<0.001), while age was not statistically different between infected and non-infected participants. Possession of shoes and defecation in toilets were associated with *S. stercoralis* infection. Children who reported having shoes (OR: 0.4; 95%CI: 0.2 – 0.9; *P*: 0.031) and defecating in toilets (OR: 0.3; 95%CI: 0.1 – 0.5; *P* <0.001) were significantly less infected with *S. stercoralis* than those who did not possess shoes and use latrines.

Reported clinical symptoms such as diarrhoea and nausea were reported more frequently in children infected with *S. stercoralis* than in infection free participants. However, the reported clinical symptoms were not significantly associated with *S. stercoralis* infection.

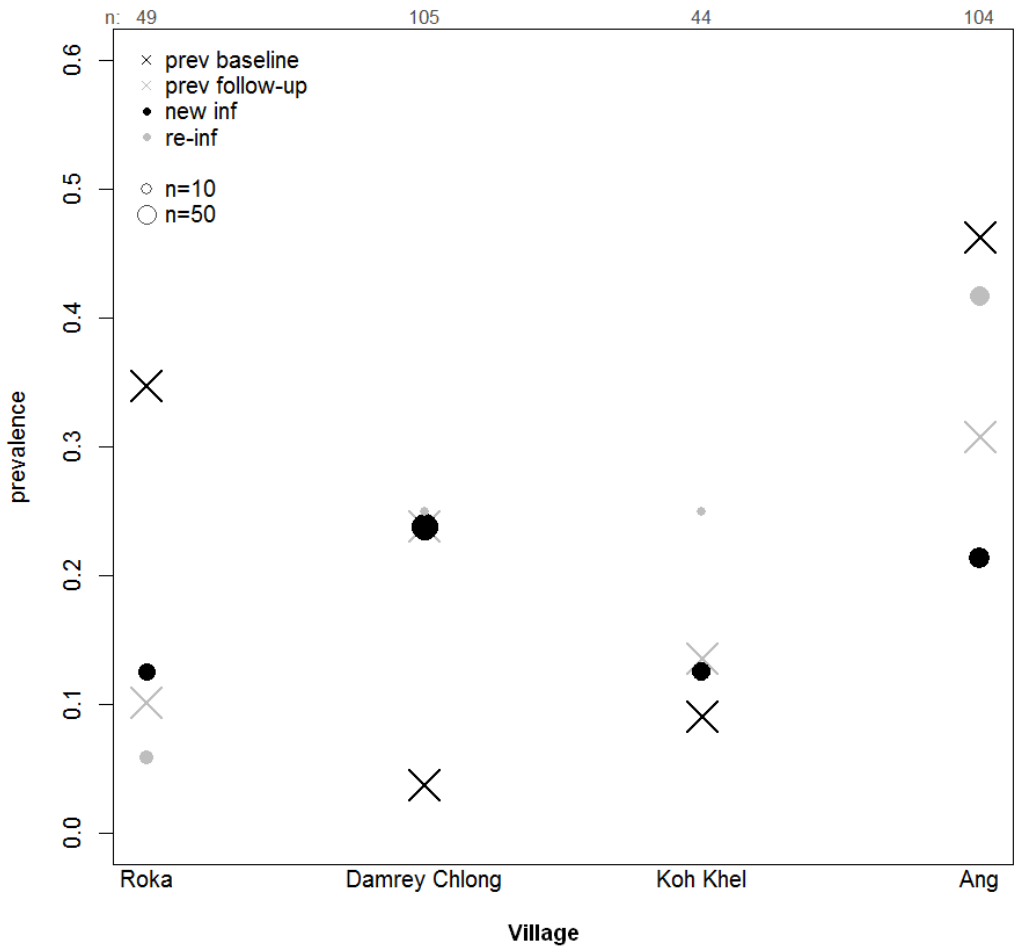

Figure 18: **Distribution of infection and re-infection rates among 302 children in different villages, Cambodia, 2009-2011**

Table 13: Risk factors for *Strongyloides stercoralis* infection in 302 children in Cambodia, 2011

	S. stercoralis N=68; n (%)	Non-*S. stercoralis* N=234 ; n (%)	OR (95% CI)	p-Value
Demographic characteristics of schoolchildren				
Gender (male)	48 (70.6)	105 (44.9)	3 (1.6 - 5.4)	<0.001
Age group				
8 - 10 years	21 (30.9)	79 (33.7)	Reference	
11 - 13 years	29 (42.6)	105 (44.9)	1 (0.5 - 2.0)	0.89
>= 14 years	18 (26.5)	50 (21.4)	1.5 (0.7 - 3.2)	0.27
S. stercoralis infection in 2009	23 (33.8)	50 (21.4)	1.9 (0.9 - 3.8)	0.07
Child has ever heard about worm (yes)	44 (64.7)	138 (59.0)	0.9 (0.5 - 1.7)	0.86
Child has ever taken Anthelminthic drug (yes)	61 (89.7)	190 (81.2)	1.8 (0.7 - 4.3)	0.21
Personal hygiene practices of schoolchildren				
Child usually defecate in toilet (yes)	24 (35.3)	147 (62.8)	0.3 (0.1 - 0.5)	<0.001
Child had washed hands after last defecating (yes)	47 (69.1)	161 (68.8)	1.1 (0.6 - 2.0)	0.76
Child had washed hands before last eating (yes)	48 (70.6)	164 (70.1)	0.9 (0.4 - 1.7)	0.69
Child usually washes hand with soap/ash (yes)	29 (42.6)	118 (50.4)	0.8 (0.4 - 1.4)	0.35
Child has shoes (yes)	57 (83.8)	218 (93.2)	0.4 (0.2 - 0.9)	0.031
Child wears shoes when going to defecate/toilet (yes)	27 (39.7)	104 (44.4)	0.9 (0.5 - 1.6)	0.73
Children's parent or guardian				
Toilet at home (yes)	14 (20.6)	108 (46.1)	0.3 (0.1 - 0.5)	<0.001
Parent/guardian reported that child has been treated for worms (yes)	56 (82.3)	205 (87.6)	0.7 (0.3 - 1.5)	0.33
Parent/guardian knows about worm/worm infection (yes)	21 (30.9)	87 (37.2)	0.9 (0.5 - 1.6)	0.63

OR: Odds Ratio; 95%CI: 95% Confidence Interval

7.5. Discussion

There is a paucity of epidemiological information on *S. stercoralis* in Cambodia, as in many other tropical, resource-poor countries [Khieu *et al.*, 2013a]. In our two-year cohort study, carried out among 302 Cambodian schoolchildren, using a rigorous *S. stercoralis* diagnostic procedure of KAP culture and Baermann technique on two stool samples per child, we observed a prevalence rate of 24.2% and 22.5% at baseline and at follow-up, respectively. Of the 73 treated *S. stercoralis* cases diagnosed at baseline, 31.5% were re-infected after two years. Of the 229 non-infected children at baseline, 19.6% were newly infected during the subsequent two years. Gender and personal hygiene were significantly associated with *S. stercoralis* infection at follow-up.

Complete data were available for 62% of the children originally enrolled in the study. Although the proportion of loss to follow-up is not unusual considering the setting combined with the relatively long follow-up period, it may lead to biased estimates. To explore potential bias, we used a multiple imputation approach to include 409 children (present at follow-up survey) with incomplete data in the analysis. The proportion of children infected and re-infected was close to the presented estimates (33.3% and 18.8%). Likewise, the risk factor estimates changed only slightly. The number of re-infected children two years after receiving ivermectin treatment was relatively small (23 cases). Hence, risk factors specifically associated with re-infection within the treatment group could not be addressed.

The prevalence and re-infection rates of *S. stercoralis* at baseline and at follow-up in the study villages were heterogeneously distributed. Unexpectedly, there was a considerable increase in prevalence in Damrey Chlong village (4.0% at baseline and 24.0% at follow-up). Furthermore, the new infection and re-infection rates in this village were high two years after infection exposure and treatment, respectively (Figure 18). However, the difference in these patterns compared to those observed in other villages was not statistically significant (small sample sizes). The four study villages are located in the same District and share similar ecological and socioeconomic conditions. The main economic activity in the villages is subsistence-level rice farming. Pigs, poultry and cattle are the most common domestic animals. In addition, the four villages were selected based on a previous report of hookworm infections (determined during a preliminary investigation prior to the study), used as a proxy for likely *S. stercoralis* transmission. To our knowledge, there was no remarkable event (e.g. flooding) that happened in Damrey Chlong village or in any of the other three villages during the study period.

The measured prevalence of *S. stercoralis* at baseline (24.2%) was similar to the prevalence at follow-up (22.5%). However, our observed infection rate is higher than those previously reported in Cambodia in studies that mostly used a single method to analyse a single stool sample [Koga-Kita, 2004; Longfils *et al.*, 2005]. Nevertheless, our findings coincide with those of a previous study (20.1%) of school-aged children around Tonlé Sap lake, northern Cambodia that used the Baermann method alone to examine one stool sample per child [Chhakda *et al.*, 2006].

Most scientific literature shows a satisfactory cure rate of ivermectin treatment [Bisoffi *et al.*, 2011; Datry *et al.*, 1994; Igual-Adell *et al.*, 2004; Khieu *et al.*, 2013a; Marti *et al.*, 1996; Naquira *et al.*, 1989; Shikiya *et al.*, 1992; Suputtamongkol *et al.*, 2011]. However, we could not identify any other study from an endemic setting where *S. stercoralis* infected individuals were re-examined to assess re-infection status [Bisoffi *et al.*, 2011; Khieu *et al.*, 2013a; Marti *et al.*, 1996; Suputtamongkol *et al.*, 2011]. To our knowledge, our study constitutes the first assessment of *S. stercoralis* re-infection two years after ivermectin treatment (100µg/kg PO, 24 hours apart) among schoolchildren in an endemic country. We found that 31.5% (one-third) of treated cases were again *S. stercoralis* positive two years later. Complete eradication of *S. stercoralis* in the intestine impossible to assess because some worms may remain circulating in blood tissue somewhere, therefore, we cannot exclude auto-infection as an explanation for some of the reported re-infected cases. While it is difficult to assess the exact number of re-infection cases due to auto-infection, the number is likely very small, as the efficacy of ivermectin two years after treatment has been reported to be as high as 97.3% in non-endemic areas [Zaha *et al.*, 2000]. Moreover, the clearance of *S. stercoralis* infection after ivermectin treatment (200µg/kg, oral single dose) is very rapid. An in-depth study of the excretion of *S. stercoralis* larvae showed that three days after ivermectin treatment, no further larvae are excreted [Schär *et al.*, 2014a].

Today, preventive chemotherapy is the mainstay of soil-transmitted helminth control [WHO, 2002]. The medicines (albendazole, mebendazole) recommended for country control programmes have a negligible impact on *S. stercoralis* infection [Montes *et al.*, 2010]. Our study offers a first insight into the benefits of mass-distribution of ivermectin; almost three-quarters of treated cases remained *S. stercoralis* infection-free for at least two years.

In conclusion, infection and re-infection rates of *S. stercoralis* among Cambodian schoolchildren were considerably high. Health education, especially on personal hygiene measures and sanitation, should be improved in the study setting. A large-scale bi-annual or annual follow-up survey should be conducted to understand the evolutionary trend of infection and re-infection rates.

Conflict of Interest

The authors declare that no competing interests exist

Author Contributions

VK, FS, SM and PO conceived and designed the study; VK collected data; FS analysed the stool specimens; HM coordinated the laboratory activities; MCC and SM coordinated the field work in Cambodia; VK, JH and PO analysed data and interpreted the results; VK and PO wrote the manuscript; PO supervised the first author in all aspect of the study; all authors have read and approved the final version of the manuscript.

7.6. Ackowledgments

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8. *Strongyloides stercoralis* is a cause of abdominal pain, diarrhea and urticaria in rural Cambodia

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8.1. Abstract

We document clinical manifestations of 21 patients heavily infected with *S. stercoralis* (more than 250 larvae in a single Baermann test) from a community in rural Cambodia, both before and three weeks after ivermectin (200µg/kg BW, single oral dose) treatment.

Out of 21 patients, 20 (95.2%), 18 (85.7%) and 14 (66.7%) reported frequent abdominal pain, diarrhea and periods of sensation of itching, respectively, during the previous six months; epigastric (11, 55.0%) and peri-umbilical (13, 65.0%) pains were most frequent. Five patients (23.8%) reported having experienced urticaria the week preceding the examination. One patient suffered from extended urticaria. Three weeks after treatment, most symptoms had been almost entirely resolved.

In rural communities of Cambodia, strongyloidiasis with high parasite load is endemic. It is associated with substantial symptoms and clinical signs, particularly abdominal pain, diarrhea and urticaria. Access to adequate diagnosis and treatment is a pressing issue that needs attention.

Keywords: *Strongyloides stercoralis*, clinical symptoms, ivermectin, Cambodia.

8.2. Introduction

Strongyloidiasis, an infection of an intestinal parasitic nematode, affects about 30-100 million people worldwide [Bethony *et al.*, 2006; Olsen *et al.*, 2009]. It is endemic in areas where sanitary conditions are poor and where the climate is warm and humid [Hall *et al.*, 1994]. The clinical manifestations of strongyloidiasis vary greatly according to infection intensity and the immune-status of the patient. It is thought that more than 50% of all infections remain asymptomatic [Cruz *et al.*, 2010; Fardet *et al.*, 2007; Foreman *et al.*, 2006]. In Cambodia, a recent study showed that 24.4% and 49.3% of schoolchildren were infected with strongyloidiasis and hookworm, respectively [Khieu *et al.*, 2013a]. Here, we report on the clinical manifestations of 21 strongyloidiasis patients from the rural province of Preah Vihear in northern Cambodia, with high numbers of *S. stercoralis* larvae in their feces.

8.3. Materials and methods

In early 2010, in a community-based survey in Rovieng district (Preah Vihear province), stool examinations were conducted for individuals in randomly selected households. Two stool samples were obtained on two consecutive days from each person and examined with Baermann [Lima *et al.*, 1961] and Koga agar plate culture (KAP) [Koga *et al.*, 1991] techniques for the presence of *S. stercoralis* larvae, as well as with a single Kato-Katz slide [Katz *et al.*, 1972] per stool sample for the detection of further helminth infections. A temporary laboratory was set-up in the local health facility to perform the stool examinations (Baermann, KAP culture and Kato-Katz methods). Patients with more than 250 larvae in one of the Baermann examinations were revisited and a detailed clinical assessment was performed. Patients were then treated with a single oral dose of ivermectin, 200µg/kg body weight. All patients were observed for one hour following treatment for the occurrence of adverse effects. Three weeks later, all patients were visited again and the clinical assessment was repeated [Khieu *et al.*, 2013a; Marti *et al.*, 1996]. All positive cases were re-treated according to the guidelines of the National

Helminth Control Program of Cambodia [CNM, 2004]. The study was approved by the Ethics Committee of the Cantons of Baselstadt and Baselland, Switzerland (EKBB, #16/10, dated 1 February 2010), and the National Ethics Committee for Health Research, Ministry of Health, Cambodia (NECHR, #004, dated 5 February 2010). Written informed consent was obtained from patients or from the parents or legal guardian of the child or appropriate literate substitutes. Specifically, we obtained a written informed consent from a patient for publication of the image. A copy of this written consent has been made available for review by the Editor-in-Chief of this journal.

8.4. Results

Of the 273 participants from seven rural villages in Rovieng district (Preah Vihear province) who provided two stool samples, 86 (31.5%) tested positive for *S. stercoralis* larvae in either the Baermann or/and KAP culture. The median age of the participants was 23 years (range: 2 – 84); 49.1% were female. Fifty-five participants (20.1%) did not attend school, while 172 (63.0%) received primary school education. Ninety participants (33.0%) possessed a toilet at home, while 101 (37.0%) reported defecating in latrines. Most participants (96.3%) had shoes and wore them while defecating. Cows (79.8%), chickens (75.5%) and pigs (67.8%) were the most commonly reported domestic animals owned by the households.

Of the 86 *S. stercoralis* cases, 21 (24.4%) were found to have high intensity *S. stercoralis* infection. The median larvae count in the Baermann examination was 790 (range: 251 - 6849). The median age of the patients was 11 years (range: 5 - 67); 23.8% were females. Eleven patients (52.4%) were younger than 16 years. Eight patients (38.1%) had no schooling, 10 (47.6%) completed primary school, and three patients (14.3%) had attended a secondary school. Seven of the patients (33.3%) were additionally infected with hookworms, with a median number of 48 eggs per gram (range: 24 – 216) as assessed by the Kato-Katz technique.

Twenty (95.2%), 18 (85.7%) and 14 (66.7%) patients reported frequent abdominal pain, diarrhea and episodes of itching sensation (uticaria), respectively, during the previous six months (Table 14). Among reported abdominal pains, epigastric (11, 55.0%) and peri-umbilical (13, 65.0%) pains were most frequent. Diarrheal episodes were characterized by liquid and semi-liquid (17, 94.4%) and greasy (13, 72.2%) stools. Five patients (23.8%) reported the presence of urticaria during the week preceding the examination. In addition, 11 patients (52.4%) reported a cough lasting longer than a week in the previous six weeks. Other symptoms included tiredness (8, 38.1%); anorexia (6, 28.6%); and pale conjunctiva (8, 38.1%), which included three patients infected additionally with hookworms. Most symptoms (abdominal pain, diarrhea and itching) were almost entirely resolved in the three-week period following ivermectin treatment. Anorexia, abdominal pain, diarrhea and urticaria were reduced statistically significant ($p<0.05$). One six-year old boy experienced vomiting within an hour after ivermectin treatment.

Three weeks after treatment, three patients (14.3%) were still *S. stercoralis* positive: a nine-year old boy had three *S. stercoralis* larvae in the Baermann test of the second stool and also a hookworm infection, revealed by the Kato-Katz (first stool sample negative); an eleven-year old girl had a few *S. stercoralis* larvae in the KAP culture of the second stool sample (first stool sample negative); and an eight-year old girl had 642 and 960 larvae in the Baermann tests, in addition to being infected by hookworms. Two of these patients reported abdominal pain and diarrhea and one patient reported experiencing cough and itching (Table 14). All three were retreated with ivermectin (200 μg/kg body weight, single oral dose).

Table 14: Clinical symptoms of patients with high intensity *Strongyloides stercoralis* infection: before and after ivermectin treatment, 2010

Clinical Examination	Before treatment	After treatment	
	N=21, n (%)	*S. stercoralis* Negative N=18, n (%)	*S. stercoralis* Positive N=3, n (%)
Tired (yes)	8 (38.1)	1 (5.5)	1 (33.3)
Anorexia/loss of appetite (yes)	6 (28.6) *	0	0
Abdominal pain (yes)	20 (95.2) *	4 (22.2)	2 (66.7)
Epigastric pain	11 (55.0)	1 (25.0)	1 (50.0)
Right hypochondrial pain	6 (30.0)	1 (25.0)	0
Left hypochondrial pain	1 (5.0)	0	0
Peri-umbilical pain	13 (65.0)	3 (75.0)	2 (100)
Under umbilical pain	2 (10.0)	0	0
Diarrhea (yes)	18 (85.7) *	3 (16.6)	2 (66.7)
Greasy	13 (72.2)	2 (66.7)	1 (50.0)
Bloody	9 (50.0)	1 (33.3)	1 (50.0)
Liquid/Semi-liquid	17 (94.4)	3 (100)	2 (100)
Constipation more than a week (yes)	3 (14.3)	2 (11.1)	0
Urticaria on body, hands or legs during one to two weeks (yes)	14 (66.7) *	2 (11.1)	1 (33.3)
Urticaria on the body and hand during one week, particularly at night (yes)	5 (23.8)	0	1 (33.3)
Cough more than one week. Most cough with sputum and mostly at night (yes)	11 (52.4)	4 (22.2)	1 (33.3)
Pale conjunctiva (yes)	8 (38.1) *	0	0
Febrile at night during 3 days (yes)	1 (4.8)	0	0

* Significant difference between before and after treatment: $p<0.05$ (McNemar test)

During a visit to the communities, a 43 year-old farmer, living in the rural eastern part of Preah Vihear province, was diagnosed with a *S. stercoralis* infection with 924 and 478 larvae present in his two Baermann examinations. Larvae and adult *S. stercoralis* were detected in KAP culture examinations of the stools (Figure 19). He was co-infected with hookworms. The patient presented with abdominal pain, diarrhea, nausea, vomiting, fever, and a pronounced and persistent skin rash, which had been present with extensive itching for more than two years. The rash was observed on the back, chest, abdomen and extremities (Figure 20a, b) and, due to frequent and intense scratching, showed signs of focal infection. Three weeks after treatment with a single oral dose of ivermectin (200 µg/kg body weight) and a single oral dose of mebendazole, the patient's rash had almost disappeared and he was free of episodes of intensive itching.

Figure 19: Mating adult *Strongyloides stercoralis* observed on Koga agar plate culture

Figure 20a

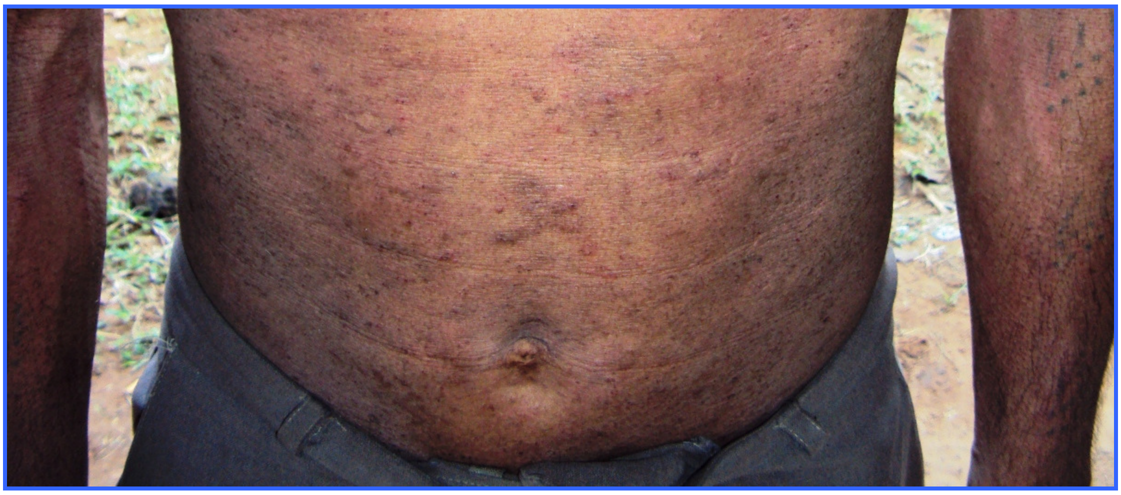

Figure 20b

Figure 20: Extended dermatitis on back (20a) and abdomen (20b) in 43 years old farmer

8.5. Discussion

Most of the patients had experienced at least one episode of abdominal pain, diarrhea and itchiness during the preceding six months. These symptoms had not been diagnosed or treated in this setting where poor and vulnerable people have only limited or even no access to health facilities. Additionally, in Cambodia, adequate diagnostic tests for strongyloidiasis are not available at public health facilities, including central level. Within the confines of our study, we could not diagnose additional underlying medical conditions such as gastritis, allergy or protozoan infections. However, it seems that other etiologies were unlikely to have played a major role in symptoms, as most symptoms dramatically resolved after ivermectin treatment.

Ivermectin, the drug of choice for strongyloidiasis [WHO, 2002], is not available in Cambodian health facilities, except for a few pharmacies in Phnom Penh. It is sold at USD 10.00 per tablet of 3 mg, which is not affordable for local people. Albendazole, the alternative drug for treatment of strongyloidiasis but with a lower efficacy, is recommended where ivermectin is not available [Montes *et al.*, 2010], but the therapeutic regimen for strongyloidiasis is not established in the guidelines of the National Helminth Control Program of Cambodia [CNM, 2004].

This report of clinical manifestations in patients with high intensity of *S. stercoralis* infection, from communities in northern rural Cambodia, documents the severity of clinical symptoms associated with *S. stercoralis* in a population living in a poor setting with virtually no access to diagnosis and treatment. Certainly, many socio-economically and environmentally similar areas exist throughout Southeast Asia and elsewhere in resource poor countries.

Competing Interests

The authors have declared that no competing interests exist.

Authors' contributions

VK carried out the field work, performed analysis and wrote the manuscript with PO. SS participated in the study design and field data collection. FS conducted the laboratory analysis. SM conceived the study and participated in design and coordination. HM participated in laboratory coordination. PO conceived and designed the study and interpreted data and wrote the manuscript together with VK. All authors contributed to manuscript revisions and approved the final manuscript.

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9. Discussion

9.1. Overview

The research studies presented in this PhD thesis were designed and performed in the dearth of virtual non-existing information on *S. stercoralis* infection in Cambodia. To understand and document the importance of *S. stercoralis* infection in Cambodia, particularly epidemiological and clinical features, five research studies were conducted among two study populations (school- and community-based) in three provinces (Kandal, Preah Vihear and Takeo province), socioeconomic and ecologically distinctly different settings, from 2009 to 2011. Moreover, we assessed the efficacy of ivermectin against *S. stercoralis* and explored the validity of diagnostic methods and the effect of sampling efforts in the observed prevalence of intestinal helminthes, especially *S. stercoralis* in schoolchildren (Chapter 4). Finally, we documented for the first time in Cambodia, and most probably for many other similar endemic setting, the re-infection of *S. stercoralis* among schoolchildren two years after ivermectin treatment (Chapter 7).

S. stercoralis is endemic in tropical, sub-tropical and temperate regions worldwide [Foreman *et al.*, 2006]. However, the global prevalence distribution of *S. stercoralis* is heterogeneous and for many potentially endemic settings information is lacking [Schär *et al.*, 2013b]. Many epidemiologic aspects of *S. stercoralis* are not well understood, such as which infection rates and intensities can typically be expected in different settings and populations, and when an individual is infected the first-time and how quickly the re-infection can occurred after successful treatment [Paula *et al.*, 2011; Schär *et al.*, 2013b]. Most available data for *S. stercoralis* infection originate from studies on other STHs (*A. lumbricoides*, *T. trichiura* and hookworms) which employ diagnostic techniques such as Kato-Katz thick smear which possesses a very low sensitivity for the detection of *S. stercoralis* infection [Olsen *et al.*, 2009; Schär *et al.*, 2013b]. Today, there are only Thailand and Brazil, the only two resource poor countries, which dispose adequate information on *S. stercoralis* infection [Schär *et al.*, 2013b].

Epidemiological studies of *S. stercoralis* in Cambodia, including large-scale prevalence, re-infection, risk factors, clinical aspects, treatment efficacy and diagnostic methods have not been conducted. Our first in-depth coprological investigations on *S. stercoralis* infection among two study populations (schoolchildren and general population) in three provinces of Cambodia, using Baermann method and KAP culture, revealed prevalence between 21.0% and 44.7%, and re-infection rate of 31.5% among schoolchildren after two years of ivermectin treatment. Prevalence of 31.4% and 14.5% was observed among participants less than 6 years old in Preah Vihear and in Takeo province, respectively. Furthermore, *S. stercoralis* infection increased with age and displayed a higher prevalence in the males than in the females in all age groups. Personal hygiene and sanitation including wearing shoes, possession and use of latrine, were significant predictors of *S. stercoralis* infection. Substantial symptoms and clinical signs, particularly diarrhea, abdominal pain, muscle pain, itchy skin and urticaria were associated with *S. stercoralis* infection. We observed that ivermectin was highly efficacious against *S. stercoralis* infection and well tolerated. KAP culture had more accuracy than Baermann technique for *S. stercoralis* diagnosis.

In the previous chapters, the discussion of each research study outcome has been given. In this chapter, a global discussion regarding the overall results of this PhD work is provided. It will focus on (1) the prevalence and re-infection rate observed, (2) the risk factors identified; (3) the clinical manifestations associated with the infection and the efficacy of ivermectin treatment; and (4) the observations on the validity of diagnostic methods and sampling efforts. Finally, we will propose several further research needs which are required to be addressed; and we will provide some, particularly for the screening, and prevention and control of *S. stercoralis* infection in Cambodia and other endemic countries.

9.2. Prevalence and re-infection

We observed different prevalence of *S. stercoralis* in three socioeconomically and ecologically different settings of Cambodia. One-firth (21.0%), a quarter (24.4%) and almost half (44.7%) of study participants from province located in southern (general population in Takeo), central-southern (pupils in Kandal) and northern (general population Preah Vihear) Cambodia, respectively, were infected with *S. stercoralis*. The difference of community-based prevalence in Takeo and Preah Vihear province was considerable. The prevalence of *S. stercoralis* in people living in Preah Vihear province was at least two times higher than in those from Takeo province (44.7% *versus* 21.0%). This is most likely due to the fact that we performed different laboratory approach: two stool samples in Preah Vihear versus one stool sample per participant in Takeo. However, the observed prevalence in Preah Vihear (44.7%) was substantially higher than in Kandal (24.4%) province where a different laboratory process was applied, i.e., two fecal samples per participant in Preah Vihear versus three stool samples per child in Kandal. Preah Vihear and Takeo province are economically and geographically different, which might influence the transmission of *S. stercoralis* in the respective province. Takeo is a flat (plain) and wet province where less than one-third (27.7%) of people are poor (living below USD 1 per day) and the province's economy consists basically of agricultural farming, fishery, rice and fruit cropping. Whereas Preah Vihear province is strongly hilly forested and extremely remote places exist where no proper roads do exist. A majority (51.8%) of Preah Vihear inhabitants are poor (living below USD 1 per day), and the main source of income in the province bases on farming and agriculture [Ministry of Planning, 2006; NIS, 2008; NIS *et al.*, 2011]. In addition, we observed that personal hygiene and sanitation of participants in these two provinces were considerably different. 40.0% of households in Takeo province possessed a latrine, while only 10.5% of household in Preah Vihear province owned toilet. Furthermore, we observed that the participants in Takeo used latrine for defecating more than those in Preah Vihear (40.0% *versus* 11.5%).

To the best of our knowledge large-scale studies on *S. stercoralis* infection and risk factors such as in Preah Vihear and Takeo province have never been reported before. This first report of prevalence of *S. stercoralis* is substantially higher than those stated in three previous reports from Cambodia [Chhakda *et al.*, 2006; Koga-Kita, 2004; Longfils *et al.*, 2005] and in reports from neighboring countries, Laos [Sayasone *et al.*, 2009] and Thailand [Nontasut *et al.*, 2005]. One of the main reasons was probably due to a rigorous diagnostic approach employed in our studies (number of stool samples, multiple diagnostic methods) than did the other studies, where they commonly used a single method to examine a single stool sample. However, a prevalence (20.2%) similar to the one found in our studies in Kandal (24.4%) and Takeo (21.0%) was observed among school-age children living in villages bordering Tonlé Sap Lake, central-northern Cambodia, using only the Baermann technique to analyze a single stool sample [Chhakda *et al.*, 2006].

Our *S. stercoralis* infection prevalence observations of Takeo and Preah Vihear province are considerably higher than those of other studies from neighbouring countries: Laos and Thailand. Sayasone and colleagues found 10.3% of Lao participants were infected with *S. stercoralis*, when a single faecal specimen was analysed by FECT technique [Sayasone *et al.*, 2009]. As for Thailand, Nontasut and colleagues, using KAP culture to analyse a single stool sample, reported a prevalence of 15.9% [Nontasut *et al.*, 2005]. Moreover, our observed prevalence is higher compared to the earlier studies using similar laboratory approach (three stool samples analyzed by KAP culture and Baermann test) in China (11.7%) [Steinmann *et al.*, 2007] and in Zanzibar (10.8%) [Knopp *et al.*, 2008]. More importantly, community-based prevalence in Preah Vihear province (44.7%) is, to our knowledge, a highest prevalence ever reported [Schär *et al.*, 2013b].

In the schoolchildren 2 years cohort study, almost one-third (31.5%) of the 73 treated *S. stercoralis* cases were *S. stercoralis* positive after two years This is a first report of re-infection rate after ivermectin treatment (2x100µg/kg PO, 24 hours apart) from a endemic setting. Most scientific literatures indicated a satisfactory cure rate after three weeks of ivermectin treatment. But, none of the study reported the re-infection status after successful ivermectin treatment [Bisoffi *et al.*, 2011; Marti *et al.*, 1996; Suputtamongkol *et al.*, 2011]. However, in our study, it is not possible to determine whether our the 23 treated *S. stercoralis* cases diagnosed in follow-up (two years after ivermectin treatment) were re-infected after successful treatment, since it is poorly understood how long ivermectin could fully eradicate *S. stercoralis*, or re-occurred by auto-infection, as these cases were not detected at three weeks after ivermectin treatment due to the low larvae excretion. Even though, however, it is difficult to assess the exact number of re-occurrent cases, it seems that this number is not high, since the efficacy of ivermectin after two years of treatment has been previously reported to be as high as 97.3% [Zaha *et al.*, 2000], and a rapid clearance of *S. stercoralis* larvae after ivermectin treatment (200µg/kg oral single dose) was recently reported. Schär and colleagues showed that ivermectin could fully eradicate *S. stercoralis* infection as quickly as three days after ivermectin treatment [Schär *et al.*, 2014a].

9.3. Risk factors of *S. stercoralis* infection

A *S. stercoralis* infection prevalence of 31.4% and 14.5% was found among children under or equal to 5 years in Preah Vihear and in Takeo province, respectively. The detection of infection in young children suggests that the infection with *S. stercoralis* (mainly through skin contact by infective larvae L_3) takes place likely in the household and villages. It is not surprising that our study participants were infected with *S. stercoralis* at very early age, since young Cambodian children usually play on the ground around the house or in the village without wearing shoes.

A different outcome regarding the association between age and *S. stercoralis* infection was observed in the two community-based surveys. A significant association was found in Preah Vihear study (compared to children less than 6 years old, all age groups were at significant higher risk for infection), while age was not a significant predictor for *S. stercoralis* infection in Takeo survey. Moreover, in Preah Vihear province, the prevalence of *S. stercoralis* infection increased with age, particularly in the first 8 years of life and reached a plateau, whereas in the southern Takeo province the infection rate started at 14.5% in young children aged less than 6 years with the peak prevalence of 28.0% in participants aged between 56 and 60 years. Age, hence, seems not to be a predominant predictor of *S. stercoralis* infection, as individuals can acquire the infection at young age and may remain infected for dedicates or the entire life [Concha *et al.*, 2005; Montes *et al.*, 2010; Prendki *et al.*, 2011].

Figure 21: Traditional Khmer instruments used for paddy work

We observed that the prevalence of *S. stercoralis* was significantly higher in males than in females in both community-based studies which is coinciding with the previous reports in Thailand [Nontasut *et al.*, 2005] and China [Steinmann *et al.*, 2007]. In addition, the infection rate in the male participants displayed a higher infection than in the females in all age groups. This is probably due to daily activities of the rural Cambodian people. More than

80% of rural Cambodian people are rice farmers, and men are mainly responsible for rice cultivation work. The paddy cultivation techniques are not all modern in rural Cambodia. The traditional Khmer rice cultivators always work in the mud of rice field without footwear during the whole rice cultivation (at least three months a season, two or three times a year). As for rural Cambodian women, they are usually responsible for the household. As housewife, they usually wear shoes when working around the household or going to the village market. Therefore, the women's exposure to soil is less intense than the exposure of male farmers whose tasks are all stages of rice cultivation.

Figure 22: Paddy cultivation activities in Cambodia

Our studies confirm that personal hygiene and sanitation including wearing shoes, possession and use of latrine, were significant predictors of *S. stercoralis* infection. Almost three-quarter of *S. stercoralis* cases in the study population (schoolchildren in Kandal) could be prevented if personal hygiene (possession and use of latrine) was improved, and 39.0% of *S. stercoralis* cases in the study area (Preah Vihear) would be reduced if everyone defecates in latrines. The cycle of *S. stercoralis* transmission could be interrupted by simply improving personal hygiene and sanitation. In countries where sanitation and human waste disposal have improved, *S. stercoralis* infection has almost non-existed [WHO, 2013].

9.4. Clinical features and treatment

The clinical presentation of strongyloidiasis is extremely variable reflecting the complex life cycle of the parasite. It also varies greatly from an immune-competent to immune-suppressed individual. Mild symptoms include gastrointestinal (nausea, intermittent vomiting, hematemesis, anorexia, diarrhea, constipation, weight loss and gastritis), pulmonary (nonproductive cough, wheezing, dyspnea, fever, throat irritation and hemoptysis) and cutaneous (itchy, urticaria, pruritus, edema, inflammation and petechiae) systems can happen in chronic strongyloidiasis. However, more than 50% of strongyloidiasis cases are asymptomatic [Cruz *et al.*, 2010; Liu *et al.*, 2009; Ly *et al.*, 2003; Siddiqui *et al.*, 2001; Sridhara *et al.*, 2008; Vadlamudi *et al.*, 2006].

In our study, we documented all reported clinical symptoms during precedent two weeks from all study participants (schoolchildren and general population). Most of *S. stercoralis* cases experienced at least an episode of diarrhea, abdominal pain, muscle pain, cough, itchiness and urticaria during the preceding two weeks or six months. These symptoms had not been diagnosed or treated in the areas where poor and vulnerable people have only limited or even no access to health facilities. Moreover, Cambodian public and private health facilities, including central level, do not dispose adequate diagnostic method and treatment for strongyloidiasis. The true clinical relevance of strongyloidiasis and the association between strongyloidiasis and other infectious diseases are not well understood. In our study, we could not diagnose other etiologies which might mimic strongyloidiasis such as gastritis, allergy or protozoan infections and so on. However, in our clinical assessment study (pre- and post-treatment) among 21 patients with high number of *S. stercoralis* larvae (Chapter 8) we found that most gastro-intestinal and skin symptoms were associated with *S. stercoralis* infection. The symptoms almost entirely resolved three weeks after ivermectin treatment. It seems that additional underlying infections were unlikely to have played a major role in symptoms. However, more in-depth investigations are required to fully assess the clinical impact of this parasite in humans.

A most important symptom of strongyloidiasis (pathognomonic sign), the so-called *larva currens*, was never seen or reported among our high intensity cases. *Larva currens* is the skin involvement, which is visualized by urticarial serpiginous eruptions. However, *larva currens* are usually transient and last for a few hours up to few days only, but could recur over weeks, months or years [Amer *et al.*, 1984; Iwamoto *et al.*, 1998].

We assessed the efficacy of ivermectin three weeks after treatment among *S. stercoralis* cases. A cure rate of 85.7% and 98.3% were observed in 21 cases with high intensity of larvae (Chapter 8) and in 107 schoolchildren cases (Chapter 4), respectively. Our high ivermectin efficacy confirms earlier reports from comparable trials [Bisoffi *et al.*, 2011; Datry *et al.*, 1994; Igual-Adell *et al.*, 2004; Marti *et al.*, 1996; Naquira *et al.*, 1989; Nontasut *et al.*, 2005; Shikiya *et al.*, 1992; Suputtamongkol *et al.*, 2011]. Today, it is not known whether ivermectin can fully eradicate *S. stercoralis* from human gastro-intestinal tract. Most scientific literatures showed a satisfactory cure rate after three weeks of ivermectin treatment, while Zaha and colleagues reported a high efficacy (97.3%) after two years of ivermectin [Zaha *et al.*, 2000]. In our cohort study of schoolchildren we found that three-quarters of schoolchildren were infection-free at two years of exposure. To assess the efficacy of treatment is difficult, particularly in populations exposed to on-going

S. stercoralis transmission. A short follow-up period might overestimate the complete cure, whereas a longer follow-up period bears the risk of a re-infection. Therefore, ideal efficacy assessments for drugs against *S. stercoralis* should be conducted in non-exposed populations.

Without appropriate therapy, *S. stercoralis* infection does not resolve and may persist for life [WHO, 2013]. Strongyloidiasis is a difficult infection to treat because, for many helminth infections, a treatment is considered sufficient if worm burden is below the level at which clinical disease develops [Krolewiecki *et al.*, 2013; Siddiqui *et al.*, 2001]. Ivermectin was highly effective and well tolerated when given orally (200 µg/kg/day) [Sridhara *et al.*, 2008]. Ivermectin is listed in WHO as drug of choice for *S. stercoralis* infection treatment [WHO, 2006]. However, ivermectin is not included in the list of essential drugs of the Ministry of Health of Cambodia. Although there are at least two big pharmacies in Phnom Penh where the drug is sold, its price is extremely high (USD 10 per tablet of 3 mg) and is prohibitive for a wide-scale use. Albendazole, the alternative drug for treatment of strongyloidiasis has a much lower efficacy (38-45%), is recommended where ivermectin is not available [Montes *et al.*, 2010]. But the therapeutic regimen for strongyloidiasis is not established in the guidelines of the National Helminth Control Program of Cambodia [CNM, 2004]. Therefore, in order to increase access to adequate treatment of strongyloidiasis cases solutions for lost cost, high quality ivermectin must be found in Cambodia.

To date, WHO's preventive chemotherapy is the recommend intervention strategy of against STHs (*A. lumbrocoides, T. trichiura* and hookworms) in resource poor countries. Today, preventive chemotherapy drug (albendazole, mebendazole) for STHs control has marginal impact on *S. stercoralis* infection [Montes *et al.*, 2010]. The main impact of preventive chemotherapy for STHs is reduction of prevalence or sustenance of decreases in transmission or worm/eggs intensity [WHO, 2006]. Yet, with its ability of "auto-infection", the only STH that can replicate within the human host [Montes *et al.*, 2010], *S.*

stercoralis load reduction would not be a successful indicator of treatment or control goal. Establishing a preventive chemotherapy guideline for *S. stercoralis* is challenging, since the current distribution of *S. stercoralis* in communities from different epidemiological studies has shown prevalence peaks in adolescent and remaining stable in adults, while the mass drug administration is targeting on preschool- and school-age children only [Krolewiecki *et al.*, 2013]. Furthermore, it is not known how quickly an individual is re-infected with *S. stercoralis* after successful treatment [Schär *et al.*, 2013b]. However, our community-based studies found that participants were highly infected with *S. stercoralis* at very young age (31.4% for Preah Vihear and 14.5% for Takeo), and that the infection prevalence was increasing with age, particularly in the first 8 years of age and reaching a plateau. In addition, the two-year cohort study observed that three-quarters of treated cases and 80.4% of healthy schoolchildren at baseline were *S. stercoralis* infection free during two years of infection exposure. Our findings provide a first insight in benefits if ivermectin could be added to mass drug distribution program in controlling the *S. stercoralis* infection.

9.5. Diagnosis and sampling efforts

Three main factors contribute to the neglect of *S. stercoralis* infection: poor or unspecific clinical symptoms, irregular larvae excretion and lack of gold standard diagnostic method [Valerio *et al.*, 2013]. A number of diagnostic tests have been employed to detect *S. stercoralis*, including parasitological, serological and molecular methods. However, all of these techniques have problems with sensitivity, specificity, availability, sophistications or need qualified technicians in endemic resource poor countries [Montes *et al.*, 2010; Olsen *et al.*, 2009; Requena-Mendez *et al.*, 2013]. Today, the detection of *S. stercoralis* larvae in the stool specimen is proof of infection [Requena-Mendez *et al.*, 2013; Siddiqui *et al.*, 2001]. Baermann [Garcia *et al.*, 2001] and KAP culture [Koga *et al.*, 1991], coprological diagnostic methods, were frequently employed in field-based epidemiological surveys on *S. stercoralis* infection [Schär *et al.*, 2013b]. However, the sensitivity and specificity of both methods is not satisfactory [Requena-Mendez *et al.*, 2013]. Hence, single diagnostic method analyses on a single stool sample, are not sufficient reach an acceptable sensitivity. To overcome this limitation, analyzing a single stool specimen by multiple diagnostic methods has been proposed [Siddiqui *et al.*, 2001].

All research studies presented in the PhD thesis employed two coprological diagnostic techniques (KAP culture and Baermann method) to detect *S. stercoralis* larvae in fecal specimens. None of the two methods could diagnose all *S. stercoralis* infection. The performance of Baermann method and KAP culture in diagnosing *S. stercoralis* infection in single, double and triple stool samples were compared (Chapter 4, 5 and 6). In all studies, when using the combination of these two techniques as "gold standard", KAP culture was more sensitive than Baermann technique, i.e., 88.4% versus 75% (Chapter 4), 81.9%, versus 76.8% (Chapter 5) and 78.5% versus 67.1% (Chapter 6). Our findings coincide with previous reports from Brazil [Ines Ede *et al.*, 2011; Marchi Blatt *et al.*, 2003], USA [Salazar *et al.*, 1995], rural Côte d'Ivoire [Glinz *et al.*, 2010], and Honduras [de Kaminsky, 1993]. However, conflicting results were stated in the previous studies conducted in south-central Côte d'Ivoire, Zanzibar, China and Uganda. They reported that Baermann method was more accurate than KAP culture [Becker *et al.*, 2011; Knopp *et al.*, 2008; Steinmann *et al.*, 2007; Stothard *et al.*, 2008].

Figure 23: Baermann technique

Despite its disadvantages (time consuming, cost and requirement of expertise in differentiating hookworms from *S. stercoralis* larvae), KAP culture (with the advantage of using a small stool specimens) was reported as superior method compared to the traditional techniques: direct smears, filter paper culture or FECT [Requena-Mendez *et al.*, 2013]. Furthermore, a high sensitivity of KAP culture was previously observed. KAP culture was able to identify more than 96% of cases [Sato *et al.*, 1995]. As for Baermann method, compare to KAP culture, was cheaper and less time consuming. But, the technique is laborious and needs a considerably larger quantity of fecal materials, which may lead to the compliant problem, especially among young children who are at high risk and have often only small quantity of stool to offer. Nevertheless, Baermann method was previously described being best since it was able to detect all cases (100%) [Steinmann *et al.*, 2007]. In our studies, neither of the tests, KAP culture or Baermann test, can detect all *S. stercoralis* infections. The sensitivity of KAP culture and Baermann method was less than 90% and 80%, respectively. Our observed prevalence increased when analyzing stool samples with a combined two diagnostic tests. None of both methods was most appropriate diagnostic test, in term of laboriousness and cost for field-based epidemiological surveys, to use in endemic resource poor countries.

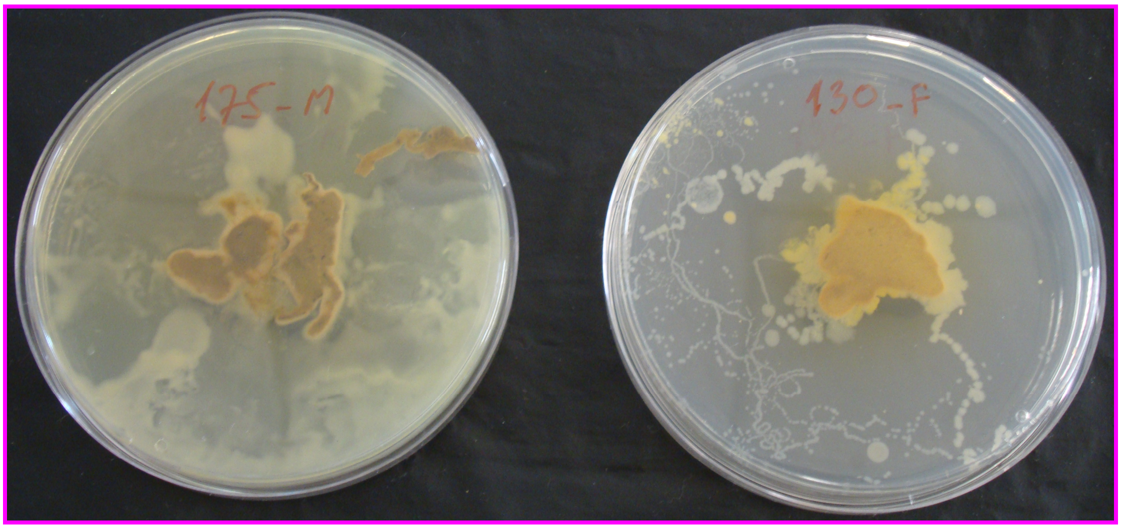

Figure 24: Koga agar plate culture

Due to irregular larvae excretion, many infected individuals might be misdiagnosed. To increase the sensitivity of diagnosis for *S. stercoralis* infection, Dreyer and colleagues suggested to analyze multiple consecutive stool specimens [Dreyer *et al.*, 1996]. Our studies analyzed single, double and triple stool sample by rigorous laboratory approach (KAP culture and Baermann method). The effects of sampling efforts (three stool samples) of our study in Kandal province were remarkable (Chapter 4). The observed prevalence of intestinal helminth infections considerably increased when three stool samples were analyzed by single or combined diagnostic methods (Figure 8), e.g., the prevalence of *S. stercoralis* (combined methods) increased from 18.6% to 24.4% when three stool samples were examined instead of one, which was close to "true prevalence" (24.8%, SD: 4.1%) estimated in a mathematical model (Table 2) [Marti *et al.*, 1993]. An increased prevalence of *S. stercoralis* when three stool samples were analyzed instead of one has been reported previously in China and Zanzibar [Knopp *et al.*, 2008; Steinmann *et al.*, 2007].

The failure to detect larvae in a stool examination does not necessarily indicate the unequivocal absence of the infection [Siddiqui *et al.*, 2001]. To make a correct diagnosis or to reach an acceptable "true prevalence", analyzing multiple stool samples by multiple parasitological diagnostic methods is needed. However, collecting big volume of multiple stool specimens on consecutive days is always a challenge. Alternative option, hence, is the pointed diagnostic methods using a small quantity of fecal specimen such as serological [Sykes *et al.*, 2011] and molecular [Mejia *et al.*, 2013; Moghaddassani *et al.*, 2011; Schär *et al.*, 2013a; Verweij *et al.*, 2009] techniques. However, these methods need further development and require further validation before they can be recommended for wider use. However, the cost and sophistication of these methods might hamper their introduction in tropical resource poor countries where *S. stercoralis* is most prevalent, particularly in Cambodia.

Whenever possible, examination of multiple stool samples with different diagnostic methods is the ideal option.

9.6. Further research needs

Based on the results and observations made in these reported studies, the following research gaps need to be address in future:

- ⇨ In order to decide whether ivermectin should be added in the preventive chemotherapy approach, a large-scale community-based study should be performed to assess the cost-effectiveness of ivermectin treatment and to identify the target population for the preventive chemotherapy strategy.
- ⇨ An evaluation on how strongyloidiasis can be added to the target disease in the national Cambodian helminth control strategy. Preventive and curative measures for *S. stercoralis* infection should be proposed and integrated into the current national soil-transmitted helminth preventive and control strategies. This strategy could stimulate similar initiatives in other endemic resource poor countries.
- ⇨ An excellent map for *S. stercoralis* infection prevalence rates was developed for Preah Vihear. Future initiative should establish the spatial distribution of *S. stercoralis* infection in the entire country of Cambodia, or the region of Southeast Asia
- ⇨ Given the fact that *S. stercoralis* has been reported in dogs and cats before, the transmission between human and animal should be investigated in detail in order to assess in how far household animal may contribute to human infection (or vice-versa).
- ⇨ Assess YLDs (Years Lost from Disability) and DALYs (Disability-Adjusted Life Years) of *S. stercoralis* infection to better understand and document the burden of infection of *S. stercoralis* in relation to other helminth infections.
- ⇨ Develop and/or validate “Gold-Standards” test using small amount of stool specimens such as serologic/immunologic or molecular diagnostic techniques for a reliable screening of *S. stercoralis* infection.

9.7. Recommendations

Based on our results, the following recommendations are proposed:

- ⇨ Implement preventive measures for *S. stercoralis* infection by integrating them into the current Cambodian National Helminth Control Program by reinforcing the primary preventive measures such personal hygiene (wearing shoes, defecating in toilet) and sanitation.
- ⇨ Ivermectin should be made available in Cambodian public and private health facilities, with an affordable price.
- ⇨ *S. stercoralis* infection screening should be included in the soil-transmitted helminth research activities in Cambodia, employing either Baermann method or Koga agar plate culture or both whenever possible.
- ⇨ *S. stercoralis* detection should be routinely performed in every stool examination in Cambodian public and private health facilities, using either Baermann method or/and Koga agar plate culture.
- ⇨ Strongyloidiasis should be evoked among patients presenting gastro-intestinal (stomach pain, diarrhea) and dermatological (urticaria, itchy) symptoms.

10. CONCLUSIONS

We conclude that *S. stercoralis* infection is highly prevalent in rural and semi-rural communities of Cambodia where appropriate diagnosis and treatment do not exist. The re-infection rate of *S. stercoralis* among schoolchildren after two years of treatment is considerably high. Certainly, similar prevalent and re-infection rates can be found in Southeast Asian countries sharing similar socioeconomic and environmental conditions with Cambodia. Thus, *S. stercoralis* infection should no longer be neglected in Cambodia and elsewhere of tropical resource poor countries.

Vulnerable individuals such as preschool- and school-aged children are highly affected. *S. stercoralis* infection displays a higher prevalence in the males than in the females in all age groups, and the prevalence increases with age. Personal hygiene and sanitation including wearing shoes, possession and use the latrine, are significant predictors of *S. stercoralis* infection. Improvement of a proper personal hygiene and sanitation could considerably reduce the infection and re-infection. Hence, health education on the primary preventive measures should be improved, both at school and community. Our findings further underline the need for re-enforce water and sanitation interventions in resource-poor settings.

In the absence of a "gold standard", the analysis of multiple stool samples with different diagnostic methods (KAP culture and Baermann technique) is required in order to reach a reliable diagnosis, especially in the epidemiological surveys on *S. stercoralis*. Moreover, an oral single dose of ivermectin (200µg/kg body weight) is highly efficacious against chronic uncomplicated strongyloidiasis with few side-effects. Finally, our studies document that the severity of clinical symptoms, including abdominal pain, diarrhea and urticaria, associated with *S. stercoralis* in a population living in a poor setting with no access to the disease control strategy. It is of concern not only in the study areas, but also in the whole country. Given its potential to produce systemic infections, the access to adequate treatment of chronic uncomplicated strongyloidiasis is an urgent need in Cambodia.

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12. Curriculum Vitae

Personal Data

First name	**Virak**
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Marital status	Married with one daughter
Nationality	Cambodian
Languages	Khmer (Mother tongue), English (very good), French (very good), Lao (very good oral)
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Education

2009 – 2013	**Doctor of Philosophy (PhD) in Epidemiology** Swiss Tropical and Public Health Institute, University of Basel, Basel, Switzerland
2002 – 2004	**Diplôme d'Études Approfondie (DEA)** Institut de la Francophonie pour la Médecine Tropicale, Vientiane, Lao PDR
1994 – 2001	**Medical Doctor (MD)** Faculty of Medicine, University of Health Sciences, Phnom Penh, Cambodia
1987 – 1993	**Diploma of Secondary School** Battambang High School, Battambang, Cambodia

Work Experiences

Feb. 09 – Present	National Helminth Control Program, National Center for Parasitology, Entomology and Malaria Control, Ministry of Health, Phnom Penh, Cambodia
Mar. 06 – Jan. 09	Epidemiology and Public Health Unit, Institut Pasteur du Cambodge, Phnom Penh, Cambodia
Aug. – Oct. 06	Consultant to CARE in Cambodia for conducting the Avian Influenza KAP (Knowledge, Attitudes & Practices) survey in Prey Veng province, Cambodia
Oct. – Dec. 05	Supervisor on mass tetanus vaccination campaign in Prasath Bakong district, Siem Reap province, Cambodia
Oct. 04 – Sept. 05	Lecturer at Institut de la Francophonie pour la Médecine Tropicale, Vientiane, Lao PDR

Professional Training

Feb. 2013	Scientific Manuscript Writing Training Course, Research Institute for Tropical Medicine, Alabang, Muntinlupa City, Philippines
Oct. 2012	Good Clinical Practice (GCP) for Investigators and Sub-Investigators, Swiss Tropical and Public Health Institute, Basel, Switzerland
Mar. 2012	Writing a journal article – and getting it published, Institute of Social and Preventive Medicine, Bern, Switzerland
Oct. 2010	Statistical Methods for Epidemiology, Institute of Social and Preventive Medicine, Bern, Switzerland
Dec. 2007	Regional Training Course on International Outbreak Response of the GOARN (Global Outbreak Alert and Response Network), Manila, Philippines, organized by WHO of Western Pacific Region Office
Mar. 2007	Course on New Development in Rabies Epidemiology, Diagnosis and Control, Pasteur Institute in Ho Chi Minh City, Vietnam
Dec. 2006	Epi-Info Training Course at National Institute for Public Health, Phnom Penh, organized by CDC Atlanta, USA
Jan. – Mar. 2005	Training at Parasitology-Mycology Laboratory of the Andrée ROSEMON Hospital of Cayenne, French-Guiana

Publications

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