Epidemiology of *Strongyloides stercoralis* on Mekong Islands in Southern Laos

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A B S T R A C T

Strongyloides stercoralis is a neglected helminth infection potentially leading to a systemic infection in immunocompromised individuals. In Lao People's Democratic Republic (Lao PDR, Laos), information on *S. stercoralis* infection is scarce. We assessed *S. stercoralis* infection and associated risk factors and symptoms on the Mekong Islands in southern Laos. On two stool samples Baermann and Kato-Katz techniques were performed to detect *S. stercoralis* larvae and concomitant helminth infections. Among 729 individuals, 41.0% were infected with *S. stercoralis*. Men were at higher risk than women (OR 1.92). Urticaria and body itching was associated with *S. stercoralis* infection (OR 2.4). Infection with *Opisthorchis viverrini* (72.2%), *Schistosoma mekongi* (12.8%), and hookworm (56.1%) were very common. Few infections with *Trichuris trichiura* (3.2%), *Ascaris lumbricoides* (0.3%) and *Taenia* spp. (0.3%) were detected. The majority of helminth infections were of light intensity in prevalences of 58.0%, 52.1%, 8.2%, 3.3% and 0.3%, for *O. viverrini,* hookworm, *S. mekongi, T. trichiura* and *A. lumbricoides* respectively. Nevertheless, heavy infection intensities were observed for *O. viverrini* (0.7%), *S. mekongi* (1.8%) and hookworm (1.7%). *S. stercoralis* is highly endemic on islands of Khong district, Champasack province, southern Laos. The national helminth control programme should no longer neglect the presence of this helminth infection.

1. Introduction

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

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

In Lao Peoples' Democratic Republic (Lao PDR, Laos) information on *S. stercoralis* infection is scarce. The diagnostic techniques used in the country, i.e., direct smears and Kato-Katz technique (Katz et al., 1972) have a very low sensitivity (Requena-Mendez et al., 2013). Therefore, *S. stercoralis* infection might be missed and underestimated diagnosis in the laboratories of Hospitals in the country. However, in 1996 the prevalence of *S. stercoralis* in Laos was estimated at 19% in Thakek and Hinboun district, Khammouane Province, central of the country by using agars plate culture method (Vannachone et al., 1998). In many parts of the country, e.g. on the Mekong islands in Champasack province, or Saravane province, water supply and sanitation facilities are absent in communities (Sayasone et al., 2007). In addition rural populations' life style and farming activities favor transmission (e.g. intense skin contact with soil). Other helminthiasis such as STH, food-borne trematodiasis

(FBT) and schistosomiasis mekongi are highly prevalent (Forrer et al., 2012; Rim et al., 2003; Sayasone et al., 2007; Sayasone et al., 2011).

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

2. Materials and Methods

2.1. Ethics statement

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

2.2. Study area and population

The study was conducted in March 2011 on three islands, i.e. Donlong, Donthan and Donlieng Island located in the Mekong River in Khong district, Champasack province, southern Laos. Donlong Island composes of four villages, namely Haulong, Longsong, Longkang and Hanglong village whereas Donthan and Donlieng islands compose of one village each namely Donthan and Donlieng village, respectively. Donlong, Donthan and Donlieng have a population of approximately 2,174 (Haulong: 567; Longsong: 543; Longkang: 510 and Hanglong: 554), 586 and 137 inhabitants, respectively. The main occupation of villagers in these three islands is rice subsistence farming, vegetable plantation, and fishing activities in the Mekong. Additionally, in Donlong a considerable number of farmers cultivate tobacco.

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

2.3. Field procedures and laboratory examinations

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

Two stool samples were collected per study participants within a five day period. Each sample was examined by using Kato-Katz thick smears technique (Katz et al., 1972) and Baermann technique (Garcia and Burckner, 2001). Pre-labeled plastic 30ml stool containers (ID numbers, name, age and date of stool collection) were handed out to each participant. They were asked to provide a full container of stool. Each morning, filled containers were collected and replaced with empty ones for stool collection on the following day. The stool samples were stored at ambient temperature and transferred to the laboratory of the Khong District Hospital within 2-3 hours post-collection where they were further processed.

Kato-Katz and Baermann tests are described in detail elsewhere (Khieu et al., 2013a; Sayasone et al., 2011). In brief, approximately 5 g of each stool sample was divided from each stool sample for performing Baermann test (Garcia and Burckner, 2001). The stool sample was placed on a gauze-lined mesh in a glass funnel equipped with a rubber tube and a clamp, and covered with de-chlorinated tap-water. After 2 hours, the water (approx. 50 ml) was centrifuged and the sediment examined under a microscope for *S. stercoralis* larvae (L1-stage). A single Kato-Katz thick smear (Katz et al., 1972) was prepared for each stool sample and examined within 1 hour of preparation. Helminth eggs were counted and recorded separately to obtain species-specific infection intensity estimates.

2.4. Data management and analysis

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

3. Results

3.1. Study population

In total, 729 individuals had complete data records (Figure 1). They originated from 247 households on the three islands: 347 (47.6%) and 382 (52.4%) individuals from Donlong and Donthan/Donlieng islands, respectively; 45.7% (333) were male; all were ethnic Laoloum. Age ranged from 2 to 95 years with a median age of 30.6 years. Among the participants, illiterate, and primary and secondary school graduate were 7.0%, 60.8% and 29.6%, respectively (Table 1). Only 2.6% had a technical/university level training. They lived in Donthan and Donlieng villages. The main occupation of the villagers was farming (61.9%) such as rice, tobacco, and vegetable farming while only few were government employees (2.5%). The socio-economic status on Donlong was significantly higher than on the other two islands ($p = 0.032$).

3.2. Strongyloides stercoralis *infection and co-infections*

The overall *S. stercoralis* infection prevalence was 41.0% (Table 2). The infection rate did not differ between the islands (Donlong 44.1% vs. Donthan/Donlieng 38.2%, $p = 0.107$). Highest infection rate was observed with *O. viverrini* (72.2%), followed by hookworm (56.1%) and *S. mekongi* (12.8%). *T. trichiura* (3.3%), *A. lumbricoides* (0.3%) and *Taenia* spp. (0.3%). Infection prevalence of *O. viverrini* (76.1% vs. 68.6%, $p = 0.024$) and *S. mekongi* (25.6% vs. 1.0%, $p < 0.001$) was significantly higher on Donlong than on the other two islands. Whereas, hookworm infection prevalence was significantly higher on Donthan/Donlieng islands (63.9% vs. 47.6%, $p < 0.001$).

Among the 729 individuals, only 11.1% were free of helminth infections. In 65.3% of the study participants two or more helminth infections were diagnosed. Multiple helminth infections were significantly more frequent on Donthan and Donlieng than on Donlong (p=0.001, Table 2).

The infection intensity of the diagnosed intestinal parasitic infections is given in Table 3. Most diagnosed helminth infections were of light intensity, e.g. 58.0% of *O. viverrini* infections. However, heavy infection intensities were found in patients with *S. mekongi* (1.8%), hookworm (1.7%) and *O. viverrini* (0.7%) infections.

3.3. Risk factors associated with Strongyloides stercoralis *infection*

The results of the risk analyses for a *S. stercoralis* infection are presented in Table 4. The most important risk factor was gender. Male compared to female study participants had a significantly higher risk for a *S. stercoralis* infection by taking into account the age of the study participants (adjusted OR 1.79, 95% CI 1.45-2.67).

S. stercoralis infection was diagnosed in participants of all ages. Children of the age group ≤ 5 years had the lowest infection prevalence (33.3%). However, in none of the older age groups the infection risk increased significantly. Interestingly, the age infection prevalence was distinctly different between male and female study participants (Fig. 2). In male participants the infection prevalence reached a peak 60% in the age between 20 and 30 years, and remained at around 50% in the older age groups. In female participants the infection reached a plateau of 38% in individuals of 10 years and remained up to 40 years, and dropped thereafter.

In our analyses none of the socio-economic risk factors such as socio-economic status, occupation and level of education was associated with *S. stercoralis* infection. Furthermore, also hygiene behaviours wearing shoes (slippers), having and using a latrine and having been treated with antihelminthic drugs in the past six months were significantly associated with *S. stercoralis* infection.

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

4. Discussion

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

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

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

In our study, we used the Baermann method on two stool samples per enrolled patients. The infection prevalence was comparable to recent reports from Cambodia (Khieu et al., 2013a; Khieu et al., 2014a; Khieu et al., 2014b), but substantially higher than infection prevalences reported from neighbouring China (Steinmann et al., 2007; Steinmann et al., 2008) and Thailand (Jongsuksuntigul et al., 2003; Sithithaworn et al., 2003). Our diagnostic procedures could have been improved by examining more stool samples per person and by adding additional diagnostic techniques. E.g. in a study in Cambodian children three stool samples were examined per child with a combination of Baermann technique and Koga Agar plate. Taken this approach as gold standard, our examination on two samples with a Baermann technique results in a sensitivity of approximately 70% and in combination with Koga Agar plate method a 93% sensitivity could have been reached (Khieu et al., 2013a). However, the substantial additional material costs and time efforts required for conducting the Koga-Agar plate must be taken into account when planning a field investigation. In our study, these factors did not allow that this method could be added in the diagnostic study procedures.

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

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

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

Most recent developments in our study area might have led to the absence of these associations. In fact, we selected the villages because the Provincial Health Office reported low numbers of households with latrine on villages on island in the Khong district. However, during our investigations we found that more than 40% of the households had a latrine.

Indeed in the last year, a number of health related intervention were undertaken in the Khong district, including general health promotion activities, and latrine construction and mass-deworming campaigns. We explain the absence of the associations with these new developments where people had access to improvements however remained infected with *S. stercoralis*.

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

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

In conclusion, *S. stercoralis* infection is highly endemic in the islands of the Khong district, Champasack province, southern Lao PDR. The results from this study and other *S. stercoralis* reports from the country should not be longer neglected by the national helminth control programme. County-wide assessments on *S. stercoralis* infection prevalence and related morbidity would be most useful to further push the agenda of an intensified integrated soil-transmitted helminth control in which *S. stercoralis* is adequately addressed.

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References

Becker, S.L., Vogt, J., Knopp, S., Panning, M., Warhurst, D.C., Polman, K., Marti, H., von, M.L., Yansouni, C.P., Jacobs, J., Bottieau, E., Sacko, M., Rijal, S., Meyanti, F., Miles, M.A., Boelaert, M., Lutumba, P., van, L.L., N'Goran, E.K., Chappuis, F., Utzinger, J., 2013. Persistent digestive disorders in the tropics: causative infectious pathogens and reference diagnostic tests. BMC.Infect.Dis. 13, 37.

Bethony, J., Brooker, S., Albonico, M., Geiger, S.M., Loukas, A., Diemert, D., Hotez, P.J., 2006. Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. Lancet 367, 1521-1532.

Forrer, A., Sayasone, S., Vounatsou, P., Vonghachack, Y., Bouakhasith, D., Vogt, S., Glaser, R., Utzinger, J., Akkhavong, K., Odermatt, P., 2012. Spatial distribution of, and risk factors for, *Opisthorchis viverrini* infection in southern Lao PDR. PLoS Negl.Trop.Dis. 6, e1481.

Garcia, L. and Burckner, D., 2001. Diagnostic medical parasitology. eds WashingtonDC: American Society for Microbiology 1-179.

Inpankaew, T., Schar, F., Dalsgaard, A., Khieu, V., Chimnoi, W., Chhoun, C., Sok, D., Marti, H., Muth, S., Odermatt, P., Traub, R.J., 2014. High prevalence of *Ancylostoma ceylanicum* hookworm infections in humans, Cambodia, 2012. Emerg.Infect.Dis. 20, 976-982.

Jongsuksuntigul, P., Intapan, P.M., Wongsaroj, T., Nilpan, S., Singthong, S., Veerakul, S., Maleewong, W., 2003. Prevalence of *Strongyloides stercoralis* infection in northeastern Thailand (agar plate culture detection). J Med.Assoc.Thai. 86, 737-741.

Katz, N., Chaves, A., Pellegrino, J., 1972. A simple device for quantitative stool thick-smear technique in schistosomiasis mansoni. Rev.Inst.Med.Trop.São Paulo 14, 397-400.

Khieu, V., Schar, F., Forrer, A., Hattendorf, J., Marti, H., Duong, S., Vounatsou, P., Muth, S., Odermatt, P., 2014a. High prevalence and spatial distribution of *Strongyloides stercoralis* in rural Cambodia. PLoS Negl.Trop.Dis. 8, e2854.

Khieu, V., Schar, F., Marti, H., Bless, P.J., Char, M.C., Muth, S., Odermatt, P., 2014b. Prevalence and risk factors of *Strongyloides stercoralis* in Takeo Province, Cambodia. Parasit.Vectors 7, 221.

Khieu, V., Schar, F., Marti, H., Sayasone, S., Duong, S., Muth, S., Odermatt, P., 2013a. Diagnosis, treatment and risk factors of *Strongyloides stercoralis* in schoolchildren in Cambodia. PLoS Negl.Trop.Dis. 7, e2035.

Khieu, V., Srey, S., Schar, F., Muth, S., Marti, H., Odermatt, P., 2013b. *Strongyloides stercoralis* is a cause of abdominal pain, diarrhea and urticaria in rural Cambodia. BMC.Res.Notes 6, 200.

MOH, 2004. Diagnosis and treatment at the district. A diagnosis and treatment guideline for the district hospital in Lao PDR. Vientiane: Ministry of Health.

Olsen, A., van, L.L., Marti, H., Polderman, T., Polman, K., Steinmann, P., Stothard, R., Thybo, S., Verweij, J.J., Magnussen, P., 2009. Strongyloidiasis--the most neglected of the neglected tropical diseases? Trans.R.Soc.Trop.Med.Hyg. 103, 967-972.

Paboriboune, P., Phoumindr, N., Borel, E., Sourinphoumy, K., Phaxayaseng, S., Luangkhot, E., Sengphilom, B., Vansilalom, Y., Odermatt, P., Delaporte, E., Etard, J.F., Rabodonirina, M., 2014. Intestinal parasitic infections in HIV-infected patients, Lao People's Democratic Republic. PLoS One. 9, e91452.

Requena-Mendez, A., Chiodini, P., Bisoffi, Z., Buonfrate, D., Gotuzzo, E., Munoz, J., 2013. The laboratory diagnosis and follow up of strongyloidiasis: a systematic review. PLoS Negl.Trop.Dis. 7, e2002.

Rim, H.J., Chai, J.Y., Min, D.Y., Cho, S.Y., Eom, K.S., Hong, S.J., Sohn, W.M., Yong, T.S., Deodato, G., Standgaard, H., Phommasack, B., Yun, C.H., Hoang, E.H., 2003. Prevalence of intestinal parasite infections on a national scale among primary schoolchildren in Laos. Parasitol.Res. 91, 267-272.

Satoh, M. and Kokaze, A., 2004. Treatment strategies in controlling strongyloidiasis. Expert.Opin.Pharmacother. 5, 2293-2301.

Sayasone, S., Mak, T.K., Vanmany, M., Rasphone, O., Vounatsou, P., Utzinger, J., Akkhavong, K., Odermatt, P., 2011. Helminth and intestinal protozoa infections, multiparasitism and risk factors in Champasack province, Lao People's Democratic Republic. PLoS.Negl.Trop.Dis. 5, e1037.

Sayasone, S., Odermatt, P., Phoumindr, N., Vongsaravane, X., Sensombath, V., Phetsouvanh, R., Choulamany, X., Strobel, M., 2007. Epidemiology of *Opisthorchis viverrini* in a rural district of southern Lao PDR. Trans.R.Soc.Trop.Med.Hyg. 101, 40-47.

Sayasone, S., Rasphone, O., Vanmany, M., Vounatsou, P., Utzinger, J., Tanner, M., Akkhavong, K., Hatz, C., Odermatt, P., 2012. Severe Morbidity Due to *Opisthorchis viverrini* and *Schistosoma mekongi* Infection in Lao People's Democratic Republic. Clin.Infect.Dis. 55, e54-e57.

Sayasone, S., Vonghajack, Y., Vanmany, M., Rasphone, O., Tesana, S., Utzinger, J., Akkhavong, K., Odermatt, P., 2009. Diversity of human intestinal helminthiasis in Lao PDR. Trans.R.Soc.Trop.Med.Hyg. 103, 247-254.

Schär, F., Inpankaew, T., Traub, R.J., Khieu, V., Dalsgaard, A., Chimnoi, W., Chhoun, C., Sok, D., Marti, H., Muth, S., Odermatt, P., 2014. The prevalence and diversity of intestinal parasitic infections in humans and domestic animals in a rural Cambodian village. Parasitol.Int. 63, 597-603.

Schär, F., Trostdorf, U., Giardina, F., Khieu, V., Muth, S., Marti, H., Vounatsou, P., Odermatt, P., 2013. *Strongyloides stercoralis*: Global Distribution and Risk Factors. PLoS.Negl.Trop.Dis. 7, e2288.

Siddiqui, A.A. and Berk, S.L., 2001. Diagnosis of *Strongyloides stercoralis* infection. Clin.Infect.Dis. 33, 1040-1047.

Sithithaworn, P., Srisawangwong, T., Tesana, S., Daenseekaew, W., Sithithaworn, J., Fujimaki, Y., Ando, K., 2003. Epidemiology of *Strongyloides stercoralis* in north-east Thailand: application of the agar plate culture technique compared with the enzyme-linked immunosorbent assay. Trans.R.Soc.Trop.Med.Hyg. 97, 398-402.

Steinmann, P., Du, Z.W., Wang, L.B., Wang, X.Z., Jiang, J.Y., Li, L.H., Marti, H., Zhou, X.N., Utzinger, J., 2008. Extensive multiparasitism in a village of Yunnan province, People's Republic of China, revealed by a suite of diagnostic methods. Am.J.Trop.Med.Hyg. 78, 760-769.

Steinmann, P., Zhou, X.N., Du, Z.W., Jiang, J.Y., Wang, L.B., Wang, X.Z., Li, L.H., Marti, H., Utzinger, J., 2007. Occurrence of *Strongyloides stercoralis* in Yunnan Province, China, and Comparison of Diagnostic Methods. PLoS.Negl.Trop.Dis. 1, e75.

Suputtamongkol, Y., Premasathian, N., Bhumimuang, K., Waywa, D., Nilganuwong, S., Karuphong, E., Anekthananon, T., Wanachiwanawin, D., Silpasakorn, S., 2011. Efficacy and safety of single and double doses of ivermectin versus 7-day high dose albendazole for chronic strongyloidiasis. PLoS Negl.Trop.Dis. 5, e1044.

Upatham, E.S., Viyanant, V., Kurathong, S., Rojborwonwitaya, J., Brockelman, W.Y., Ardsungnoen, S., Lee, P., Vajrasthira, S., 1984. Relationship between prevalence and intensity of *Opisthorchis viverrini* infection, and clinical symptoms and signs in a rural community in north-east Thailand. Bull.World Health Organ. 62, 451-461.

Vannachone, B., Kobayashi, J., Nambanya, S., Manivong, K., Inthakone, S., Sato, Y., 1998. An epidemiological survey on intestinal parasite infection in Khammouane Province, Lao PDR, with special reference to *Strongyloides* infection. Southeast Asian J.Trop.Med.Public Health 29, 717-722.

WHO, 2002. Prevention and control of schistosomiasis and soil-transmitted helminthiasis. World Health Organisation Technical Report Series 912.

Figure Legend

Figure 1: Study diagram

Figure 2: Age prevalence distribution by sex of *Strongyloides stercoralis* infection in villagers from Southern Laos

Table 1: Demographic characteristics of the study participants

Characteristic	**Overall**	**Locality**		**x^2**	***p*-value**
	n (%)	**Donlong n (%)**	**Donthan/Donlieng n (%)**		
N	729	347 (47.6)	382 (52.4)		
Age (years)					
Mean (range)	30.6 (2-95)	28.1 (2-81)	32.8 (2-95)		
Sex					
Female	396 (54.3)	187 (53.9)	209 (54.7)		
Male	333 (45.7)	160 (46.1)	173 (45.3)	0.049	0.824
Educational level					
Illiterate	51 (7.0)	27 (7.8)	24 (6.3)		
Primary school	443 (60.8)	231 (66.6)	212 (55.5)		
High school	216 (29.6)	89 (25.7)	127 (33.3)		
Technical school/University	19 (2.6)	0	19 (5.0)	25.053	<0.001
Occupation					
Farmer	451 (61.9)	216 (62.3)	235 (61.5)		
Student	212 (29.1)	101 (29.1)	111 (29.1)		
Child	48 (6.6)	29 (8.4)	19 (5.0)		
Government employee	18 (2.5)	1 (0.3)	17 (4.5)	15.934	0.001
Socio-economic status					
Most poor	146 (20.0)	55 (15.9)	91 (23.8)		
Very poor	147 (20.1)	69 (19.9)	78 (20.4)		
Poor	145 (19.8)	82 (23.6)	63 (16.5)		
Less poor	149 (20.4)	73 (21.0)	76 (19.9)		
Least poor	142 (19.4)	68 (19.6)	74 (19.4)	10.575	0.032

Table 2: Prevalence of helminth infections among villagers in the islands of Khong district, Champasack province (n=729)

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

Table 3: Intensity of helminth infections among villagers in the islands of Khong district, Champasack province (n=729)

Parasites	**Light** n (%)	**Moderate** n (%)	**Heavy** n (%)
Trematodes			
Opisthorchis viverrini	423 (58.0)	98 (13.4)	5 (0.7)
Schistosoma mekongi	60 (8.2)	20 (2.7)	13 (1.8)
Nematodes			
Ascaris lumbricoides	2 (0.3)	0	0
Trichuris trichiura	24 (3.3)	0	0
Hookworm	380 (52.1)	17 (2.3)	12 (1.7)

Table 4: Association among *Strongyloides stercoralis* infection and risk factors in the islands of Khong district, Champasack province

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

na not applicable