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Soil-Transmitted Helminthiasis in Laos: A Community-Wide Cross-Sectional Study of Humans and Dogs in a Mass Drug Administration Environment

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Abstract. We conducted a community cross-sectional survey of soil-transmitted helminthiasis in humans and dogs in four provinces in northern Laos. We collected and tested human and dog fecal samples and analyzed results against sociodemographic data. The prevalence of *Ascaris lumbricoides*, *Trichuris trichiura*, hookworm, and *Strongyloides stercoralis* was 26.1% (95% confidence interval [CI] = 23.7–28.4%), 41.5% (95% CI = 38.8–44.1%), 46.3% (95% CI = 43.3–49.0%), and 8.9% (95% CI = 7.4–10.4%), respectively. We observed strong heterogeneity for helminthiasis by ethnicity, province, and wealth status, which coincided with a risk profile demonstrating that Mon-Khmer persons and the poorest households are highly vulnerable. *Necator americanus* was the dominant hookworm species infecting humans and *Ancylostoma ceylanicum* was the only *Ancylostoma* species detected. Hookworm prevalence in village dogs was 94%, and the dominant species was *A. ceylanicum*. *Necator americanus* was also detected in dogs. It appears that dogs have a role in human hookworm transmission and warrant further investigation.

INTRODUCTION

Southeast Asia accounts for almost one-third of all cases of human ascariasis, trichuriasis, and hookworm infections worldwide.[1,2] Pre-school and school age children and pregnant women are considered at high risk because of the negative impacts of infection in these groups, including anemia, malabsorption, and decreased linear growth, adverse birth outcomes, and cognitive impairment.[3] Health outcomes are generally density dependent, whereby high-intensity infections lead to more severe clinical outcomes.[3,4] Infection with multiple soil-transmitted helminths (STHs) is common in poor tropical countries where these helminths are endemic,[4] and synergistic polyparasitic interactions may lead to higher worm burdens, thereby exacerbating the problem.[4,5] In Southeast Asia, those infected with an STH commonly harbor at least one other concurrent infection.[6]

Laos has one of the highest prevalences of STHs in Southeast Asia and polyparasitism is common. Rim and others[7] provided the evidence base for the introduction of a mass drug administration (MDA) program that achieved nationwide coverage in 2006.[8] Eight provinces have biannual treatment of 6–11-year-old children with a single dose of 500 mg of mebendazole, and the remaining nine provinces have an annual treatment.[8,9] Coverage in the highly endemic provinces was estimated to be greater than 98% in 2008 and greater than 93% for coverage across the whole country.[8] Activity of mebendazole against *Ascaris lumbricoides* remains high and historic cure rates are consistently greater than 90%.[10] However, recent studies have raised doubts about the efficacy of mebendazole in clearing or reducing the intensity of hookworm infection and to a lesser degree *Trichuris trichiura*.[10–13] In addition, evidence exists in China and Southeast Asia that peak hookworm prevalence and intensity occurs during adulthood,[14–16] indicating that targeting school age children alone may have limited impact in reducing environmental contamination with hookworm eggs.

Necator americanus is the predominant hookworm species infecting people in the Mekong River sub-region.[13,17,18] However, historical evidence suggests that the zoonotic hookworm species *Ancylostoma ceylanicum* is an important human pathogen in Southeast Asia. This particular hookworm species has been associated with clinical disease[19] but has been overlooked as a human pathogen for the past 50 years. Recent surveys conducted in Thailand and Laos indicate the potential significance of zoonotic transmission of this species.[17,18,20]

We report the results of a cross-sectional epidemiological survey of humans and dogs to investigate the patterns and risks of STHs in communities in four provinces of northern Laos. Furthermore, we report the results of a study of a randomly selected subset of hookworm-positive fecal samples to determine the hookworm species infecting humans and dogs.

MATERIALS AND METHODS

Ethics. Informed written consent was obtained from all human adult participants and from parents or legal guardians of minors. All participants were free to withdraw from the study at any time during the consultation with no further obligation. The study protocol was reviewed and approved by the Murdoch University Human Ethics Committee (Project no. 2008/266) and the Lao Ministry of Health National Ethics Committee for Health Research (No. 239/NECHR) before commencing this study.

For studies involving dogs and pigs, protocols were reviewed and approved by the Murdoch University Animal Ethics Committee (project no. R2108/07), which adheres to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. The Lao Department of Livestock and Fisheries does not at this time have a

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committee to review and approve scientific research protocols involving animals.

Study sites. Laos is an ethnically diverse country with 49 distinct ethnic groups classified into four ethno-linguistic families (Lao-Tai, Mon-Khmer, Hmong-Mien, and Sino-Tibetan), comprising 67%, 24%, 8%, and 1% of the population, respectively.[21] The study was conducted in four provinces in northern Laos (Oudomxay, Luangprabang, Huaphan, and Xiengkhuang) (Figure 1), where all four ethno-linguistic families are represented. One district in each province (Xay, Xiengngeun, Viengxay, and Pek Districts, respectively) was randomly selected for inclusion in this study. All provinces selected are categorized as highly endemic for STH and in which 6–11-year-old children receive biannual mebendazole treatment.[8,22] Mass drug administration was delivered in November and December 2008 to all four provinces included in our study (Aratchige PE, unpublished data), approximately 1–3 months before we began the study. Coverage was estimated to be 98%.[8] No detailed data on the timing of treatment of children in individual schools was available (Aratchige PE, unpublished data).

Survey design. The human survey was conducted in the dry season during January–March 2009 to maximize study participation and minimize negative impacts on seasonal labor demands. Six accessible villages in each district were randomly selected from official listings provided by provincial government offices, and villages were excluded if a four-wheel drive vehicle could not access them. Sample size calculation was performed by using simple random sampling based on estimated prevalence data for cysticercosis and taeniasis (Conlan JV and others, unpublished data); 14 households were randomly selected in each village from the official list of households provided by the village chief by using a random number table. All household members ≥ 6 years of age were asked to participate. Village chiefs were given advance notice

FIGURE 1. Study sites in northern Laos, 1 = Xay District, Oudomxay Province; 2 = Xiengngeun District, Luangprabang Province; 3 = Pek District, Xiengkhuang Province; 4 = Viengxay District, Huaphan Province.

of the survey timing and meetings were conducted in villages the day before to select households. In cases where a household refused to participate, the village chief selected a household with similar characteristics. A household questionnaire was administered to the head of each household with his/her family present to assess the house characteristics, assets owned, ownership of animals, age of each household member, ethnicity and education levels, and literacy of the male and female heads of household. Persons were also asked about their defecation practices.

Field procedures and sample collection. Questionnaires were pre-tested with persons who did not otherwise participate in the survey. Labeled plastic bags for a single fecal sample were distributed to household members to minimize risk of sample mix up and stool samples were collected from the participants the next day. Approximately 1 gram of fecal material was transferred into separate tubes containing 8 mL of 10% formalin and 80% ethanol, for microscopy and molecular analysis, respectively.

Dog fecal samples were collected with the permission of owners in the same villages as the human survey described above during January–March 2009. Samples were collected digitally and collected into two preservation solutions, 10% formalin and 80% ethanol, for microscopy and molecular analysis, respectively. Demographic data and the age and sex were recorded with the sample. Samples were sent to Vientiane at ambient temperature and subsequently stored at 4°C before processing.

Laboratory analysis. Formalin-preserved human feces was transported at room temperature to Khon Kaen University (Khon Kaen, Thailand) where samples were analyzed by using the standard formalin-ether-concentration technique and microscopy. Formalin-preserved dog feces was transported to Murdoch University (Perth, Western Australia, Australia) at ambient temperature and examined for helminth eggs by using the saturated sodium nitrate flotation technique and microscopy.

A subset of ethanol-preserved human and dog samples that were hookworm egg positive were randomly selected from the total pool of positive samples stratified by province and sent to Murdoch University at ambient temperature. Total DNA was extracted directly from feces by using the automated Maxwell® 16 Tissue DNA Purification Kit (Promega, Madison, WI) as per manufacturer's instructions.

A fragment encompassing the internal transcribed spacer 1, 5.8S, and internal transcribed spacer 2 regions of *N. americanus* (485 basepairs) and *Ancylostoma* spp. (380 basepairs) were amplified as described[20] with some modifications. Briefly, a reaction volume of 25 μL contained 1 unit of Tth Plus DNA polymerase in Tth Plus reaction buffer supplemented with 2.5 mM $MgCl_2$ (Fisher Biotech, Perth, Western Australia, Australia), 0.5 μM of each primer (RTHW1F and RTHW1R), 0.2 mM of each dNTP, and 1 μL of template DNA. Initial polymerase chain reactions (PCRs) were carried out using undiluted template; those samples that failed to amplify were retested using template diluted 1:4 in DNA-grade water. Samples were heated at 94°C for 5 minutes, followed by 40 cycles at 94°C for 30 seconds, 64°C for 30 seconds, 72°C for 30 seconds, and a final extension at 72°C for 7 minutes.

All amplified bands of the correct size were subject to DNA sequencing. The PCR products were purified by using the Agencourt AMPure XP DNA Purification and Cleanup

Kit (Beckman Coulter Australia, Gladesville, New South Wales, Australia) according to manufacturer's instructions. Purified products were sequenced in both directions by using the Big Dye Terminator System Version 3.1 (Applied Biosystems, Foster City, USA) on an ABI 373 x1 capillary sequencer.

Sequence chromatograms were edited by using FinchTV Version 1.4.0 (Geospiza Inc., Seattle, WA). Hookworm sequences were aligned and compared with previously published sequences of *A. duodenale* (GenBank accession nos. EU344797, AJ001679, AJ001594), *N. americanus* (AF217891, AJ001680), *A. ceylanicum* (DQ780009, DQ831517), *A. caninum* (AM850106, DQ438079), *A. braziliense* (DQ438054), and sequences generated from positive controls of *A. duodenale* and *A. ceylanicum* by using CLUSTALX version 2.0.[23]

Data management and statistical analysis. Questionnaire and laboratory test result data were entered into a spreadsheet (Excel®; Microsoft Corporation, Redmond, WA), and subsequent analysis was carried out by using STATA/IC version 10 (Stata Corporation, College Station, TX). The socioeconomic status of each household was calculated by using principal component analysis of household assets[24,25] after missing values were replaced by the mean of the respective asset for that ethnic group. All assets were dichotomous. Households were ranked into wealth quintiles according to their cumulative standardized asset scores. Missing data on literacy of household members caused by death, divorce, or other factors were replaced with the mean for that village and rounded to zero or one, illiterate or literate. The final analysis only considered persons with complete parasitologic data.

Age was stratified into five groups to reflect the mass drug administration target group (6–11 years), adolescents and young adults (12–19 years), adults (20–34 years), older adults (35–49 years), and elderly (≥ 50 years). Prevalence of *A. lumbricoides*, *T. trichiura*, and hookworm was calculated as the proportion of egg-positive results in the sampled population. Prevalence of *Strongyloides stercoralis* was calculated as the proportion of rhabditiform larvae detected in the sampled population. In the human study, Pearson's chi-square test was used to analyze associations between infection status and sex, location, ethnicity, age group, wealth status, defecation site, and literacy of the male and female heads-of-household. Multiple logistic regression analysis was used to calculate odds ratios and 95% confidence intervals (CIs) to determine risk associations between helminth infections and each population characteristic and the risk of co-infection with another STH. Explanatory variables that were significant ($P < 0.05$) or of borderline significant (cut-off $P \leq 0.10$) in univariate analyses were included in a multivariate random effects logistic regression model adjusting for the effect of household clustering. Risk factors were calculated for the entire survey population, and a sub-population analysis was performed for school age children (6–11 years of age) and women of childbearing age (defined as women 15–49 years of age).

For the dog study, the Kruskal-Wallis rank test was used to test for an association between dog age and hookworm infection. Pearson's chi-square test was used to analyze associations between hookworm infection status and sex and location.

RESULTS

Human study cohort, participation rates, and population structures. A total of 1,579 persons in 333 households from the 24 selected villages were eligible to participate in this survey. Of these persons, 1,358 (86.0%) persons from 330 households were included in the final analysis. Of these households, the village chief in a single Hmong-Mien village in Xiengkhuang Province selected eight participating households. The Lao-Tai and Mon-Khmer ethno-linguistic families had the highest proportion of participation with 95.9% and 89.5% of eligible persons, respectively, fully compliant. The Hmong-Mien ethno-linguistic family had the lowest proportion of participation with 72.8% of eligible persons compliant. The final survey population consisted of 564 (41.5%) Lao-Tai, 526 (38.7%) Mon-Khmer, and 268 (19.7%) Hmong-Mien. The median age was 24.0 years (range = 6–91 years). By ethnicity, the median ages were 28.0, 22.5, and 19.0 years for Lao-Tai, Mon-Khmer and Hmong-Mien, respectively ($\chi^2 = 21.6$, $P < 0.001$, by Kruskal-Wallis rank test).

Characteristics of the survey population stratified by province are shown in Table 1. Significant differences were observed for all characteristics with the exception of the sex and age category. Mon-Khmer were the predominant ethno-linguistic family in Oudomxay and Luangprabang Provinces, Lao-Tai were the predominant ethno-linguistic family in Huaphan Province, and Hmong-Mien were the predominant ethno-linguistic family in Xiengkhuang Province (Table 1). The survey population in Oudomxay Province had predominantly most or very poor persons (30.2% and 37.5%), Luangprabang Province had predominantly least poor persons (42.6%), Huaphan Province had predominantly poor persons (46.8%), and Xiengkhuang Province had predominantly less poor persons (36.6%). The highest rates of open defecation were observed in Oudomxay and Luangprabang Provinces, as were the highest rates of illiteracy of male and female heads of households (Table 1). Mon-Khmer persons were significantly more likely to defecate in the open ($\chi^2 = 182.9$, $P < 0.001$), have an illiterate male ($\chi^2 = 138.3$, $P < 0.001$) or female ($\chi^2 = 249.5$, $P < 0.001$) head of household, and belong to a most poor or very poor household ($\chi^2 = 298.6$, $P < 0.001$).

Prevalence and risks of STH infections. The overall prevalence of infection with any STH tested by the formalin-ether concentration technique was 70.6%; the prevalence of *A. lumbricoides*, *T. trichiura*, hookworm, and *S. stercoralis* were 26.1%, 41.5%, 46.3%, and 8.9%, respectively (Table 2). The species-specific prevalence of STH by population characteristic are described in detail in Table 2. There was no significant difference ($P > 0.05$) in prevalence between male and females for all parasite species with the exception of *S. stercoralis*, where the prevalence in males was more than double that of females. There was a significant difference in province-specific prevalence for all parasite species; Xiengkhuang Province had the lowest prevalence for all STHs, and Luangprabang Province had the highest prevalence for all species except hookworm. Prevalence of *A. lumbri*coides, *T. trichiura*, and hookworm decreased significantly with increasing household wealth, and *S. stercoralis* increased significantly with increasing wealth.

There was a significant difference between age groups for *A. lumbricoides*, and a peak prevalence was observed in persons 20–34 years of age. *Trichuris trichiura* prevalence decreased significantly with increasing age up to 49 years and showed a slight increase in the oldest cohort, whereas *S. stercoralis* increased significantly with increasing age up to 49 years. The prevalence of helminthiasis was significantly

Table 1
Survey population characteristics stratified by province, Laos

Characteristic	Total		Oudomxay		Luangprabang		Huaphan		Xiengkhuang		χ^{2}*	*P*
	No.	%	No.	%	No.	%	No.	%	No.	%		
Sex												
F	686	50.5	202	52.6	177	50.3	152	48.1	155	50.7		
M	672	49.5	182	47.4	175	49.7	164	51.9	151	49.4	1.4	0.702
Ethno-linguistic family												
Lao-Tai	564	41.5	58	15.1	93	26.4	278	88.0	135	44.1		
Mon-Khmer	526	38.7	298	77.6	228	64.8	0	0.0	0	0.0		
Hmong-Mien	268	19.7	28	7.3	31	8.8	38	12.0	171	55.9	964.4	< 0.001
Wealth status												
Most poor	250	18.4	116	30.2	52	14.8	38	12.0	44	14.4		
Very poor	255	18.8	144	37.5	28	8.0	41	13.0	42	13.7		
Poor	291	21.4	26	6.8	57	16.2	148	46.8	60	19.6		
Less poor	295	21.7	37	9.6	65	18.5	81	25.6	112	36.6		
Least poor	267	19.7	61	15.9	150	42.6	8	2.5	48	15.7	491.1	< 0.001
Age category (years)												
6–11	290	21.4	95	24.7	63	17.9	59	18.7	73	23.9		
12–19	288	21.2	81	21.1	78	22.2	65	20.6	64	20.9		
20–34	296	21.8	84	21.9	67	19.0	77	24.4	68	22.2		
35–49	270	19.9	75	19.5	81	23.0	58	18.4	56	18.3		
≥ 50	214	15.8	49	12.8	63	17.9	57	18.0	45	14.7	15.4	0.219
Defecation site												
Latrine	888	65.4	262	68.2	182	51.7	247	78.2	197	64.4		
Open	470	34.6	122	31.8	170	48.3	69	21.8	109	35.6	53.4	< 0.001
Male head of household												
Illiterate	232	17.1	109	28.4	93	26.4	8	2.5	22	7.2		
Literate	1,126	82.9	275	71.6	259	73.6	308	97.5	284	92.8	124.7	< 0.001
Female head of household												
Illiterate	543	40.0	241	62.8	136	38.6	76	24.1	90	29.4		
Literate	815	60.0	143	37.2	216	61.4	240	76.0	216	70.6	131.00	< 0.001

* Calculated across all groups and provinces.

greatest in the Mon-Khmer ethno-linguistic family for all STHs, and *A. lumbricoides* and *T. trichiura* infection were significantly greater in the cohort who defecated in the open. Infection with *S. stercoralis* was not associated with literacy of the male or female head-of-household, whereas *A. lumbricoides* and *T. trichiura* infections were significantly greater in households with an illiterate male or female head-of-household. Hookworm infection was significantly greater in households with an illiterate female head-of-household but not with a male head-of-household.

The significant risk factors associated with STH infection after controlling for household clustering at the community level and in high-risk sub-populations are shown in Table 3. At the community level, there was strong evidence of spatial, ethnicity, and wealth variation in persons with an STH infection. Study participants living in Luangprabang and Huaphan Provinces had an increased risk of ascariasis, trichuriasis, or any STH infection than residents in Oudomxay Province. Study participants from Xiengkhouang Province had a reduced risk of ascariasis or strongyloidiasis, and persons from Huaphan Province had an increased risk of hookworm infection. Persons in the wealthier quintiles had a reduced risk of having an STH infection with the exception of strongyloidiasis. Persons of Mon-Khmer ethnicity had increased risk of ascariasis, trichuriasis, or any STH infection than persons of Lao-Tai ethnicity, whereas persons of Hmong-Mien ethnicity had a moderately increased risk of hookworm infection but a marked reduction in risk of trichuriasis or strongyloidiasis. At the community level, open defecation was a significant risk for trichuriasis, and men had a slightly increased risk of having any STH infection.

For sub-group analysis of 6–11 year old children, a significant increase in risk of ascariasis, trichuriasis, strongyloidiasis, or any STH infection was observed in Luangprabang Province. Children in Huaphan Province were also more likely to have trichuriasis than children in Oudomxay Province, and children from least poor households had a reduction in risk of ascariasis, trichuriasis, hookworm, or any STH infection. Literacy of the heads of household were also significant, whereby children from households with a literate male head had a marked reduction in risk of ascariasis, and children from a household with a literate female head were less likely to have a hookworm infection. Boys had an increased risk of hookworm infection compared with girls.

Women of childbearing age from Luangprabang and Huaphan Provinces were at increased risk of ascariasis and trichuriasis. Furthermore, women from Huaphan and Luangprabang Provinces were also at increased risk of hookworm and strongyloidiasis, respectively. Women of Mon-Khmer ethnicity had an increased risk of ascariasis, trichuriasis, or any STH infection. Conversely, women from the least poor households had a reduced risk of ascariasis, trichuriasis, hookworm, or any STH infection. Women who reported open defecation had an increased risk of trichuriasis.

Patterns of STH polyparasitism. Overall prevalence of co-infection with *A. lumbricoides* and *T. trichiura* was 16.6% (95% CI = 14.6–18.5%), prevalence of co-infection with *A. lumbricoides* and hookworm was 14.7% (95% CI = 12.8–16.6%), prevalence of co-infection with *T. trichiura* and hookworm was 23.0% (95% CI = 20.7–25.2%), and prevalence of co-infection with all three STHs (*A. lumbricoides*, *T. trichiura*, and hookworm) was 9.6% (95% CI = 8.1–11.2%).

Table 2
Unadjusted prevalence of soil-transmitted helminths by population characteristics, Laos

Characteristic	*Ascaris lumbricoides* %	*Ascaris lumbricoides* 95% CI	*Trichuris trichiura* %	*Trichuris trichiura* 95% CI	Hookworm %	Hookworm 95% CI	*Strongyloides stercoralis* %	*Strongyloides stercoralis* 95% CI	Any STH* %	Any STH* 95% CI
Survey population	26.1	23.7–28.4	41.5	38.8–44.1	46.3	43.6–49.0	8.9	7.4–10.4	70.6	68.2–73.1
Sex										
F	28.0	24.6–31.4	40.8	37.2–44.5	43.7	40.0–47.5	5.4	3.7–7.1	68.1	64.6–71.6
M	24.1	20.9–27.3	42.1	38.4–45.9	49.0	45.2–52.7	12.5	10.0–15.0	73.2	69.9–76.6
χ^2	2.7		0.2		3.7		21.1		4.3	
P	0.103		0.628		0.053		< 0.001		0.038	
Province										
Oudomxay	24.5	20.2–28.8	45.6	40.6–50.6	50.8	45.8–55.8	7.6	4.9–10.2	75.8	71.5–80.1
Luangprabang	40.6	35.5–45.8	60.8	55.7–65.9	41.8	36.6–46.9	18.8	14.7–22.8	81.5	77.5–85.6
Huaphan	30.0	25.0–35.1	52.2	46.7–57.8	51.6	46.0–57.1	6.6	3.9–9.4	77.8	73.2–82.5
Xiengkhouang	7.2	4.3–10.1	2.9	1.0–4.8	40.5	35.0–46.1	1.6	0.2–3.1	44.1	38.5–49.7
χ^2	98.4		259.0		13.7		64.8		136.7	
P	< 0.001		< 0.001		0.003		< 0.001		< 0.001	
Household wealth status										
Most poor	37.6	31.6–43.6	53.6	47.4–59.8	59.2	53.1–65.3	7.6	4.3–10.9	86.4	82.1–90.7
Very poor	31.8	26.0–37.5	49.8	43.6–56.0	57.2	51.1–63.4	6.7	3.6–9.7	82.0	77.2–86.7
Poor	30.6	25.3–35.9	41.2	35.5–46.9	43.0	37.2–48.7	5.2	2.6–7.7	68.0	62.7–73.4
Less poor	15.6	11.4–19.8	29.8	24.6–35.1	41.4	35.7–47.0	10.8	7.3–14.4	60.0	54.4–65.6
Least poor	16.5	12.0–21.0	35.2	29.4–41.0	33.0	27.3–38.6	14.2	10.0–18.5	59.6	53.6–65.5
χ^2	54.2		43.2		52.4		17.8		78.5	
P	< 0.001		< 0.001		< 0.001		0.001		< 0.001	
Age (years)										
6–11	25.9	20.8–30.9	48.3	42.5–54.1	43.8	38.0–49.5	4.5	2.1–6.9	70.3	65.1–75.6
12–19	29.2	23.9–34.4	44.4	38.7–50.2	41.7	35.9–47.4	6.9	4.0–9.9	71.2	65.9–76.4
20–34	30.4	25.1–35.7	37.2	31.6–42.7	48.6	42.9–54.4	10.5	7.0–14.0	71.3	66.1–76.5
35–49	23.3	18.3–28.4	35.9	30.2–41.7	49.6	43.6–55.6	12.2	8.2–16.2	72.2	66.8–77.6
≥ 50	19.6	14.3–25.0	41.1	34.5–47.8	48.6	41.8–55.3	11.2	7.0–15.5	67.3	61.0–73.6
χ^2	10.0		12.3		5.5		14.3		1.6	
P	0.041		0.015		0.237		0.006		0.810	
Ethnicity										
Lao-Tai	17.7	14.6–20.9	36.2	32.2–40.1	39.7	35.7–43.8	9.9	7.4–12.4	61.9	57.9–65.9
Mon-Khmer	39.4	35.2–43.5	60.6	56.5–64.8	50.6	46.3–54.9	11.2	8.5–13.9	85.2	82.1–88.2
Hmong-Mien	17.5	13.0–22.1	14.9	10.6–19.2	51.9	45.8–57.9	2.2	0.5–4.0	60.4	54.6–66.3
χ^2	78.6		164.0		17.0		18.9		87.8	
P	< 0.001		< 0.001		< 0.001		< 0.001		< 0.001	
Defecation site										
Latrine	21.3	18.6–24.0	35.4	32.2–38.5	44.8	41.5–48.1	9.2	7.3–11.1	65.8	62.6–68.9
Open	35.1	30.8–39.4	53.0	48.4–57.5	49.1	44.6–53.7	8.2	5.8–10.8	79.8	76.1–83.4
χ^2	30.5		39.3		2.3		0.3		29.1	
P	< 0.001		< 0.001		0.128		0.564		< 0.001	
Male head of household										
Literate	23.9	21.4–26.4	37.9	35.1–40.8	45.8	42.9–48.7	8.7	7.1–10.4	68.5	65.8–71.2
Illiterate	36.6	30.4–42.9	58.6	52.2–65.0	48.7	42.2–55.2	9.5	5.7–13.3	81.0	76.0–86.1
χ^2	16.2		34.0		0.6		0.1		14.6	
P	< 0.001		< 0.001		0.423		0.737		< 0.001	
Female head of household										
Literate	22.9	20.1–25.8	36.3	33.0–39.6	40.9	37.5–44.2	9.2	7.2–11.2	64.5	61.2–67.8
Illiterate	30.8	26.9–34.6	49.2	45.0–53.3	54.5	50.3–58.7	8.5	6.1–10.8	79.7	76.4–83.1
χ^2	10.3		22.2		24.4		0.2		36.3	
P	0.001		< 0.001		< 0.001		0.643		< 0.001	

* Including all four STH infections.
STH = soil-transmitted helminth; CI = confidence interval.

Patterns of polyparasitism by population characteristics are shown in Figure 2. The prevalence of co-infection was significantly lower in Xiengkhuang Province than in the other three provinces, and the prevalence of co-infection with *A. lumbricoides* and *T. trichiura* was 30.1% in Luangprabang Province. This prevalence was significantly higher than that in the other provinces. The prevalence of polyparasitism was significantly greater in Mon-Khmer participants for all co-infections, and prevalence of polyparasitism uniformly decreased with increasing household wealth status. No significant differences were observed for the prevalence of polyparasitism across age groups (Figure 2).

Patterns of polyparasitism in children was similar to community patterns as a whole; children in Luangprabang Province had a significantly greater prevalence of dual infections with *A. lumbricoides* and *T. trichiura* and triple infections with *A. lumbricoides, T. trichiura*, and hookworm (Figure 2A). Mon-Khmer children had significantly higher prevalence of all co-infections with the exception of dual infection with *A. lumbricoides* and hookworm (Figure 2B). Furthermore, prevalence of polyparasitism in Mon-Khmer children and Luangprabang children was markedly greater than the community level prevalence. Children from most poor households had a higher prevalence of dual infections with *A. lumbricoides*

Table 3

Risk factors significantly ($P < 0.050$) associated with soil-transmitted helminth infections at the community level and in vulnerable sub-populations (children 6–11 years of age and women of childbearing age [15–49 years of age]) in Laos, as determined by multiple logistic regression modeling controlling for household clustering

Parasite	Population	Risk factor*	Adjusted OR	95% CI	*P*
Ascaris lumbricoides†	Community wide	Luangprabang Province	5.08	2.95–8.72	< 0.001
		Huaphan Province	5.55	2.62–11.76	< 0.001
		Less poor	0.36	0.19–0.69	0.002
		Least poor	0.27	0.13–0.54	< 0.001
		Mon-Khmer ethnicity	4.11	2.16–7,82	< 0.001
	6–11-year-old children	Luangprabang Province	7.84	2.29–26.84	0.001
		Least poor	0.12	0.02–0.57	0.008
		Mon-Khmer ethnicity	6.51	1.30–32.62	0.023
		Literate male head of household	0.45	0.20–0.99	0.048
	Women of childbearing age	Luangprabang Province	9.02	3.06–26.64	< 0.001
		Huaphan Province	24.49	4.92–121.87	< 0.001
		Less poor	0.14	0.04–0.54	0.004
		Least poor	0.23	0.07–0.82	0.024
		Mon-Khmer ethnicity	7.04	1.94–25.55	0.003
Trichuris trichiura§‡	Community wide	Luangprabang Province	4.11	2.29–7.39	< 0.001
		Huaphan Province	5.46	2.58–11.56	< 0.001
		Xiengkhuang Province	0.12	0.04–0.32	< 0.001
		Least poor	0.46	0.21–0.99	0.048
		20–34 years of age	0.46	0.29–0.73	0.001
		35–49 years of age	0.42	0.27–0.67	< 0.001
		Mon-Khmer ethnicity	2.40	1.27–4.55	0.007
		Hmong-Mien ethnicity	0.36	0.17–0.76	0.007
		Open defecation	1.62	1.05–2.51	0.030
	6–11-year-old children	Luangprabang Province	12.13	3.04–48.49	< 0.001
		Huaphan Province	5.51	1.33–22.84	0.019
		Least poor	0.15	0.03–0.83	0.029
	Women of childbearing age	Luangprabang Province	2.78	1.28–6.02	0.010
		Huaphan Province	12.29	4.10–36.88	< 0.001
		Mon-Khmer ethnicity	4.13	1.71–9.95	0.002
		Open defecation	3.06	1.63–5.76	0.001
Hookworm¶§	Community wide	Huaphan Province	1.89	1.01–3.53	0.045
		Least poor	0.42	0.24–0.76	0.004
		Hmong-Mien ethnicity	1.74	1.04–2.90	0.033
	6–11-year-old children	Male	2.26	1.05–4.89	0.038
		Least poor	0.13	0.02–0.68	0.016
		Literate female head of household	0.26	0.09–0.81	0.019
	Women of childbearing age	Least poor	0.26	0.10–0.67	0.005
Strongyloides stercoralis#¶	Community wide	Male	2.87	1.85–4.45	< 0.001
		Luangprabang Province	2.56	1.44–4.53	0.001
		Xiengkhuang Province	0.17	0.06–0.51	0.002
		20–34 years of age	2.66	1.30–5.47	0.008
		35–49 years of age	2.51	1.23–5.11	0.011
		≥ 50 years of age	2.19	1.03–4.67	0.042
		Hmong-Mien ethnicity	0.26	0.09–0.69	0.007
	6–11-year-old children	Luangprabang Province	17.73	2.21–142.33	0.007
	Women of childbearing age	Luangprabang Province	2.68	1.05–6.87	0.040
Any STH#	Community wide	Male	1.42	1.06–1.90	0.020
		Luangprabang Province	3.07	1.67–5.63	< 0.001
		Huaphan Province	3.81	1.80–8.06	< 0.001
		Poor	0.36	0.17–0.73	0.005
		Less poor	0.33	0.16–0.68	0.003
		Least poor	0.22	0.10–0.49	< 0.001
		Mon-Khmer ethnicity	2.90	1.54–5.50	0.001
	6–11-year-old children	Luangprabang Province	17.45	1.89–160.51	0.012
		Huaphan Province	13.14	1.31–131.91	0.029
		Less poor	0.09	0.01–0.83	0.034
		Least poor	0.03	0.00–0.39	0.008
		Mon-Khmer ethnicity	12.04	1.31–110.88	0.028
	Women of childbearing age	Huaphan Province	4.99	1.69–14.77	0.004
		Least poor	0.30	0.09–0.95	0.040
		Mon-Khmer ethnicity	3.04	1.24–7.44	0.015

*Reference comparators: Province, Oudomxay Province; wealth status, most poor; ethnicity, Lao-Tai; Age group, 6–11 years of age.
†Community-wide OR adjusted for province, wealth status, ethnicity, age group, defecation site. and male and female head-of-household literacy; 6–11-year-old children OR adjusted for province, wealth status, ethnicity, defecation site, and male and female head-of-household literacy; and women of childbearing age OR adjusted for province, wealth status, ethnicity, and defecation site.
‡Community-wide OR adjusted for province, wealth status, ethnicity, age group, defecation site, and male and female head-of-household literacy; 6–11-year-old children OR adjusted for province, wealth status, ethnicity, and male head-of-household literacy; and women of childbearing age OR adjusted for province, wealth status, ethnicity, defecation site, and male and female head-of-household literacy.
§Community-wide OR adjusted for sex, province, wealth status, ethnicity, and female head-of-household literacy; 6–11-year-old children OR adjusted for sex, wealth status, ethnicity, and male and female head-of-household literacy; and women of childbearing age OR adjusted for wealth status, ethnicity, defecation site, and female head-of-household literacy.
¶Community-wide OR adjusted for sex, province, wealth status, ethnicity, and age group; 6–11-year-old children OR adjusted for province; and women of childbearing age OR adjusted for province.
#Community-wide OR adjusted for sex, province, wealth status, ethnicity, defecation site, and male and female head-of-household literacy; 6–11-year-old children OR adjusted for province, wealth status, ethnicity, defecation site, and male head-of-household literacy; and women of childbearing age OR adjusted for province, wealth status, ethnicity, defecation site. and male and female head-of-household literacy.
OR = odds ratio; CI = confidence interval; STH = soil-transmitted helminth.

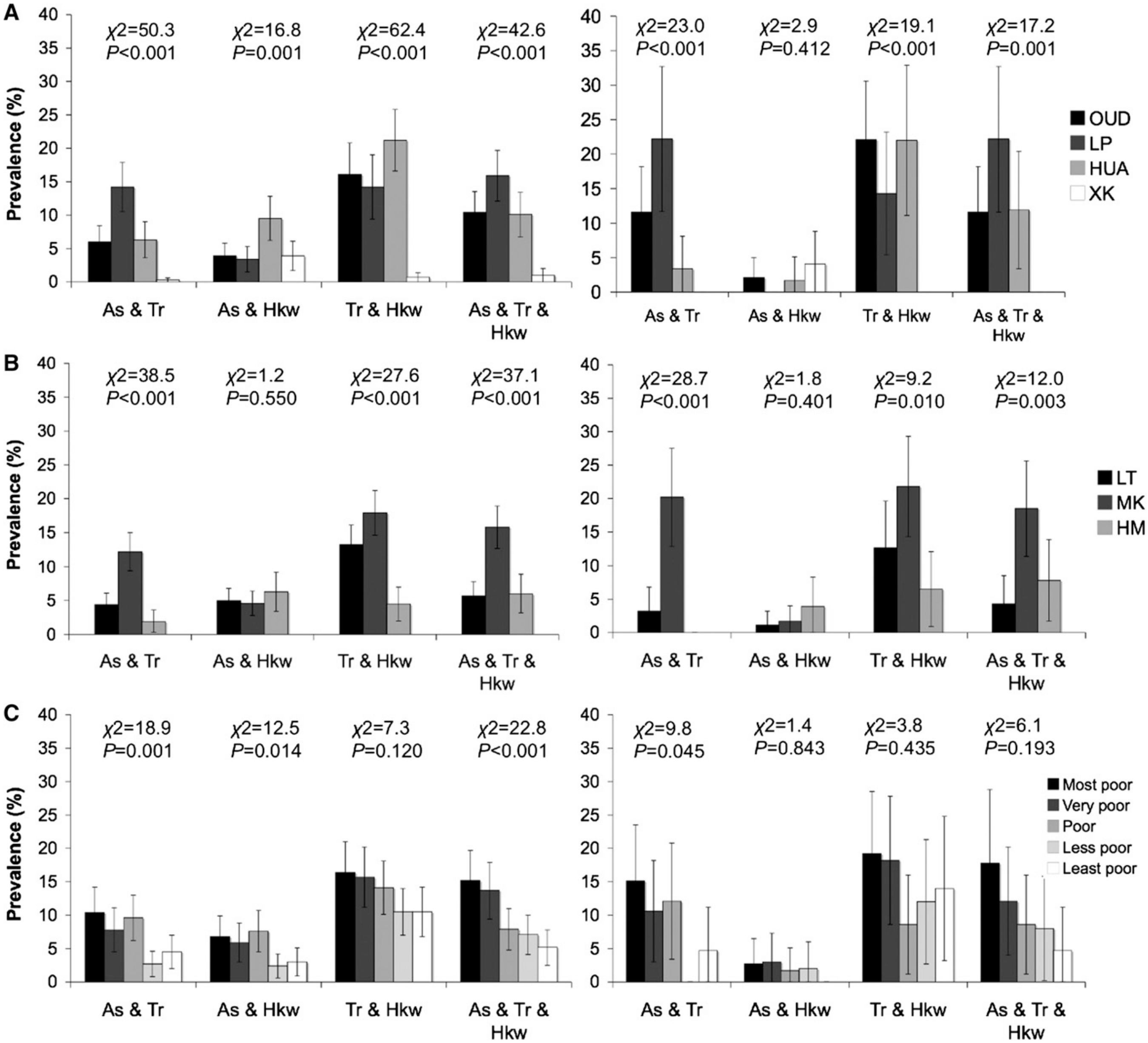

FIGURE 2. Prevalence of dual and triple infections of soil-transmitted helminthes at the community level (left column) and children 6–11 years of age (right column) by (**A**) province, (**B**) ethnicity, and (**C**) wealth status, Laos. Error bars represent 95% confidence intervals. As = *Ascaris lumbicoides*; Tr = *Trichuris trichiura*; Hkw = Hookworm; OUD = Oudomxay; LP = Luangprabang; HUA = Huaphan; XK = Xiengkhuang; LT = Lao-Tai; MK = Mon-Khmer; HM = Hmong-Mien.

and *T. trichiura* than children from less and least poor households, and there was a uniform but insignificant decrease in prevalence of triple infections with *A. lumbricoides, T. trichiura*, and hookworm from most poor households to least poor households (Figure 2C).

Associations between different helminth co-infections are shown in Table 4. The strongest associations were between *A. lumbricoides* with *T. trichiura* and hookworm with *S. stercoralis*. However, significant associations were also observed between *A. lumbricoides* and hookworm, *T. trichiura* and hookworm, and *S. stercoralis* and *T. trichiura*.

Human hookworm characterization. Forty-six fecal samples were randomly selected from the 629 human hookworm microscopy-positive samples and subjected to PCR and sequencing analysis to identify the species. The PCR amplification and sequencing was successful for 17 (37.0%) samples, and an additional 15 samples had a PCR product that failed to sequence or sequenced a non-specific product. Of the 17 characterized samples, all were identified as a single infection with either *N. americanus* (14 of 17, 82.4%) or *A. ceylanicum* (3 of 17, 17.6%). All three *A. ceylanicum*-positive samples originated in Oudomxay Province from Mon-Khmer persons, and the 14 *N. americanus*- positive samples originated in Oudomxay (2), Luangprabang (6), and Huaphan Provinces (6). None of the samples from Xiengkhuang Province showed a conclusive PCR result.

Dog hookworm characterization. Thirty-two, 30, 11, and 32 dog samples were collected from Oudomxay, Luanprabang,

TABLE 4

Significant associations ($P < 0.050$) between the different soil-transmitted helminths infecting persons in northern Laos, as determined by multiple logistic regression modeling controlling for household clustering

Parasite	Co-infection (risk factor)	Adjusted OR	95% CI	*P*
*Ascaris lumbricoides**	*T. trichiura*	2.97	2.11–4.18	< 0.001
	Hookworm	1.86	1.35–2.56	< 0.001
*Trichuris trichiura**	*A. lumbricoides*	2.87	1.99–4.13	< 0.001
	Hookworm	2.04	1.48–2.83	< 0.001
Hookworm†	*A. lumbricoides*	1.70	1.25–2.30	0.001
	T. trichiura	1.83	1.38–2.42	< 0.001
	S. stercoralis	2.41	1.53–3.79	< 0.001
Strongyloides stercoralis‡	*T. trichiura*	1.55	1.01–2.40	0.045
	Hookworm	2.28	1.47–3.52	< 0.001

*Adjusted for province, wealth status, age group, ethnicity, defecation site, and male and female head-of-household literacy.
†Adjusted for sex, province, wealth status, ethnicity, and female head-of-household literacy.
‡Adjusted for sex, province, wealth status, age group, and ethnicity.
OR = odds ratio; CI = confidence interval.

Huaphan, and Xiengkhuang Provinces, respectively, and analyzed by saturated sodium nitrate flotation and microscopy. Four villages in Huaphan Province had no dogs. All dogs were free to roam, scavenge, and defecate without hindrance in their respective villages, and 60% were female and 40% were male. A total of 94 (89.5%) of 105 dogs had hookworm eggs detected by microscopy. Prevalence in the four provinces was 93.5%, 90.0%, 90.9%, and 84.4%, respectively, and no significant difference was detected ($\chi^2 = 1.5$, $P = 0.672$). Dog age was not associated with hookworm egg detection ($\chi^2 = 2.0$, $P = 0.657$, by Kruskal-Wallis test). However, prevalence in male dogs (97.6%) was significantly greater than in female dogs (84.1%) ($\chi^2 = 4.9$, $P = 0.027$).

Twenty-three (24.5%) of 94 fecal samples positive for hookworm were analyzed by PCR and sequencing to identify the species. The PCR sequencing protocol was able to successfully amplify and characterize 18 (78.3%) of these samples. Single-species amplification of DNA from *A. ceylanicum*, *A. caninum*, *A. braziliense*, and *N. americanus* were detected in 7 (38.9%), 2 (11.1%), 1 (5.6%), and 1 (5.6%) dogs, respectively. Dual species amplification of DNA from *A. ceylanicum* and *A. caninum* was detected in 4 (22.2%) dogs, and dual species amplification of DNA from *A. ceylanicum* and *N. americanus* was detected in 3 (16.7%) dogs. Overall, *A. ceylanicum* was the most prevalent hookworm species detected in village dogs (14 [77.8%] of 18). Dogs infected with *A. ceylanicum* or *A. caninum* were detected in all four provinces, *A. braziliense* was detected only in Luangprabang Province, and *N. americanus* was detected in two villages in Oudomxay Province and two villages in Luangprabang Province. In Oudomxay Province where *N. americanus* was detected in dogs, all residents of one village reported open defecation, and all residents of the other village reported latrine use. Residents of both villages in Luangprabang Province where *N. americanus* was detected in dogs predominantly defecated in the open (65 and 68%, respectively, reported open defecation).

DISCUSSION

Despite the MDA program providing a high coverage of mebendazole treatment in school age children since 2006 in Laos,[8] we found a high prevalence of STHs in school children and in the general community. Unfortunately, there is a dearth of good-quality data with which we can compare our results. However, we have been able to provide a detailed analysis of patterns of infection, and we are the first to report specific risk factors for STH infections from four highly prevalent provinces in northern Laos.

This study has a number of important limitations. First, we collected a single stool sample and tested by using a single diagnostic protocol without egg count data. We used the formalin-ether concentration method, and this test used alone has poor sensitivity, 35.5%, 51.1%, and 43.8%, for hookworm, *T. trichiura*, and *A. lumbricoides*, respectively[26] indicating that our results are an underestimation of true prevalence. However, our preservation protocol involved a 1:8 w/v ratio of stool to formalized buffer compared with a 1:5 w/v ratio in the study of Glinz and others,[26] making it difficult to compare diagnostic performance, particularly for detection of hookworm eggs. Variable intensities of STH infections also affect interpretation of diagnostic performance data between regions, whereby high intensity infections are more sensitively detected. More accurate prevalence results would have been obtained if we used multiple diagnostic protocols in a single study.

Second, the sample size was relatively small and this was evident in the large confidence intervals in the risk factor analysis. The World Health Organization guidelines for evaluating STH at the community level recommend that 200–250 compliant persons in an ecologically homogenous environment, including climate, humidity, soil type, and temperature, is sufficient for an accurate measure of prevalence. If the assumption of ecologic homogeneity holds true at the district level, then our sample size was sufficient. Although the CIs for the risk analysis were wide, we were still able to detect a significant risk for STH infection for several population level explanatory variables.

Third, we had a high non-participation rate for the Hmong-Mien ethnic group. This rate was most likely a result of sociocultural aversion to providing fecal samples and discussing personal health details with doctors from a different ethnic group. We believe this finding could be improved in the future with a more consultative process and involvement of Hmong-Mien doctors in the survey.

Persons of Mon-Khmer ethnicity are a highly vulnerable group with respect to STH infection in northern Laos. This group was more likely to defecate in the open, be poor, and to have an illiterate male or female head of household. These findings were reflected in the high prevalence of STH infection and the increased risk in comparison with other ethnic groups. Mon-Khmer school age children were more than six times more likely to have an *A. lumbricoides* infection and 12 times more likely to have any STH infection than Lao-Tai children.

Of particular note was the high prevalence of *A. lumbricoides* in Mon-Khmer children and poor households so soon after the MDA program. Mebendazole is recognized as having good activity against *A. lumbricoides*, and recent and historical data consistently show *A. lumbricoides* cure rates in excess of 90% with a single 500-mg dose of mebendazole.[10,11] The prepatent period for *A. lumbricoides* is approximately 2–3 months,[27] and we would therefore not expect the cases we detected to be a result of re-infection or larvae transiting through the body at the time of treatment. The high prevalence of *A. lumbricoides* in the children in Luangprabang Province and Mon-Khmer children (50.8% and 47.1%, respectively) indicates that these children may be overlooked in the system, possibly as a consequence of not being enrolled in school, absenteeism, or other sociocultural beliefs affecting use of therapy.

School enrollment in Laos is highly variable, and low enrollment rates have been observed for the poorest households and for non-Lao Tai ethnic groups.[28] In addition to low enrollment rates, non-Lao Tai children who are enrolled in school face language problems because the curriculum is taught exclusively in the Lao language, and labor demands on primary school children are highest for economically disadvantaged households in rural areas,[28] possibly resulting in limited attendance and absenteeism. Our findings are consistent with those of Noke and Bundy,[29] who found that children in Jamaica with the heaviest infections came from the most socioeconomically disadvantaged households and were the least likely to comply with a treatment program based on screening. Compliance with the MDA program in Laos is undoubtedly a complex and multifaceted issue. However, education and out-reach programs targeting non-Lao Tai ethnic groups may help to address this issue. Interestingly in our study, children with a literate male head of household had a marked decrease in risk of ascariasis.

A recent review of STHs in Southeast Asia[30] singles out the MDA program in Laos as a success and cites a reduction in STH prevalence in a small pilot survey from one school in each of four northern provinces[9] in comparison with a large study conducted in 2000–2002.[7] The pilot study[9] failed to disclose the school locations, ethnicity, and relative wealth status of the children tested and compares pooled data, whereas Rim and others[7] stratified prevalence data by province. The review by Jex and others[30] and the study by Phommasack and others[9] represent an over-interpretation of the data and specifically fail to take into account the spatial, ethnic, and wealth heterogeneity of STH infection in the population in Laos and the different risk profiles that we encountered in our study. We cannot readily compare the data from our study with the work of Rim and others[7] from 2000–2002 because study sites differed, but we can use it to approximate overall trends. In 2001, Rim and others[7] found 65.2% and 62.6% of children in Luangprabang and Oudomxay Provinces, respectively, had evidence of *A. lumbricoides* infection by cellophane-thick smear compared with our finding of 50.8% and 30.5%, respectively. Similarly in 2002, Rim and others[7] found that 72.0% and 50.6% of children in Huaphan and Xiengkhuang Provinces, respectively, were infected with *A. lumbricoides* compared with our finding of 18.6% and 4.1%, respectively. The overall trends presented here and elsewhere indicate that the program is having a measure of success, but robust monitoring will be required to prevent the most vulnerable persons from missing treatment.

Almost all Mon-Khmer children with ascariasis had concurrent infections with either *T. trichiura*, hookworm, or both, as was the case for the poorest children, indicating that health and development consequences were potentially greater in these groups. Recent studies in the Philippines demonstrated that low-to-moderate intensity multiple infections were associated with anemia,[5,31] although schistosomiasis seemed to be the dominant controlling infection, as was observed in a similar study in Brazil.[32] These results have not been supported by studies conducted in Africa, where schistosomiasis is also prevalent,[33,34] although these later studies could be complicated by administration of drugs to control helminthiasis. Less clear again is the impact of multiple infections on cognitive function. Multiple studies indicate that STH infections have a detrimental effect on cognitive performance and educational outcomes in school children.[35] Of particular note are recent data from geographically diverse regions indicating that light and moderate polyparasitic infections have a detrimental effect.[36,37] In the Laos context, polyparasitic STH infections are highly prevalent in high-risk groups and may have a strong role in perpetuating the cycle of poverty.

Few studies have sought to determine the species of hookworm causing human or dog infections in Laos. The results we present indicate that *N. americanus* is the predominant species causing human hookworm disease in northern Laos, and that *A. ceylanicum* is the predominant species causing dog infections. Our results are in contrast to those from recently published studies from a single village in Savannakhet Province in southern Laos where *Ancylostoma* species and *Trichostrongylus colubriformis* were more prevalent then *N. americanus*.[17,38] We observed a high failure rate in the PCR for human hookworm and this could be caused by a variety of reasons. It could be the result of PCR inhibition, which has been observed in fecal samples, or more complex factors such as high prevalence of hookworm infection with low worm burdens. Sato and others[17] found a strong correlation between PCR success and eggs per gram of feces, whereby low egg count samples, indicative of low worm numbers, were more likely to fail in a PCR. This finding could account for PCR failure in our study. A similarly strong correlation between egg count and cycle threshold values in real-time PCR for *N. americanus* has been observed.[39] With further validation and incorporation of *A. ceylanicum* and *Trichostrongylus* spp. specific primer sets, the latter method may be a more appropriate molecular tool because real-time PCR affords greater sensitivity than conventional PCR. The presence of *T. colubriformis* in northern Laos could also account for the discrepancy between our microscopy and PCR results; *N. americanus* and *Ancylostoma* eggs cannot be differentiated from *T. colubriformis* by microscopy,[17] and our PCR protocol could not detect *T. colubriformis* DNA.

The molecular diagnosis and subsequent identification of hookworm species was far more successful in fecal samples of dogs than those of humans and possibly reflects a higher worm burden in dogs. The three *A. ceylanicum* human cases we detected by PCR had a worm count sufficient to produce enough eggs to be detected by conventional PCR. *Ancylostoma ceylanicum* is often associated with poorly established infection in the human gut, which leads to low worm numbers, low egg output, and absence of blood loss symptoms.[40,41] Patent *A. ceylanicum* infections with high worm numbers have caused significant human disease, including blood loss,[19] intestinal pain and discomfort[41,42] and cognitive impairment.[42] That we found that almost one-fifth of PCR-positive samples were *A. ceylanicum* is significant because this finding represents a parasite species that will not be controlled by a MDA program targeting only humans. Moreover, all but four of the surveyed villages had a relatively large unmanaged dog population representing a strong infection pressure on human inhabitants. Our data also shows that village dogs have a role in *N. americanus* epidemiology in northern Laos. From our data, we cannot definitively say if dogs are a transport host or if they are harboring a patent infection, but experimental data from Japan has documented patent *N. americanus* infection in young dogs exposed to 1,000 larvae.[43] If the human population is exerting a strong and continuous infection pressure on dogs, then there remains the possibility of spill-over into the dog population and zoonotic transmission between host species. Because we found that almost one-quarter of PCR-positive dogs harbored *N. americanus* eggs, we believe further investigations are warranted to discount patent infection and zoonotic transmission.

We present the first study to describe the patterns of STH infection in the context of MDA in northern Laos. Our evidence serves to highlight an important limitation of the MDA program. Poor compliance, absenteeism, or delivery failure results in poor uptake of mebendazole treatment by children of the Mon-Khmer ethno-linguistic family and children from the poorest households. The efficacy of mebendazole in controlling *T. trichiura* and hookworm infections may also need to be examined in Laos. This suggestion is consistent with studies on efficacy conducted elsewhere.[10–13] At the community level, we have conclusively demonstrated that Mon-Khmer persons are highly vulnerable, as are persons from the poorest households. There is a need for effective outreach programs designed in conjunction with improvements to sanitation and health services and delivery of improved therapy, possibly albendazole plus ivermectin,[11] to reach the most vulnerable persons. The emerging trend of the Lao MDA program is positive but far from conclusive, and we strongly question the use of mebendazole as the only therapy. Because of the lack of good-quality parasitologic field data from northern Laos, we do not concur with other authors[9,30,44] that MDA success is adequately measured by high rates of drug delivery. A thorough appraisal of the MDA program in Laos is warranted, and a robust monitoring program should be urgently initiated, taking into account the role of dogs in the natural history of human hookworm infection.

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