Incidence and Risk Factors of Hookworm Infection in a Rural Community of Central Thailand

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Abstract. A cohort study to identify incidence and risk factors of hookworm infection was conducted in a rural community, central Thailand from November 2005 to February 2007. Stool specimens were examined for hookworm eggs using wet preparation, Kato thick smear, and water-ethyl acetate sedimentation technique. The incidence rate of hookworm infection was 7.5/100 person-years. The independent risk factors for acquiring hookworm infection were barefoot walking (incidence rate ratio [IRR] = 4.2, 95% confidence interval [CI] = 1.2–14.5) and raising buffaloes around the house (IRR = 4.8, 95% CI = 1.9–11.8). Sequencing of internal transcribed spacer 1 (ITS1)–5.8S–ITS2 region of the ribosomal RNA gene were performed for identifying species of hookworm. *Necator americanus* was the most common hookworm identified in this population. *Ancylostoma duodenale* and *A. ceylanicum* were also detected. Our data suggest transmission of both human and animal hookworms in this community. Thus, prevention and control strategies of hookworm infection should cover both human and animal infection.

INTRODUCTION

Human hookworm infection caused by *Necator americanus* and *Ancylostoma duodenale* has been reported worldwide, especially among people in tropical and subtropical countries with low socioeconomic status. It has been estimated that 576 million people around the world are infected with the regions of the highest prevalence of hookworm infection being reported in sub-Saharan Africa, the Pacific Islands, India, East and South Asia, Latin America, and the Caribbean.^{1,2} *Necator americanus* has been found to be more predominant worldwide than *A. duodenale*. In addition, animal hookworms of dogs and cats, *A. ceylanicum* and *A. caninum* have been occasionally reported in humans.³

High-risk groups suffering from hookworm disease are children and pregnant women.⁴ Infected persons who have chronic intestinal blood loss could further develop iron-deficiency anemia and hypoalbuminemia.^{5,6} Additionally, heavy infection in children causes stunted growth and impairment of intellectual and cognitive development.^{7,8} In the past decade, prevention and control of hookworm infection has been globally neglected because most individuals with light to moderate hookworm infection generally are asymptomatic. In an attempt to control the morbidity, the World Health Organization (WHO) and Japanese International Cooperation Agency launched the project of soil-transmitted control first in Asia in 2004 and scaled-up to East and West Africa in 2005 and 2006, respectively. The school-based deworming programs could effectively reduce the infection of soil-transmitted helminthes in children^{1,9} but could miss positive adult cases. In a few studies, *Ascaris* and *Trichuris* prevalence and intensity go down with age, hookworm prevalence and intensity go up with age.^{2,3}

In Thailand, hookworm infection is still a major public health problem, especially in rural areas. Survey studies in different regions of Thailand showed that hookworm infection in rural communities ranged from 5% to 46.2%.^{10–13} Of these populations, children were also at high risk with a prevalence of

19.6–22.2%.¹¹ Most studies of hookworm infection in Thailand were conducted using a cross-sectional study design. This type of study might not be suitable for finding causal inference because it only provides the outcome and its associated characteristics at a specific point in time. Because hookworm infection is a chronic disease, a cohort study may be more appropriated for identifying the true risk factors. In November 2005, we conducted a survey for hookworm infection at Tungsor Hongsa Village, a rural community in Chachoengsao Province, central Thailand that showed a prevalence of hookworm infection of 12%. To determine the incidence and associated risk factors of hookworm infection, a cohort study was performed from November 2005 to February 2007. In addition, species determination of hookworm was also performed using polymerase chain reaction (PCR) and DNA sequencing. This study provides the most recent information on risk factors associated with hookworm infection in central Thailand and facilitates the development of more effective intervention strategies.

MATERIALS AND METHODS

Study area and population. The research protocol was reviewed and approved by the Ethics Committee of the Royal Thai Army Medical Department. Informed consent was obtained from the enrolled participants or the parents of the enrolled children < 15 years of age. The study was conducted at Tungsor Hongsa community, Chachoengsao Province, 228 km east of Bangkok, Thailand, which comprised 788 individuals. All participants recruited in this study were through household visits. A baseline survey of hookworm infections was carried out in this community in November 2005. Of 585 stool specimens, 70 (12.0%) were positive for hookworm eggs using three methods, including wet preparation, Kato thick smear, and water-ethyl acetate sedimentation technique. For Kato thick smear, 50–60 mg of feces was used and slides were read within 30 minutes. Treatment of hookworm infection was performed using 100 mg mebendazole twice daily for 3 days. Health education for the prevention of hookworm infection was also provided. In February 2007, 778 individuals still lived in this community, and the remainder migrated to work in other areas. Those who were negative for hookworm eggs from the baseline survey in November 2005 ($N = 515$)

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were invited to enroll in a follow-up survey in February 2007. Those who received anthelmintic drugs such as albendazole or mebendazole 2 weeks before the study were excluded. Examination for hookworm eggs in the follow-up survey was performed using the same methods as in the baseline survey.

Species identification of hookworm. Fecal samples positive for hookworm eggs were subjected to DNA extraction and PCR according to the protocols described by Traub and others.¹⁴ Positive PCR products corresponding to the expected amplicon sizes for *N. americanus* (485 bp) and *Ancylostoma* spp. (380 bp) internal transcribed spacer 1 (ITS1)-5.8S-ITS2 region of ribosomal RNA (rRNA) gene were purified from gel using QIAquick Gel Preparation Kit (Qiagen, Hilden, Germany) according to the recommendations of the manufacturer. The DNA sequencing was conducted by Macrogen Inc., Seoul, Korea. Chromatograms were manually checked and edited using Sequencher version 4.0.5 (Gene Codes Corp., Ann Arbor, MI). Subsequently, nucleotide sequences of ITS1-5.8S-ITS2 region of rRNA gene of hookworm obtained in this study were aligned and compared with the previously published sequences of GenBank database using BioEdit version 7.0.1.

Questionnaires. To determine the risk factors for hookworm infection, standardized questionnaires were used. Enrolled participants were interviewed face-to-face covering demographic data, socioeconomic conditions, and sanitary behaviors, i.e., hand washing, toilet use, habit of walking barefoot, including the kinds of animals raised around the housing area.

Statistical analysis. Incidence rate per 100 person-years of observation were calculated for each demographic category and potential risk factor variables. Possible risk factors for hookworm infection were analyzed using incidence rate ratios (IRR) and their 95% confidence intervals (CIs). The χ^2 test was used to compare proportions. A Poisson regression model with random effects and robust standard error was performed for univariate and multivariate analysis to assess the independent association of the risk factors and hookworm infection using STATA/SE for version 9.2 (StataCorp LP, College Station, TX). Clustering by household was included in the analysis. The maximum likelihood method was used to estimate frailty variance.

RESULTS

Study population. In February 2007, there were 778 individuals living in this village, 568 (72.1%) stool samples were collected. The overall prevalence of hookworm infection was 10.2% (58 of 568). Of 568 subjects, 352 individual were eligible for data analysis in the cohort study because these enrolled subjects were those of 515 hookworm-negative individuals in the base-line survey in 2005. The response rate of the cohort study was 68.3% (352 of 515). The characteristics of the enrolled subjects are shown in Table 1. Of 352, 142 (40.3%) were male and 210 (59.7%) were female. The median age was 23.9 ± 18.6 . The prevalence of hookworm infection was significantly different among age groups ($P < 0.041$). No significant difference was found among gender, educational level, and occupation groups.

Incidence and risk factors of hookworm infection. The incidence was estimated during November 2005–February 2007. Of 352 participants, 33 (9.4%) had hookworm infection. Wet preparation, Kato thick smear, and water-ethyl acetate

TABLE 1
Characteristics of the enrolled subjects and incidence of hookworm infection

Characteristics	Enrolled subjects	No. of hookworm-infected person (%)	P value
Gender			
Male	142	14 (9.9)	0.798
Female	210	19 (9.1)	
Age group (y)			
≤ 15	160	13 (8.1)	0.453
> 15	191	20 (10.5)	
Year of education (y)			
0–4	179	17 (9.6)	0.562
> 4	146	14 (9.5)	
Occupation			
Agriculture	119	13 (10.9)	0.546
Others	203	18 (8.9)	

sedimentation technique could identify 18 (54.5%), 29 (87.9%), and 32 (97.0%) cases of hookworm infection, respectively. The incidence rate of hookworm infection was 7.5/100 person-years. Univariate and multivariate Poisson regression analysis for the risk factors of acquiring hookworm infection are shown in Table 2. Univariate analysis showed that persons who walked barefoot and raised buffaloes nearby were 2.8 and 6.5 times at greater risk of getting hookworm infection, respectively. No association was found between age group, gender, level of education, using latrines, raising dogs, cats, pigs, chicken, ducks, fish, and cows around the housing area and hookworm infection. Using the multivariate Poisson regression model, we found that barefoot walking had 4.2 (95% CI = 1.2–14.5) times after adjusted for age, raising cats, and buffaloes, while raising buffaloes nearby owners' houses had 4.8 (95% CI = 1.9–11.8) times greater risk of getting hookworm infection after adjusting for barefoot walking and raising cats.

Identification of hookworm species. Of the 58 hookworm egg-positive specimens, 50 (86.2%) were successfully amplified and genetically characterized on the basis of its DNA sequence at the ITS1-5.8S-ITS2 region of the rRNA gene. Of these, 46 (92%), 2 (4%), and 1 (2%) individuals were shown to harbor single infections with eggs of *N. americanus*, *A. ceylanicum*, and *A. duodenale*, respectively. A single individual (2%) harboring a mixed infection of *N. americanus* and *A. ceylanicum* was identified.

DISCUSSION

In this community, hookworm transmission to humans was continuing as shown by the incidence rate of hookworm infection that occurred at 7.5/100 person-years. Our results showed that the prevalence of hookworm infection in 2007 was 10.2%, which was relatively stable, compared with 12.0% from the baseline survey in 2005. Our results might have underestimated the percentage, because we examined only a single stool sample from each participant. Light hookworm infections might have been under-diagnosed using the Kato thick smear method and formalin-ethyl acetate sedimentation. Additionally, the follow-up rate of 68.3% in this study could have affected the incidence rate, because the majority of those who did not participate in the follow-up survey in 2007 were the young adult farmers who temporarily moved out of the village to find jobs during the dry season.

Risk factors of hookworm infection have been identified from several cross-sectional studies in many countries.

TABLE 2
Univariate and multivariate analysis for risk factors of hookworm infection*

Factor	Person-years of exposure	No. of hookworm infection (incidence rate/100 persons-year)	Crude IRR (95% CI)	P value	Adjusted IRR† (95% CI)	P value
Gender						
Female	262.2	19 (7.9)	1			
Male	177.4	14 (7.3)	0.9 (0.4–1.8)	0.780		
Age (y)						
> 15	238.9	20 (8.4)	1		1	
≤ 15	199.4	13 (6.5)	0.8 (0.4–1.4)	0.401	1.0 (1.0–1.1)	0.105
Education level (y)						
> 4	182.2	14 (7.7)	1			
0–4	223.6	17 (7.6)	0.98 (0.5–1.9)	0.94		
Wash hands before meal						
No	259.6	20 (7.8)	1			
Yes	155.0	12 (7.7)	1.0 (0.5–1.9)	1.000		
Wash hands after defecated						
No	99.82	10 (10.0)	1			
Yes	314.8	22 (7.0)	0.7 (0.3–1.5)	0.320		
Defecated in toilets						
No	64.9	6 (9.2)	1			
Yes	350.9	26 (7.4)	0.8 (0.3–1.7)	0.530		
Barefoot walking						
No	120.0	4 (3.3)	1		1	
Yes	295.8	28 (9.5)	2.8 (1.0–7.8)	0.046	4.2 (1.2–14.5)	0.023
Raising animals						
Dogs						
No	106.2	7 (6.6)	1			
Yes	284.68	22 (7.7)	1.3 (0.4–4.1)	0.650		
Cats						
No	264.2	24 (9.1)	1		1	
Yes	126.0	5 (4.0)	0.4 (0.1–1.3)	0.079	0.5 (0.2–1.4)	0.179
Pigs						
No	345.9	26 (7.5)	1			
Yes	45.0	3 (6.7)	0.8 (0.2–4.0)	0.820		
Cows						
No	261.0	17 (6.5)	1			
Yes	129.8	12 (9.2)	1.5 (0.7–3.1)	0.340		
Buffaloes						
No	375.8	23 (6.1)	1		1	
Yes	15.0	6 (40.0)	6.5 (2.4–17.3)	< 0.001	4.8 (1.9–11.8)	0.001

* IRR = incidence rate ratio; 95% CI = confidence interval.

† Adjusted for all other variables in the model including age, barefoot walking, and raising cats and buffaloes around houses.

Acquiring hookworm infection is directly related to exposure to soil where filariform larvae, the infective stage, live in and penetrate human skin.^{4,15,16} Poor personal hygiene, particularly defecation practices^{16–19} and sanitation,^{17,20} have been reported as risk factors of hookworm infection. Hookworm infection has been associated with low socioeconomic status¹⁶ and low educational attainment.^{1,4,16,21} On the other hand, economic development and reduced poverty,¹ knowledge of soil-transmitted helminths,¹⁵ regular use of anthelmintic drugs, good socioeconomic status,²¹ and good hygiene and sanitation¹⁷ provided protection.

In contrast to other studies,^{2,3,22} we found that the incidence of hookworm infection in children and adults were not significantly different. This could be explained by a shared environment and also similar health behaviors. Indeed, the villagers exposed to soil by walking barefoot were 4.2 times at greater risk of hookworm infection. In addition, those who raised buffaloes around the house were 4.8 times at greater risk of hookworm infection. Buffaloes usually bury themselves in mud pools located around the owners' houses. The moist soil or mud is suitable for the development and growth of filariform larvae.^{23–25} Thus, the areas with buffaloes could maintain the infectious larvae for a period of time. The owners acquired the infection because they did not wear shoes or other protection

while working with buffaloes. Other farm animals such as pigs were also reported to be risk factors of hookworm infection.¹⁶ However, our study showed no association between these animals and hookworm infection.

In the past, animal hookworm infections in humans have been overlooked because of a lack of molecular tools to identify hookworm species using eggs in stool specimens. Hookworm species can be identified from adult worm expulsion, which is not a convenient task. In this study, different species of hookworm were identified using PCR and analysis of DNA sequences. Because we used unpreserved stool samples, kept at 4°C for hookworm DNA extraction, DNA could have degraded during storage. As a result, only 86% of the positive samples showed PCR positive. In this study community, the most predominant human hookworm worldwide, *N. americanus*, was identified in 92% of hookworm-positive individuals, followed by *A. ceylanicum* and *A. duodenale*. Only one case with *A. duodenale* infection was found in this area, which supports a geographical restriction of this species. In the southern part of Thailand, a very low prevalence of *A. duodenale* (0.1%) was reported in school children, compared with 99.9% *N. americanus*.¹¹ Recently, a study of people living in temple communities in Bangkok identified *N. americanus* and *A. ceylanicum*, but not *A. duodenale* infection.¹⁴

Both experimental and natural infections with *A. ceylanicum* have been described in humans associated with severe abdominal discomfort²⁶ and in heavy infections, anemia.²⁷ In Thailand, Areekul and others²⁸ found 16% of hookworm-positive individuals to harbor adult worms of *A. ceylanicum* by autopsy, often in mixed and in light infections with *N. americanus*. In Bangkok, Traub and others, found 2 of 7 hookworm egg-positive individuals living in close proximity to semi-domesticated dogs to harbor *A. ceylanicum* using PCR-restriction fragment length polymorphism (RFLP). A survey study of adult hookworms in cats revealed that *A. ceylanicum* had a prevalence of 92%, followed by *A. caninum*, 23%.²⁹ Using molecular tools by PCR-RFLP, dogs in temple communities in Bangkok harbored a striking number of hookworms (58.1%) composing *A. ceylanicum* (77%) and *A. caninum* (9%).^{14,30} These data provide evidence that hookworm infection is endemic in stray or semi-domesticated dogs and cats in some areas of Thailand, especially those with no access to regular deworming programs. Dogs were common domestic animals in households in the studied community, sometimes roaming freely and defecating around the neighborhood. Thus, housing areas are likely to be contaminated with dog feces, with transmission exacerbated by areas with mud and damp soils that favor the development of the infectious stage. Thus, zoonotic awareness should be a concern because semi-domesticated dogs could be a potential source of transmission. To show the true prevalence of hookworm species, further molecular studies to identify specific species of hookworm should be done in different regions of Thailand.

In conclusion, our study revealed the transmission of both human and animal hookworms in this community. Thus, control measures should be applied for both humans and animals. From our information, avoiding exposure to soil by wearing shoes or some protection, especially when working with buffaloes, should be promoted. In addition, deworming of dogs should be considered.

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