PREVALENCE AND ASSOCIATED RISK FACTORS OF INTESTINAL PARASITES IN HUMANS AND DOMESTIC ANIMALS ACROSS BORDERS OF THAILAND AND LAO PDR: FOCUS ON HOOKWORM AND THREADWORM

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Abstract. Hookworm and threadworm infections are major public health problems in developing countries. A cross sectional study comprising 843 participants (346 males and 497 females) was conducted in three populations: i) Thai residents (TR) of Ubon Ratchathani Province, Thailand; ii) Laotian immigrant workers (LI) in the same province; and iii) Laotian residents (LR) in Champasak Province, Lao PDR. Participants were interviewed based on a structured questionnaire regarding their health status. Stool samples from participants and 300 samples from domestic animals (277 dogs and 23 cats) living in the participants households were collected and examined for parasitic infection using a formalin-ether concentration and a Harada-Mori filter paper culture techniques. Approximately one-third of TR and LI populations and domestic animals in Thailand were positive for parasitic infections, while almost half of LR population and domestic animals were positive. We confirmed by PCR and DNA sequencing a case of *Ancylostoma ceylanicum* infection in a Thai man. We also observed infections of other parasites, such as *Taenia* spp and *Opisthorchis viverrini*. Multivariate analysis indicated that risk factors for hookworm infection were population group and walking barefoot. Factors associated with threadworm infection were population group, adult male, lack of previous antiparasitic treatment and of knowledge of parasitic infection, and failure to wash hands after contact with domestic animals. Our results highlight the high prevalence of both hookworm and threadworm infections especially among LI population and domestic animals in both countries. Our findings emphasize the need for public health intervention to control the spread of parasitic infections in Thailand and Lao PDR.

Keywords: domestic animal, hookworm, parasite, risk factor, threadworm, zoonosis

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INTRODUCTION

Hookworm and threadworm *(Strongyloides stercoralis)* are soil transmitted helminths (STHs) or nematodes of medical and veterinary importance (Stephenson *et al*, 2000; Conlan *et al*, 2011). The infections can lead to iron deficiency anemia and malnutrition in people who live in poverty, especially in the tropical and subtropical regions of Asia and Latin America. Approximately 600-740 million people suffer from hookworm infection globally, while threadworm infection has been found in as many as 50-100 million people worldwide (Siddiqui and Berk, 2001; Hotez, 2011). It is estimated that patients infected with these worms have disability-adjusted life years (DALYs) resulting in a loss of 1.8-22.1 million life years (Hotez, 2011). Moreover, infections in children may affect their growth and development (Pawlowski *et al*, 1991). Although most of the infections do not result in severe symptoms, in the longer term it accumulates hidden impacts on health and well being.

Healthcare in Thailand and Lao PDR has been improving over time, but studies of STH infections have reported that the prevalence of hookworm infection in different regions of Thailand varies from 4.3% to 22.2% (Anantaphruti *et al*, 2002; Kaewpitoon *et al*, 2015a,b). Threadworm infection has a high prevalence in the northeastern region of Thailand and its endemic area is usually found alongside hookworm infection. Recently, a study in Lao PDR showed the prevalence of hookworm and threadworm infection of 46.3% and 8.9% respectively (Conlan *et al*, 2012).

Thailand and Lao PDR share a long border, and citizens from both sides, sometimes with their domestic animals, frequently cross the border for trade, tourism, residence, to receive medical care, and for employment. There is limited data from cross sectional studies for STHs in Thailand and Lao PDR. Starting in 2015, the formation of ASEAN Economic Community (AEC) is expected to increase further cross-border movement of workers and tourists within and between the ten member nations. This study aimed to determine the prevalence and associated factors for hookworm and threadworm infections in i) Thai residents (TR) in Ubon Ratchathani Province, Thailand; ii) immigrant workers (LI) in Ubon Ratchathani Province from Lao PDR; and iii) Laotian residents (LR) in Champasak Province, Lao PDR. At the same time, we analyzed fecal samples from domestic animals (dogs and cats) obtained from the same participants households.

MATERIALS AND METHODS

Study population and data collection

We conducted a cross-sectional study of 843 participants (346 males and 497 females) from Warin Chamrab (W), Khong Chiam (K) and Sirindhorn (S) Districts, Ubon Ratchathani Province, Thailand (Fig 1) and from Phonthong District (P), Champasak Province, Lao PDR, including 300 domestic animals (277 dogs and 23 cats) from the same households using a random sampling technique. All participants completed consent forms before enrolling in the study. All human studies were reviewed and approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Thailand (IRB No. 429/57) and by the National Ethics Committee for Health Research, National Institute of Public Health, Ministry of Health of Lao PDR (NIOPH/NECHR No. 02/2015). Participants were interviewed by research personnel using

a structured questionnaire to obtain information regarding sociodemographic characteristics, lifestyle, medical history, knowledge of parasites and information concerning their domestic animals. Fecal samples were collected for two participants and one domestic animal per house according to standard procedures for parasitic diagnosis.

Laboratory diagnosis

Fecal samples from participants and their domestic animals were examined under a light microscope using formalin-ether concentration (FECT) and Harada-Mori filter paper culture techniques (Harada and Mori, 1955; Tun *et al*, 2015). Microscopic examination of fecal slides was cross-checked by two parasitologists and a third examiner if there were conflicting results. PCR diagnostic confirmations were conducted of a fecal sample from one TR and four LIs who were infected with hookworm by using primers and amplification conditions previously described (Traub *et al*, 2008). We also conducted PCR and DNA sequencing using a new set of primers: NECFW1 (5′ CATAACTTGTGTG GTGTGGTACCT 3′) and NECRE1 (5′ ACACATCCACATGGCGAACATCG 3′). DNA sequencing results were confirmed with GenBank database. Nucleotide sequences obtained from this study were deposited in DDBJ/EMBL/GenBank with accession numbers KU855046-KU855050.

Statistical analysis

Data were entered into an Excel database and double checked in order to validate all data before analytical processing. Frequency distribution of sociodemographic characteristics, behavioral characteristics and medical history data were evaluated. Differences in distribution of covariates for infected and uninfected subjects were evaluated by chi-square test. Differences in mean values for covariates of interest among infected and uninfected subjects were assessed using Student's *t*-test. Logistic regression procedures were employed to calculate odds ratio (OR) of potential risk factors associated with parasitic infection. All statistical analyses were performed using SPSS 17.0 (IBM, Armonk, NY).

RESULTS

Of the 843 participants (346 males and 497 females), 50.9% were from TR population, 23.4% LI and 25.7% LR. The average age was 43.8 years. The majority of the subjects were farmers who earned less than THB 5,000 (USD 140) per month (Table 1).

Approximately one-third of TR and LI populations and their domestic animals were positive for parasitic infections. Among LR almost half of the population and their domestic animals were found to be positive. Threadworm and hookworm were major parasitic infections in humans and domestic animals, with the highest prevalence of threadworm infection (19.3%) found in LI population, followed by LR (15.7%) and TR (14.0%). The highest prevalence of hookworm infection was found in LR group (17.5%), followed by LI (11.2%) and TR (4.4%). In domestic animals from the same households, 35.7% and 39.7% of TR and LR samples, respectively, while the prevalence of threadworm was 0.6% and 1.7%, respectively (Fig 1).

In five cases of hookworm infection, we were able to culture larvae and extract DNA for PCR and DNA sequencing. One case of *Ancylostoma ceylanicum* infection was confirmed in TR population and four cases of *Necator americanus* infection were confirmed in LI population.

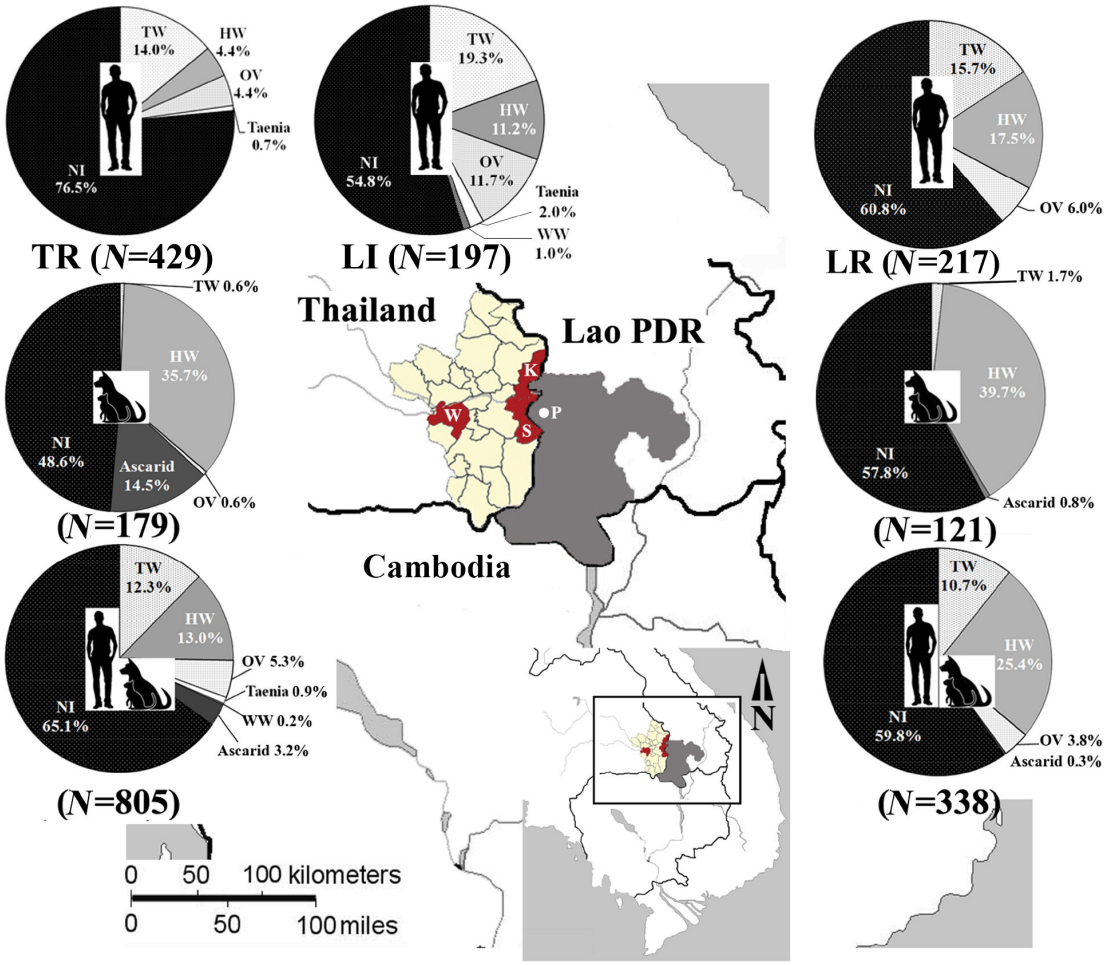

Fig 1–Prevalence of gastrointestinal helminthes in human and domestic animal populations beside border of Thailand (pie charts, left) and Lao PDR (pie charts, right). The study was conducted in Khong Chiam (K), Sirindhorn (S) and Warin Chamrab (W) Districts in Ubon Ratchathani Province, Thailand and in Phonthong District (P) in Champasak Province, Lao PDR. Black thick lines indicate borders separating Thailand, Lao PDR and Cambodia. Insets are locations of study sites. Ascarid is parasite in genus *Toxocara*. HW, hookworm; NI, no infection; OV, *Opisthorchis viverrini*; TW, threadworm *(Strongyloides stercoralis)*; WW, whipworm *(Trichuris trichiura)*.

Family members and domestic animals from the same household in TR and LR groups were found to be positive for hookworm infections in six (3.4%) and 14 (11.6%) families, respectively. There were eight and five families with members in the same household having threadworm infection among TR and LR group, respectively (data not shown). In addition, results of both human and domestic animals fecal examination detected eggs of *Taenia* spp and *O. viverrini*, while Ascarid spp were detected in only domestic animals (Fig 1).

Table 1
Sociodemographic characteristics of subjects in Ubon Ratchathani Province, Thailand and Champasak Province, Lao PDR.

Characteristics	TR (n = 429)	LI (n = 197)	LR (n = 217)
	n (%)	n (%)	n (%)
Gender			
Male	211 (49.2)	53 (26.9)	82 (37.8)
Female	218 (50.8)	144 (73.1)	135 (62.2)
Age (years)			
≤ 15	33 (8.0)	7 (4.2)	14 (6.6)
16-30	41 (9.9)	39 (23.2)	34 (16.0)
31-45	124 (29.9)	54 (32.1)	49 (23.1)
46-60	160 (38.6)	43 (25.6)	80 (37.7)
≥ 61	57 (13.7)	25 (14.9)	35 (16.5)
Marital status			
Single	49 (11.6)	15 (7.9)	36 (16.8)
Married	359 (85.1)	162 (85.3)	176 (82.2)
Divorced	14 (3.3)	13 (6.8)	2 (0.9)
Education (years)			
No	6 (1.4)	89 (47.8)	26 (12.0)
6	297 (71.6)	73 (39.2)	71 (32.9)
9	63 (15.2)	16 (8.6)	46 (21.3)
> 9	49 (11.8)	8 (4.3)	73 (33.8)
Occupation			
None/student	34 (8.3)	9 (4.8)	40 (20.4)
Laborer	154 (37.7)	104 (55.0)	11 (5.6)
Farmer	182 (44.5)	71 (37.6)	74 (37.8)
Other	39 (9.5)	5 (2.6)	71 (36.2)
Income (Baht)			
≤ 5,000	197 (50.8)	86 (50.6)	107 (51.2)
5,001-10,000	93 (24.0)	57 (33.5)	77 (36.8)
>10,000	98 (25.3)	27 (15.9)	25 (12.0)

TR, Thai residents in Ubon Ratchathani Province, Thailand; LI, Laotian immigrant workers in Ubon Ratchathani Province; LR, Laotian residents in Champasak Province, Lao PDR.

Multivariable logistic regression analysis indicated that risk factor of hookworm infection was walking barefoot. Participants (TR, LR and LI groups) who sometimes walked barefoot had a 3.79-fold (95% CI: 1.32-10.89) increased odds of getting hookworm infection, while those who always walked barefoot had a 4.19-fold increase (95% CI: 1.37-12.87), with LI and LR group having a 2.25-fold (95% CI: 1.11-4.54) and 4.91-fold (95% CI: 2.70-8.93) increased odds, respectively compared with the TR group (Table 2). Another risk factor for hookworm infection was that of not using a latrine (OR = 2.44, 95% CI: 0.87-6.81). It is important to

Table 2
Risk factors for hookworm and threadworm infections in Ubon Ratchathani Province, Thailand and Champasak Province, Lao PDR.

Factor	*n*	Hookworm infection					Threadworm infection				
		Infected	Unadjusted		Adjusted[a]		Infected	Unadjusted		Adjusted[a]	
		%	OR	95% CI	OR	95% CI	%	OR	95% CI	OR	95% CI
Population											
TR	429	4.4	1.00	Reference	1.00	Reference	14.0	1.00	Reference	1.00	Reference
LI	197	11.2	2.71	(1.43-5.14)	2.25	(1.11-4.54)	19.3	1.47	(0.94-2.30)	2.12	(1.19-3.79)
LR	217	17.5	4.58	(2.57-8.17)	4.91	(2.70-8.93)	15.7	1.14	(0.72-1.80)	0.95	(0.56-1.62)
Gender											
Male	346	9.0	0.92	(0.57-1.48)	-	-	20.8	1.91	(1.32-2.79)	2.32	(1.48-3.63)
Female	497	9.7	1.00	Reference	-	-	12.1	1.00	Reference	1.00	Reference
Age (years)											
≤ 20	73	5.5	0.62	(0.22-1.82)	-	-	5.5	0.29	(0.10-0.82)	0.16	(0.04-0.67)
21-45	322	12.7	1.57	(0.97-2.54)	-	-	18.9	1.16	(0.79-1.70)	1.24	(0.80-1.93)
≥ 46	400	8.5	1.00	Reference	-	-	16.8	1.00	Reference	1.00	Reference
Always or occasional use of latrine											
Yes	775	9.0	1.00	Reference	1.00	Reference	15.1	1.00	Reference	-	-
No	29	20.7	2.63	(1.04-6.67)	2.44	(0.87-6.81)	31.0	2.53	(1.13-5.69)	-	-
History of treatment for anti-parasitic infection											
Yes	497	8.0	1.00	Reference	-	-	13.5	1.00	Reference	1.00	Reference
No	276	11.2	1.45	(0.88-2.37)	-	-	19.2	1.53	(1.03-2.26)	1.84	(1.16-2.91)
Knowledge of parasite											
Yes	126	6.3	1.00	Reference	-	-	7.1	1.00	Reference	1.00	Reference
No	652	10.6	1.75	(0.82-3.73)	-	-	17.6	2.78	(1.37-5.65)	2.69	(1.29-5.63)
Always or occasional hand wash after contacting with domestic animals											
No animal	99	5.1	1.00	Reference	-	-	11.1	1.00	Reference	1.00	Reference
Yes	408	9.6	1.99	(0.76-5.18)	-	-	14.0	1.30	(0.65-2.58)	1.48	(0.69-3.21)
No	238	12.2	2.61	(0.98-6.95)	-	-	20.6	2.07	(1.03-4.18)	2.84	(1.29-6.29)

Occupation											
None/Student	83	6.0	1.00	Reference	-	-	12.0	1.00	Reference	-	-
Laborer	269	8.9	1.53	(0.56-4.14)	-	-	16.7	1.47	(0.70-3.06)	-	-
Farmer	327	10.1	1.75	(0.66-4.63)	-	-	16.5	1.44	(0.70-2.97)	-	-
Other	115	11.3	1.99	(0.68-5.81)	-	-	14.8	1.27	(0.55-2.93)	-	-
Walking with barefeet											
No	114	3.5	1.00	Reference	1.00	Reference	14.0	1.00	Reference		
Sometimes	567	9.3	2.84	(1.01-8.00)	3.79	(1.32-10.89)	17.1	1.26	(0.71-2.24)	-	-
Always	147	14.3	4.58	(1.53-13.76)	4.19	(1.37-12.87)	12.2	0.86	(0.42-1.76)	-	-

[a]Adjusted for all other covariates in the model. TR, Thai residents in Ubon Ratchathani Province, Thailand; LI, Laotian immigrant workers in Ubon Ratchathani Province; LR, Laotian residents in Champasak Province, Lao PDR.

note that because of the small sample size, this association did not reach a statistically significant value.

As regards threadworm infection, LI group had a 2.12-fold (95% CI: 1.19-3.79) increased odds of being infected, while LR group does not show any statistically significant difference compared to TR group. Males had a 2.32-fold increased odds of threadworm infection (95% CI: 1.48-3.63) compared to females. Younger people had fewer risks of infection than participants who were of working age. Subjects who did not have a history of parasitic treatment had a 1.84-fold (95% CI: 1.16-2.91) increased odds of infection than people who had been previously treated for anti-parasitic infection. Participants who did not have knowledge about parasitic diseases had a 2.69-fold (95% CI: 1.29-5.63) increased odds of infection. Those who did not wash their hands after contacting domestic animals had a 2.84-fold (95% CI: 1.29-6.29) increased odds of threadworm infection, while no statistical significance was found in the group of people who washed their hands after having contact with their domestic animals compared with participants who did not keep domestic animals.

DISCUSSION

The present study highlights a cross sectional investigation of parasitic infections in people of Thailand and Lao PDR as well as their pets in the same households. Statistically significant independent risk factors for threadworm infection among TR, LI, and LR groups are male gender, no history of anti-parasitic treatment, lack of knowledge of parasitic diseases, and no hand washing after contact with domestic animals. Threadworm infection in the three test groups was much higher

than that of domestic animals. Interestingly, an opposite result was observed with hookworm infection.

Geographically, the results demonstrate that threadworm infection in humans in the three test districts (K, S and W) of Thailand were higher than that of hookworm infection. A previous study (Boonjaraspinyo *et al*, 2013) showed a prevalence of threadworm infection in rural communities of northeast Thailand lower (9.5%) than our results (33.3%). This may be due to the sensitivity of the diagnostic method. The Harada-Mori filter paper culture technique makes use of the ability of threadworms to enter into a free-living cycle and the larger numbers of worms are easier to detect. This makes the technique more sensitive than simple smear and FECT used for threadworm diagnosis (Siddiqui and Berk, 2001).

On the other hand, the prevalence of hookworm infection in Phonthong District (P) of Lao PDR was higher than threadworm infection. Conlan *et al* (2012) found hookworm infection in 46.3% of the population in Lao PDR, but we found a lower prevalence (17.5%) in P. A previous survey of Jiraanankul *et al* (2011) reported a prevalence of hookworm infection in a rural community of Thailand of 10.2%, while our study was 4.4%. These more recent lower prevalences of hookworm infection may be due to the promotion and improvement of health and sanitation in Thailand and Lao PDR. The results of hookworm infection in both human and domestic animals, in particular of *A. ceylanicum*, suggests a close contact between humans and domestic animals and a potential zoonotic disease transmission (Traub *et al*, 2005). The finding of *A. ceylanicum*, a zoonotic hookworm infection in TR group is in agreement with a previous study (Phosuk *et al*, 2013). However, more studies are needed using molecular biology techniques to identify other parasite species shared between humans and domestic animals.

Humans, dogs and cats can be infected with *O. viverrini* by eating raw fish containing infective metacercariae (Kaewpitoon *et al*, 2015a). The prevalences of *O. viverrini* infection in the study area in northeastern Thailand (16.1%) and Lao PDR (6.0%) were lower than that of previous studies in northeastern Thailand (> 20%) and Khammouane Province, Lao PDR (54.8%) (Kaewpitoon *et al*, 2015a; Saiyachak *et al*, 2016), which is probably due to praziquantel treatment during the period before our specimen collection. In addition, *O. viverrini* infection in domestic animals in northeastern Thailand was reported in Khon Kaen Province (Enes *et al*, 2010), but there has been no previous study conducted in Ubon Ratchathani Province.

Our results of the prevalences of parasitic infection emphasize that i) there was potential for zoonotic infection between domestic animals and humans living in the same households (Traub *et al*, 2005); and ii) there was a potential for transmission of a new population of human parasites across a common border. An in-depth analysis with broader sampling sites in both sides of the Thai-Lao border using PCR-based techniques along with epidemiological data is of fundamental importance to gain a better knowledge of parasitic diseases in human and domestic animal populations in these areas.

Our study confirmed several risk-associated factors of hookworm and threadworm infections. For instance, in the case of hookworm infection, risk of infection in LR population was higher than in TR, and that the risk of infection in LI population was higher than the TR

when adjusted for all other confounding variables. This may be due to the policy of parasitic infection prevention and control in Thailand, which has been in operation since 2002 (Wongsaroj *et al*, 2009). Our study found that hookworm infection in a population could be prevented if personal hygiene was improved (*viz*, use of latrine and not walking barefoot), which is in agreement with previous observations by Strunz *et al* (2014) and Mahmud *et al* (2013) on the association of human life style and hookworm transmission. With regards to threadworm infection, the higher risk of adult males is not surprising given the behavioral characteristics, personal hygiene, and occupation. Furthermore, our results indicated that younger people had lower risks of infection than people who were of working age, which is in agreement with a study carried out in neighboring Cambodia (Khieu *et al*, 2014).

Despite the high prevalence of hookworm in domestic animals of both countries, we observed a low prevalence of threadworm in domestic animals. These findings are in line with other studies reported that threadworm infections are less common in adult dogs compared with puppies (Itoh *et al*, 2003; Goncalves *et al*, 2007). This might be explained by the immature immune systems of puppies. Additionally, it has been documented that poor hygienic conditions, temperature, and humidity are all associating factors (Itoh *et al*, 2003; Goncalves *et al*, 2007). Skin penetration of infective larva relies on host skin temperature (Sakura and Uga, 2010).

In conclusion, our study identifies a number of risk factors associated with parasitic infections in three groups of human populations as well as their domestic animals from both sides of the Thai-Lao border. We also report the findings of multiple species of parasites capable of infecting both human and domestic animals. Almost half of the entire study populations of both humans and domestic animals were infected with hookworm and threadworm. This suggests that people and animals in these areas and elsewhere where there are infected people or animals are at risk. Therefore, further investigations of broader sampling sites along both sides of the border using molecular techniques along with epidemiological data will be of fundamental importance for disease control and prevention.

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