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Prevalence and clinical aspects of human *Trichostrongylus colubriformis* infection in Lao PDR

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ABSTRACT

There have been few studies on human trichostrongyliasis in Southeast Asia, information on its clinical manifestations is also sparse. Trichostrongyliasis occurs predominantly in areas where poor hygiene is common especially where human/animal feces are used as a fertilizer, thereby contaminating vegetables and stream water. The intimate coexistence of domestic animals and humans explains the prevalence of *Trichostrongylus* infection in such areas. The goal of the current study was to determine the prevalence of trichostrongyliasis among villagers in Thakamrien village, Sonkon district, Savannakhet province, Laos, and to investigate potential relationships between clinical features, laboratory data, and severity of infection. Of 272 villagers examined, 160 (58.8%) were determined positive for helminthic infections by fecal examination, and 59 (36.9%) of these were infected with *Trichostrongylus*. Only 58 cases were in the inclusion criteria of the study and then underwent further assessment, including a questionnaire on personal behaviors, physical examination, and laboratory tests. Villagers in the trichostrongyliasis group were more likely than the control group to have consumed fresh vegetables, not washed their hands before meals or after using the toilet, and to have had close contact with herbivorous animals (goats and cows). Similarly, villagers in the trichostrongyliasis group were more likely than the control group to have a history of loose feces, rash, or abdominal pain; however, no obvious clinical symptoms were observed during physical examination of the trichostrongyliasis patients. The degree of infection was determined by both fecal egg counts and quantification of adult worms after deworming. Laboratory data were evaluated for any relationship with severity of infection. No significant differences were found in laboratory values between the trichostrongyliasis and control groups, with most values being within normal limits; however, both groups had high eosinophil counts. This study demonstrated that the useful clinical characteristics of trichostrongyliasis patients include history of loose feces, rashes, and abdominal pain, as well as in personal behaviors, such as the regular consumption of fresh vegetables, lack of hand washing, and close contact with cattle.

1. Introduction

Trichostrongylus colubriformis, a zoonotic nematode, normally lives in the digestive tracts, especially the small intestines, of domesticated and wild herbivorous animals, with the parasite's head embedded in the mucosa. Human infection occurs incidentally, by the ingestion of vegetation contaminated with infective 3rd-stage larvae. *T. orientalis* the most common species found in humans, while various other species are known to infect animals. Human infection is uncommon, but human trichostrongyliasis has occasionally been reported (Beaver et al., 1984; Boreham et al., 1995). A few trichostrongyliasis studies have been reported sporadically in Southeast Asia (Panasoponkul et al., 1985; Rim et al., 2003; Yong et al., 2007; Sato et al., 2010). Information on the disease in Southeast Asia, including its clinical presentation in humans, which is sometimes not mentioned in standard textbooks, is therefore sparse. The current study sought to determine the prevalence of *Trichostrongylus* infection among

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Table 1
Distribution of *Trichostrongylus* infection (23 cases) and mixed infection with other helminthes (35 cases) in different ages groups and sexes among participants.

Age groups	Single infection of *T. colubriformis*		Mixed infection of *T. colubriformis*		Total	Mixed infection with other helminths		
	Male	Female	Male	Female		Hookworms	*Taenia* spp.	MIF
11–20	1	1	2	4	8	3	2	2
21–30	1	1	1	0	3	0	0	1
31–40	4	2	2	5	13	0	2	7
41–50	0	5	8	3	16	4	2	9
51–60	2	4	2	6	14	2	1	7
61–70	0	1	0	1	2	0	0	1
71–80	1	0	1	0	2	0	0	1
Total	9	14	16	19	58	9	7	28

MIF: minute intestinal flukes.

villagers in Savannakhet province, Laos, and investigate clinical symptoms, including laboratory data, linked to this disease.

Lao people favor consumption of raw or incompletely cooked foods with unique Lao dishes made of raw fish, beef, and pork. This habit is largely responsible for the spread of opisthorchiasis, sarcocystiasis, and taeniasis (Giboda et al., 1991). The lack of appropriate sanitary latrines is also a source of contamination and transmission of soil-transmitted helminthiases by *Ascaris*, *Trichuris*, and hookworm (Rim et al., 2003). The intimate coexistence of domestic animals and humans largely explains the prevalence of *Trichostrongylus* infection in the area (Giboda et al., 1991; Sato et al., 2010). The current study aimed to determine the prevalence of trichostrongyliasis among villagers in Thakamrien village, Sonkon district, Savannakhet province, Laos, and investigate clinical features and laboratory data linked to severity of infection.

2. Materials and methods

2.1. Fecal examination and culture

The study was approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University (MUTM 2011-002-01). A total of 272 villagers (160 female, 112 male) from Thakamrien village, Sonkon district, Savannakhet province, were enrolled into the study. After the study details were explained, all study participants provided written informed consent to participate. Each participant provided about 5 g of feces for examination. Participants were interviewed about their personal behaviors using a standard questionnaire, and underwent a physical examination to assess clinical presentations potentially linked to trichostrongyliasis. Fecal specimens were processed at the field-work site using the modified cellophane thick smear method (Katz et al., 1972). Each entire slide was examined under a microscope and all helminth eggs were counted as eggs per gram (EPG). Participants positive for *Trichostrongylus*-like eggs were selected for further investigation by fecal culture (Harada and Mori, 1951). Hatched larvae were collected in 70% ethanol for identification to species by PCR assay. Participants negative for parasite infection were selected to form the control (negative) group, provided they met similar sexes and ages criteria to the positive group. The participants in the control group were selected as close matches for those in the positive group.

2.2. Hematological and biochemical laboratory investigations

Ten milliliters of blood was drawn from each participant for initial laboratory investigation, hematocrit, total and differential white blood cell counts including biochemical tests for albumin, total protein and globulin.

2.3. Anthelmintic treatment and collection of expelled worms

All *Trichostrongylus*-infected participants were treated with a single dose of albendazole (400 mg). Purgation using 60 ml of saturated magnesium sulfate solution was only performed for single trichostrongyliasis. All fecal samples were collected and washed several times with tap water until the supernatant came clear. The sediments were inspected with the naked eye for macroparasites, and examined under a stereomicroscope for microparasites. Adult *Trichostrongylus* worms found in positive participants were collected in 10% formalin for morphological identification using staining. The participants were followed up post-treatment for whole day fecal examinations over 3 consecutive days.

2.4. PCR assay

PCR with specifically designed primers was used to confirm *Trichostrongylus* species (Sato et al. (2010). The ITS2 of the *Trichostrongylus* spp. was amplified using primer set jhTsp (forward): 5′-TTATGTGCCACAAATGAAGA-3′ and NC2 (reverse) 5′-TTAGTTTCTTTTTCCTCCGCT-3′. The result of the specifically amplified PCR products was observed and sequenced. The sequences were aligned and compared with *Trichostrongylus* spp. sequence data in GenBank using BioEdit (Hall, 1999).

Table 2
Relationship of personal behaviors, complaints and clinical features of trichostrongyliasis cases compared with control cases.

Items	*Trichostrongylus* infection		Chi-square
	Positive cases	Control cases	
Personal behaviors			
Close contacting with herbivorous animals	57 (54.1)	40 (42.9)	Cal = 5.24**
Consuming fresh and raw vegetables	46 (48.5)	41 (38.5)	Cal = 1.81*
Washing hands before meals	36 (39.6)	35 (31.4)	Cal = 2.33*
Wasingh hands after toilet usage	36 (39.6)	35 (31.4)	Cal = 2.33*
Complaints			
Loose fecal excretion	9 (14.5)	17 (11.5)	Cal = 6.29**
Rash appearance	30 (29.6)	23 (23.4)	Cal = 5.98**
Nausea	17 (15.0)	10 (11.9)	Cal = 0.76[a]
Vomiting	11 (9.5)	6 (7.5)	Cal = 0.66[a]
Loss appetite	13 (12.8)	10 (10.2)	Cal = 0.01[a]
Lassitude	39 (39.0)	31 (31.0)	Cal = 0.00[a]
Abdominal pain	35 (31.8)	22 (25.2)	Cal = 1.62*
Flatulence	37 (34.6)	25 (27.4)	Cal = 0.95[a]
Clinical features			
Fever	17 (15.0)	10 (11.9)	Cal = 0.76*
Pallor	1 (0.5)	0 (0.4)	Cal = 0.80*
Generalized abdominal pain	2 (1.7)	1 (1.3)	Cal = 0.15*

[a] NS.
* $p < 0.250$.
** $p < 0.100$.