J Trop Med Parasitol. 2011;34:54-61.

ORIGINAL ARTICLE

Available online at www.ptat.thaigov.net

Zoonotic Helminth Contamination of the Environment in Rural Villages of Southern Lao PDR

Wanna Maipanich[1], Kittipong Chaisiri[1], Tippayarat Yoonuan[1], Megumi Sato[2], Marcello Otage Sato[3], Tiengkham Pongvongsa[4], Boungnong Boupha[5], Kazuhigo Moji[6], Jitra Waikagul[1]

[1] *Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, Thailand;*
[2] *School of Health Sciences, Faculty of Medicine, Niigata University, Japan;*
[3] *Laboratorio de Parasitologia, Escola de Medicina Veterinaria Zootecnia, Universidade Federal do Tocantins, Araguaina, Tocantins, Brazil;*
[4] *Station of Malariology, Parasitology and Entomology, Savannakhet Province, Lao PDR;*
[5] *National Institute of Public Health, Ministry of Health, Lao PDR;*
[6] *Research Institute for Humanity and Nature, Japan*

Abstract

Helminth infections are common among humans and animals in Savannakhet Province, Lao PDR. To assess the level of such environmental contamination, surface soil samples, houseflies, and vegetables, were collected from Ban Lahanam and Ban Thakhamlaine villages, in Songkhone District. Surface soil samples contained one ascarid egg, as well as free-living nematode larvae. The external body surfaces of the houseflies carried adult *Haplorchis taichui* (otherwise known as minute intestinal fluke), as well as opisthorchid and taeniid eggs, nematode larvae, and mites. Three third-stage angiostrongylid larvae were found on pre-washed vegetables purchased at a local market. Taeniid, oxyurid, trichurid and strongylid helminths eggs were detected on other vegetable samples. Such findings indicate this environment was contaminated with several different helminth species at various stages of development, highlighting the risk of parasitic zoonotic infections for humans and domestic animals. Communities should therefore be offered advice on how to improve standards of sanitation.

Keywords: zoonotic helminth infection, environmental contamination, Lao PDR

Introduction

Inhabitants of rural areas in a number of developing countries continue to display high prevalence rates of helminthic infection. Zoonotic parasitic infections, especially, tend to occur in areas where animals and humans have close contact. In some remote areas of Lao PDR, humans and animals often share the same living space, being kept either under the same roof or within reachable distance. Household environments are easily contaminated with animal dung, making living conditions unsanitary. Pathogens in

Correspondence:
Jitra Waikagul,
E-mail: <jitra.wai@mahidol.ac.th>

fecal matter also accumulate in soil and can be carried elsewhere by flies, animals and humans. Animal dung is frequently used as a fertilizer for homegrown vegetables, which in turn can result in the contamination of food with pathogenic organisms. These vegetables can later become a source of infection for humans, most commonly with zoonotic diseases. Our previous studies found several helminthes to be prevalent in the village of Ban Lahanam in Savannakhet, including *Opisthorchis viverrini*, *Haplorchis taichui*, hookworm, *Taenia* sp and *Trichostrongylus colubriformis* [1-3]. To evaluate the potential risk of infection, this study sought to detect helminth objects present in the environment by examining surface soil samples, houseflies and vegetables – which are the suspected sources of helminthiases transmission – in the villages of Ban Lahanam and Ban Thakhamlaine in the Songkhone District of Savannakhet, Lao PDR.

Materials and methods

Study sites

Our previous studies confirmed the southern Laotian province of Savannakhet to be an endemic area for several species of zoonotic helminths, thus making it a suitable site for this study [1-3]. Ban Thakhamlaine and Ban Lahanam, two villages in the Songkhone District, were chosen. Both are adjacent without any clear boundary; located at latitude 16° 16′ 0″ N and longitude 105° 16′ 0″ E. Household and villager demographics, as well as environmental surroundings, are almost identical (Fig 1). A small morning market located near the health office was used. Three types of samples were collected from the study areas: surface soil, houseflies, and vegetables. They were transported to the laboratory of the Faculty of Tropical Medicine, Mahidol University, in Bangkok, for examination.

Fig 1 The two study areas.

Soil samples

Surface soil was found to play a crucial role in the infection/reinfection of community members [4,5]. Samples of surface soil were collected from various sites around each house, including the septic tank (n = 11) and the playground or animal-keeping area (n = 15). In the chosen areas, a sample of surface soil, measuring 1 sq ft with no greater depth than 1 cm, was taken and kept in an individually labeled plastic bag. All samples were kept at room temperature and subsequently transferred to Bangkok, where they were examined by sugar flotation method. In the laboratory, 1 g of the sample was placed in a centrifuge tube with 10% sodium hypochlorite. The mixture was shaken to detach parasites from soil particles, and then centrifuged for five minutes at 2,000 rpm. After discarding the supernatant fluid and mixing the sediment with other media (sugar solution with a specific gravity of 1.200), the mixture was left at room temperature for two hours before microscopic examination [6].

Housefly samples

Houseflies were caught in a small restaurant and around households in the two villages. Each fly was covered with a single plastic bag and combined into one container for each catching site. These were then stored in a cooler box. Thirty flies were kept in a capped bottle containing 15 ml formalin-detergent (FD) solution (10 ml formalin and 50 ml detergent dissolved in 440 ml water), and sent to Mahidol University. In the laboratory, the preserved flies, in their preservative solution, were washed in an ultrasonic cleaner (Elma Transsonic Digital D-7700) at level five for 15 minutes at room temperature [7]. All sediment was observed under a light microscope for the presence of helminth eggs or larvae.

Vegetable samples

A total of 1,320 g of vegetable samples were taken, including some types frequently eaten raw (holy basil, hairy basil, spring onion, coriander, Chinese cabbage, lettuce, spiritweed/long coriander, kitchen mint, and heart leaf). These were collected from garden plots around the villages or purchased from the morning market. They were cut into small pieces and soaked in FD solution for 30 minutes, then washed in an ultrasonic cleaner at level five for 15 minutes at room temperature. The sediments were then examined under a light microscope for the presence of helminth eggs and larvae.

Results

Soil samples

Since our investigation was conducted during the raining season in Lao PDR, the soil samples collected were saturated with rainwater. One ascarid egg containing a degenerated larva was found in a sample collected near a septic tank in Ban Lahanam. In other soil samples, several unknown species of possible free-living nematode larvae were found.

Housefly samples

Houseflies were observed to be carrying opisthorchid eggs, taeniid eggs and mites on their body surfaces. The eggs of opisthorchid and taeniid worms were found either as a single egg or as a cluster of 10-20 eggs (Table 1). Unexpectedly, one sample of flies collected from the local restaurant at Ban Thakhamlaine village contained adult *Haplorchis taichui* worms, which were identified by their characteristic fan-shape spines on the ventral sucker (Fig 2) [8]. It is possible the presence of *H. taichui* on the body surface of these flies might have occurred after feeding on a duck's intestinal contents in the garbage. In rural areas here, food prepared from duck's flesh and its internal organs is very common.

Vegetable samples

In the vegetable samples collected (Fig 3), one strongylid worm egg was detected in a garden plot where cattle excreta were used as a fertilizer. The egg was oval, with a thin shell and contained a morula-stage embryo. Vegetables bought from the morning market were contaminated with nematode larvae and adults, as well as several mites and insects. *Taeniid* eggs were found in

Table 1 Parasites found on the external body surfaces of houseflies in Ban Lahanam and Ban Thakhamlaine, Songkhone District, Savannakhet, Lao PDR, 10-12 August 2010.

Site of collection	No. exam	No. of minute intestinal flukes		*Taenia* egg	Larvae**	Mites***
		egg	adult			
Around household	92	97, 2*	-	-	3	3
Local restaurant	166	68, 1*	3 (*H. taichui*)	38	23	23

* cluster of eggs (each cluster contained 10-20 eggs); ** unknown species of nematode larvae; *** ectoparasites of mammals and avians

Table 2 Parasites discovered on vegetables collected from the village of Ban Thakhamlaine, 10-12 August 2010.

Source	Amount exam (g)	No. of parasites discovered			Others
		egg	larvae	adult	
Vegetable plot					
holy basil & hairy basil	200	strongylid (1)	-	free-living nematode ♀ (1)	several mites & and insects
Local market					
spring onion & coriander	335	trichostrongylid (1)	3 rd stage larvae *Angiostrongylus* sp* (3), unknown nematode (3)	free-living nematode ♀ (3), ♂ (2)	mites (2)
holy basil	100	-	-	-	mites (7)
Chinese cabbage	245	-	unknown nematode (7)	free-living nematode ♀ (1)	insect larvae & adult (7), mite (1)
lettuce	235	-	unknown nematode (12)	-	mites (2)
spiritweed/long coriander	35	*Taenia* sp (5)	unknown nematode (172)	-	mite (1)
kitchen mint	50	*Taenia* sp (1)	unknown nematode (4)	-	mites (7)
heart leaf	120	*E.v* (1), *T.t* (1)	-	-	insect larva (1)

* larvae with constriction at tail-end; *E.v* = *Enterobius vermicularis*; *T.t* = *Trichuris trichiura*

the spiritweed and kitchen mint. Oxyurid and trichurid eggs were detected in heart leaf, which is often eaten raw by the local populace. One trichostrongylid egg and nematode larvae were detected on spring onions and coriander bought from the local market (Table 2). Of the six

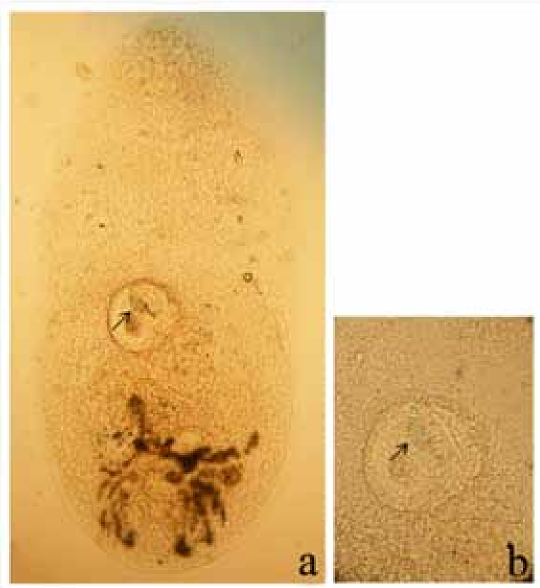

Fig 2 ***Haplorchis taichui*** **discovered on external body surface of houseflies: a) adult worm; b) ventral sucker with fan-shaped spines.**

Fig 3 The examined vegetables: a) holy basil; b) hairy basil; c) spring onion and coriander (sold bunched together); d) Chinese cabbage; e) lettuce; f) spiritweed/ long coriander; g) kitchen mint; h) heart leaf.

Fig 4 ***Angiostrongylus*** **sp 3rd stage larva isolated from vegetables: a) whole larva; b) well-developed chitinous rod in the buccal cavity; c) constriction at the tail-end.**

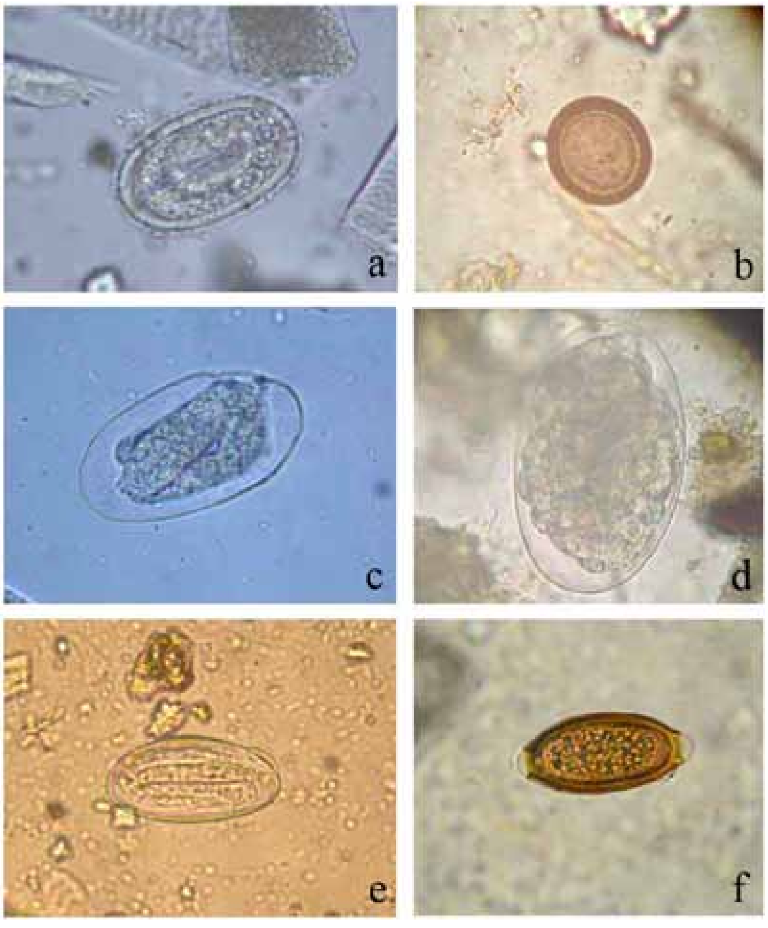

Fig 5 **Helminth eggs in the collected samples: a) *Ascaris* sp with degenerated larva; b) *Taenia* sp; c) Strongylid; d) *Trichostrongylus* sp; e) *Enterobius vermicularis*; f) *Trichuris trichiura*.**

larvae found, three had characteristics specific to third-stage *Angiostrongylus* larvae: a well-developed chitinous rod in the buccal cavity and a constricted tail (Fig 4). Morphologically, none of the recovered adult worms resembled any species of human or animal pathogenic nematode, so may be free-living nematodes in the soil.

Taeniid eggs contaminating the houseflies and soil samples might be *Taenia* or *Echinococcus*, since the study site is a ruminant-raising area.

Discussion

We confirmed that the soil, housefly and vegetable samples taken from the study villages were highly contaminated with helminthes (Tables 1, 2). This suggests a high risk of infection for villagers and animals in the local area. Angiostrongyliasis is a medically important helminthic disease, since it causes meningoencephalitis and other severe symptoms. Generally, the intermediate hosts of *Angiostrongylus* are animals, such as mollusks, freshwater prawns, terrestrial crabs, and frogs [9,10]. Vegetables have also been described as a possible source of angiostrongyliasis [11-14]. In this study, we were able to isolate the infectious larval stage of *Angiostrongylus* sp on vegetables bought from the local market. It is possible, therefore, that villagers are at risk of contracting angiostrongyliasis infections, not just from eating raw snail intermediate hosts, but by simply consuming raw vegetables. In these remote parts, populations are at high risk of *Angiostrongylus* infection, since they believe that home-grown vegetables are clean, chemical-free, and safe to eat raw, sometimes even without washing. This belief may increase the risk of infection in some areas. Surprisingly, houseflies were found to carry many parasitic objects. They were confirmed as having a role as a mechanical vector for helminthic infections, capable of mediating disease transmission within the community. Also, it has been reported that helminthic objects, protozoan cysts and bacteria are carried by houseflies from place to place, and from animal to human and vice versa, in many countries [15-19]. Flies are not just unpleasant pests to humans, but also medically significant animals that transmit infectious diseases.

In a previous study by Sato *et al* [3], the prevalence rate of *Trichostrongylus* infection in Ban Lahanam Village was rather high (21.2%), leading us to expect to find many infective-stage larvae in the environment. However, only one trichostrongylid egg was found on the spring onions and coriander. As this study took place during the rainy season, many parasitic objects in the soil might have been washed away by the rain. The numbers found may increase if samples are taken and examined during the dry season. To confirm this hypothesis, further investigations should be conducted to compare levels of helminthic-object contamination in the environment at different times of the year. Environmental factors are very important when considering mechanisms of parasitic transmission, especially in times of unpredictable climate change and increasingly frequent natural disasters. In rural, parasitic-disease-endemic areas, humans and animals are surrounded by pathogens and share the same unsanitary environment. To prevent disease transmission, appropriate health education about sources and transmission methods of infection, as well as about safe food handling, preparation and storage, should be provided to local communities.

Acknowledgements

The authors wish to thank all staff members of the Lahanam Health Office, and the villagers from the study areas for providing facilities and general assistance. This research was supported by the RIHN research project "Environmental Changes and Infectious Diseases in Tropical Asia", a grant awarded to Kazuhiko Moji.

References

1. Sato M, Pongvongsa T, Sa-nguankiat S, Yoonuan T, Dekumyoy P, Kalambaheti T, *et al*. a. Copro-DNA diagnosis of *Opisthorchis viverrini* and *Haplorchis taichui* infection in an endemic area of LAO PDR. Southeast Asian J Trop Med Public Health. 2010;41:28-35.

2. Sato M, Sa-nguankiat S, Yoonuan T, Pongvongsa T, Keomoungkhoun M, Phimmayoi I, *et al*. b. Copro-molecular identification of infections with hookworm eggs in rural Lao PDR. Trans R Soc Trop Med Hyg. 2010;104:617-22.
3. Sato M, Yoonuan T, Sa-nguankiat S, Nuamtanong S, Pongvongsa T, Phimmayoi I, *et al*. Human *Trichostrongylus colubriformis* infection in a rural Laotian village. Am J Trop Med Hyg. 2011;84:52-4.
4. Kobayashi A, Katakura K, Hamada A. The fate of *Ascaris* eggs applied to the soil under various conditions. In: Yokogawa M, *et al*, editors. Collected papers on the control of soil-transmitted helminthiases Vol III. Tokyo: Hoken Kaikan; 1986. p. 15-9.
5. Kagei N. Techniques for the measurement of environmental pollution by infective stage of soil-transmitted helminthes. In: Yokogawa M, *et al*, editors. Collected papers on the control of soil-transmitted helminthiases Vol II. Tokyo: Hoken Kaikan; p. 27-46.
6. Maipanich W, Waikagul J, Viusessuk K. Efficacy of three floating media in separating *Ascaris* eggs from soil. Mahidol Univ J. 1996;3:157-60.
7. Maipanich W, Sa-nguankiat S, Pubampen S, Kusolsuk T, Rojekittikhun W, Castelli F. House flies: potential transmitters of soil-transmitted-helminth in an unsanitary community. J Trop Med Parasitol. 2008;32:14-22.
8. Radomyos P, Sirivichayakul C, Waikagul J, Krudsood S, Takeuchi T, Kojima S, *et al*. Atlas of medical parasitology with 434 colour illustrations. 1st ed. Bangkok: Medical Media Co. Ltd.; 2004.
9. Yii CY, Chen CR, Hsieh HC, Shih CC, Cross JH, Rosen L. Epidemiologic studies of eosinophilic meningitis in southern Taiwan. Am J Trop Med Hyg. 1975;24:447-54.
10. Lai CH, Yen CM, Chin C, Chung HC, Kuo HC, Lin HH. Eosinophilic meningitis caused by *Angiostrongylus cantonensis* after ingestion of raw frogs. Am J Trop Med Hyg. 2007;76:399-402.
11. Beaver PC, Jung RC, Cupp EW. Clinical parasitology. 9th ed. Philadelphia: Lea & Febiger; 1984.
12. Desowitz RS. Ova and parasites. Maryland: Harper & Row; 1980.
13. Muller R. Worms and disease: a manual of medical helminthology. London: William Heinemann Medical Books; 1975.
14. Slom TJ, Cortese MM, Gerber SI. An outbreak of eosiniphilic meningitis caused by *Angiostrongylus cantonensis* in travelers returning from the Caribbean. N Engl J Med. 2002;346:668-75.
15. Getachew S, Gebre-Michael T, Erko B, Balkew M, Medhin G. Non-biting cyclorrhaphan flies (Diptera) as carriers of intestinal human parasites in slum areas of Addis Ababa, Ethiopia. Acta Tropica. 2007;103:186-94.
16. Khan AR, Hug F, Disease agents carried by flies in Dacca city. Bangladesh Med Res Counc Bull. 1978;4:86-93.
17. Monzon RB, Sanchez AR, Tadiaman BM, Najos OA, Valencia EG, de Rueda RR, *et al*. A comparison of the role of *Musca domestica* (Linnaeus) and *Chrysomya megacephala* (Fabricius) as mechanical vectors of helminthic parasite in a typical sum area of Metropolitan Manila. Southeast Asian J Trop Med Public Health. 1991;22:222-8.
18. Sulaiman S, Sohadi AR, Yunus H, Iberahim R. The role of some cyclorrhaphan flies as carriers of human helminths in Malaysia. Med Vet Entomol. 1998;2:1-6.
19. Umeche N, Mandah LE. *Musca domestica* as a carrier of intestinal helminths in Calabar, Nigeria. East Afr Med J. 1989;66:349-52.