Tropical Medicine and Health Vol. 37 No. 2, 2009, pp. 55-61

Original article

Parasite egg contamination of water and air in a suburban area of Hanoi, Vietnam

Shinichi Noda[1*], Nguyen Thi Viet Hoa[2], Shoji Uga[3], Le Khanh Thuan[2], Yoshiki Aoki[4] and Yasunori Fujimaki[4]

Received 13 February, 2009 Accepted 8 May, 2009 Published Online 12 June, 2009

Abstract: Contamination of water and air by soil-transmitted helminth eggs was investigated in a small village located in the suburbs of Hanoi, Vietnam. Water samples were collected from 29 households, two schools, two kindergartens, one restaurant, three ponds and 23 ditch sites during the rainy season. Water samples were also collected at the same places, except for one household and one restaurant, during the dry season. The water samples collected from households, schools, kindergartens and restaurant were comprised of well-water and rain-water. These samples included both water filtrated with sand and gravel and non-filtrated water. Two-liter water samples were examined for helminth eggs by either a centrifugation or flotation method. The contamination of air by helminth eggs was assessed by the method of Kroeger *et al.* (1992). Eggs in air were trapped on adhesive tapes hanging in rooms and in the area around 29 houses, two schools, two kindergartens, one restaurant and 18 utility poles.

Out of 63 water samples collected from households in the rainy season, helminth eggs were found in four water samples; one from non-filtrated well-water and three from filtrated well-water samples. The one non-filtrated water sample contained six eggs of *Toxocara* sp., while the three filtrated water samples contained one egg each of *Trichuris* sp., *Trichiuris* sp. and *Taenia* sp. No eggs were found in the water samples collected from schools, kindergartens or the restaurant in the rainy season. All water samples collected from ponds and ditches in the rainy season contained many helminth eggs. The eggs found were *Ascaris* sp., *Trichuris* sp., *Toxocara* sp., *Ascaridia galli*, hookworm, *Taenia* sp. and *Fasciola* sp. Examination of the adhesive tapes hanging in the air showed that four sites were contaminated by helminth eggs, i. e. one site near the house, two sites near the school and one site at a utility pole. The species of eggs found were *Trichuris* sp., *Ascaridia galli* and *Taenia* sp. The number of eggs found on tapes was only one or two. In the dry season, a few samples of well-water and rain-water collected from the residential area were contaminated with helminth eggs, and all samples collected from ponds and ditches contained many eggs of various species similar to those collected in the rainy season. The present study clearly indicates that, in our study area, the villagers were subject to infection from soil-transmitted helminthes directly and indirectly through water.

Key words: Intestinal parasite; *Ascaris*; *Trichuris*; Water; Air; Contamination; Vietnam

INTRODUCTION

We carried out an epidemiological study on soil-transmitted helminth infections in a village located in the suburbs of Hanoi, Vietnam. The main object of this study was to identify factors influencing the transmission of intestinal helminth eggs. Vietnam is a highly endemic area for soil-transmitted helminth infection [1]. In our study area, 76% of school children were infected with the parasite. Among the helminth parasites, the most frequently detected was *Trichuris trichiura* (67%) followed by *Ascaris lumbricoides* (34%), and hookworm (3%) [2].

In the study area, well water is used for daily life, and rain water is also used in some households. The village is surrounded by cultivated fields and isolated from other towns and villages. The sewage from households flows into roadside ditches and ends up in the village ponds or the ditches in cultivated fields. Human and animal excrement is

[1] Research Center for the Pacific Islands, Kagoshima University, Kagoshima, Japan
[2] National Institute of Malariology, Parasitology and Entomology, Hanoi, Vietnam
[3] Department of Medical Technology, Faculty of Health Sciences, Kobe University, Hyogo, Japan
[4] Department of Parasitology, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan
*Corresponding author:
Research Center for the Pacific Islands, Kagoshima University
1-21-24 Korimoto, Kagoshima 890-8580, Japan
E-mail: snoda@cpi.kagoshima-u.ac.jp

used as fertilizer in the cultivated fields. Under these circumstances, the water contaminated with helminth eggs may play an important role as a source of parasitic infections. In the present study, we attempted to determine the contamination level of helminth eggs in water and air samples.

MATERIALS AND METHODS

Study area and period

The survey was conducted from June to August 2004 (rainy season) and from December 2004 to February 2005 (dry season). The study area, Ha Hamlet, is located in the suburbs of Hanoi, Vietnam. The population of the hamlet is 4,100 (1,010 households), with about three-fourths of the people engaged in agriculture.

Water sampling and laboratory analyses

Sixty-three water samples (two liters each) were collected from 29 households. Samples consisted of water pumped from a well, rain water stored in the tank, a mixture of well and rain water in the tank, and three types of water filtrated with sand and gravel. Water samples from the tank were collected after agitating the water in the tank. Well and filtrated water samples were also collected from two schools, two kindergartens and one restaurant. Water samples were not collected from one household and one restaurant in the rainy season. Water samples from three ponds and 23 points in ditches were collected after agitating the collection sites.

Each water sample was transferred to a two liter beaker, 10 g NaOH added, and the mixture left until the next day. After removing the supernatant, the remaining fluid was transferred to a 50 ml centrifuge tube and centrifuged at 3,000 rpm for 10 minutes. If the volume of the sediment was small, it was observed under a microscope. If the volume of the sediment was large, a flotation method was applied for the detection of eggs. Proper volume of saturated $NaNO_3$ was added to the centrifuge tube and centrifuged at 1,000 rpm for 5 minutes. Saturated $NaNO_3$ was added to the top of the centrifuge tube, and a grease-free slide glass was placed carefully on the top to contact the surface of the fluid. After 20 minutes, the slide glass was removed, and a cover glass was placed on the slide glass for observation under a microscope. Several drops of saturated $NaNO_3$ were added again to the centrifuge tube, and a new slide glass was placed on the top. After 10 minutes, the slide glass was removed, and a cover glass was placed for observation.

Detection of helminth eggs in the air

Contamination of air by helminth eggs was assessed by the method of Kroeger *et al.* (1992). To detect helminth eggs in the air, transparent adhesive strips (Bang dinh trong, Tan Hoang Long Company, Hanoi, Vietnam) were used as egg-traps [3]. The detection of helminth eggs in the air was attempted at 159 points; 53 points inside and 88 points outside of houses, schools, kindergartens and a restaurant, and 18 points near utility poles. Transparent adhesive strips (5 cm wide and 15 cm long) were attached on walls or pillars of 29 houses, two schools, two kindergartens, one restaurant, and six utility poles at a height of one meter. These adhesive strips were left for two weeks outside and for one month inside. Four slide glasses were placed on each of the removed adhesive strips, and the edge of each slide glass was cut to separate them. All slides were examined under a microscope.

RESULTS

Water contamination with helminth eggs

In the rainy season, a small number of helminth eggs (*Trichuris* sp., *Toxocara* sp. and *Taenia* sp.) were found in four out of 29 households (13.8%) but not in the water from schools, kindergartens and a restaurant (Tables 1 and 2). A low level of contamination with parasite eggs in households was also observed in the dry season, i. e. a small number of helminth eggs (*Ascaris* sp., *Trichuris* sp. and *Toxocara* sp.) were found in three out of 28 households (10.7%) but not in the water from schools and kindergartens (Tables 3 and 4).

All water samples from ponds and ditches were contaminated with helminth eggs during both the rainy and dry seasons (Table 1, 2, 3 and 4). Eggs of *Ascaris* sp., *Trichuris* sp., *Ascaridia galli*, *Toxocara* sp., and *Taenia* sp. were isolated from the water of ponds. Eggs of *Ascaris* sp. were the most abundant in number, followed by *Trichuris* sp. in the water of ponds. A large number of helminth eggs were found from the water of ditches. Eggs of *Ascaris* sp., *Trichuris* sp., *Ascaridia galli*, *Toxocara* sp., hookworm, *Taenia* sp. and *Fasciola* sp. were isolated from the water of ditches. About one half and two thirds of water collected from ditches contained more than a hundred eggs of *Ascaris* sp. in the rainy and dry seasons, respectively. Two water samples (D16 and 20) in the rainy season contained more than a thousand eggs of *Ascaris* sp. (Table 2). These two water collection sites were located in the center of the dwelling area.

Air contamination with helminth eggs

In the rainy season, a small number of helminth eggs were found at four points outside; one point in the house

Table 1. Contamination of water by helminth eggs in the rainy season

Place	Source of samples collected	Type of samples	No.of samples examined	No.of samples with eggs (%)
Household	Well	Original	21	1 (4.8)
		Filtrated	29	3 (10.3)
	Rain	Original	7	0
		Filtrated	2	0
	Well+Rain	Original	2	0
		Filtrated	2	0
School	Well	Original	1	0
		Filtrated	4	0
Kindergarten	Well	Original	2	0
		Filtrated	2	0
Restaurant	Well	Filtrated	2	0
Pond	Pond	Agitated	6	6 (100)
Ditch	Ditch	Agitated	23	23 (100)
Total			103	33

Table 2. Number of helminth eggs recovered from water samples in the rainy season

Place	Source of samples collected	Type of samples	No. of eggs detected / 2 liters of water						
			Ascaris sp.	*Trichuris* sp.	*Ascaridia galli*	*Toxocara* sp.	hookworm	*Taenia* sp.	*Fasciola* sp.
Household	Well	Original	0	0	0	6	0	0	0
	Well	Filtrated	0	1	0	0	0	0	0
	Well		0	1	0	0	0	0	0
	Well		0	0	0	0	0	1	0
Pond	P1	Agitated	2	2	0	0	0	2	0
	P1		6	0	0	0	0	0	0
	P2		27	0	2	2	0	2	0
	P2		8	0	0	0	0	0	0
	P3		17	6	0	0	0	0	0
	P3		15	7	0	0	0	1	0
Ditch	D1	Agitated	90	20	0	0	0	1	6
	D2		62	30	0	0	0	0	0
	D3		185	10	0	0	0	0	0
	D4		221	9	0	0	0	0	0
	D5		33	27	0	0	0	0	0
	D6		66	4	0	0	0	0	0
	D7		284	9	0	2	0	3	0
	D8		2	3	1	0	0	0	0
	D9		5	7	0	0	0	0	0
	D10		21	0	0	0	0	0	0
	D11		11	0	0	0	0	0	0
	D12		70	53	0	0	0	0	0
	D13		220	39	40	0	0	0	0
	D14		82	39	0	0	0	0	0
	D15		172	40	0	3	0	10	0
	D16		2352	407	7	0	0	3	0
	D17		698	454	27	8	0	1	0
	D18		2	0	0	0	0	0	0
	D19		2	0	1	0	0	0	0
	D20		1019	113	14	0	0	0	0
	D21		630	90	0	467	50	0	0
	D22		770	46	7	0	0	2	0
	D23		407	26	0	0	0	0	0
Total			7479	1443	99	488	50	26	6

Table 3. Contamination of water by helminth eggs in the dry season

Place	Source of samples collected	Type of samples	No.of samples examined	No.of samples with eggs (%)
Household	Well	Original	19	2 (10.5)
		Filtrated	28	0
	Rain	Original	5	1 (20.0)
		Filtrated	1	0
	Well+Rain	Original	2	0
		Filtrated	2	0
School	Well	Original	1	0
		Filtrated	3	0
Kindergarten	Well	Original	2	0
		Filtrated	2	0
Pond	Pond	Agitated	6	6 (100)
Ditch	Ditch	Agitated	22	22 (100)
Total			93	31

Table 4. Number of helminth eggs recovered from water samples in the dry season

Place	Source of samples collected	Type of samples	No. of eggs detected / 2 liters of water						
			Ascaris sp.	*Trichuris* sp.	*Ascaridia galli*	*Toxocara* sp.	hookworm	*Taenia* sp.	*Fasciola* sp.
Household	Well	Original	6	0	0	0	0	0	0
	Well		0	1	0	0	0	0	0
	Rain		0	0	0	1	0	0	0
Pond	P1	Agitated	33	7	0	0	0	0	0
	P1		37	0	0	0	0	0	0
	P2		74	14	0	0	0	0	0
	P2		25	1	0	0	0	0	0
	P3		3	2	0	0	0	0	0
	P3		5	2	0	0	0	0	0
Ditch	D1	Agitated	7	6	0	0	0	0	0
	D2		118	19	0	5	0	2	0
	D3		306	18	0	0	0	0	0
	D4		445	27	0	0	0	0	0
	D5		262	61	0	0	0	0	0
	D6		313	14	0	0	0	0	0
	D7		143	94	0	87	0	2	0
	D8		336	27	0	0	0	0	0
	D9		444	42	0	0	0	0	0
	D10		181	67	12	10	0	2	0
	D11		34	8	0	4	0	2	0
	D12		38	14	0	0	0	18	0
	D13		14	5	0	0	0	0	0
	D14		0	3	0	2	0	13	0
	D15		84	18	0	0	0	0	0
	D16		412	132	0	0	0	0	0
	D17		239	5	0	0	0	0	0
	D18		0	1	0	4	0	70	0
	D19		164	54	8	54	0	2	0
	D20		366	15	0	0	0	3	0
	D21		441	159	0	0	0	7	0
	D23		399	3	0	7	0	3	0
Total			4929	819	20	174	0	124	0

Table 5. Species and number of helminth eggs collected from adhesive tapes in the rainy season

Site of collection	No.of sites examined	Place of adhesive tape	No.of tapes	No.of tapes on which eggs were trapped (%)	No.of eggs detected				
					Ascaris sp.	*Trichuris* sp.	*Ascaridia galli*	*Toxocara* sp.	*Taenia* sp.
Household	29	Inside	29	0					
		Outside	58	1 (1.7)	0	0	0	0	2
School	2	Inside	17	0					
		Outside	17	2 (11.8)	0	0	2	0	0
Kindergarten	2	Inside	6	0					
		Outside	12	0					
Restaurant	1	Inside	1	0					
		Outside	1	0					
Utility pole	6	Outside	18	1 (5.6)	0	0	1	0	0
Total	40	Inside	53	0					
		Outside	106	4 (3.8)	0	1	3	0	2

(*Taenia* sp.), two points around the school (*Trichuris* sp. and *Ascaridia galli*) and one point at a utility pole (*Ascaridia galli*), but not at points inside (Table 5). No helminth eggs were found from points inside or outside in the dry season.

DISCUSSION

It is easy to imagine that raw wastewater increases the risk of helminthic infection. Studies to determine the risk associated with wastewater used for agricultural purposes in Morocco indicated that raw wastewater use led to a high risk of helminthic infection [4, 5]. Similarly, a significant increase in risk of *Ascaris*, hookworm and *Trichuris* infection was observed in farming communities irrigating with wastewater in India [6], and direct exposure to untreated wastewater was associated with an excess risk of *Ascaris* infection in children in Mexico [7]. Meanwhile, the importance of drinking water as a source of helminth infection was shown by epidemiological studies. In the population living on a low-country tea plantation of Sri Lanka, people who drank only boiled water and washed their hands before meals were relatively unlikely to be infected [8]. Four significant environmental factors influencing helminthiasis among Pacific Island school children were identified: inadequate water supply, availability of school canteens, regular water/sanitation maintenance regimes, and overcrowded classrooms [9]. In Belize, villagers who drank treated water and owned electric appliances had a low risk for helminthic infection [10]. An epidemiological study on children in Brazil showed the protective effect of availability of water in a washbasin as well as better hygiene, sanitation and socioeconomic status [11]. In Uzbekistan, the risk of reinfection by parasite was 30% lower in the hygiene promotion group (medicine provided and hygiene activities promoted) than in the treatment group (medicine provided but no hygiene promotion activities) [12].

Helminth eggs have in fact been found in various wastewater sources. Helminth eggs were isolated at high rates from sewage water samples in Turkish farming villages where inhabitants were highly infected with soil-transmitted helminthiases [13]. In Iran, wastewater samples obtained from both entry wastewater and effluent of treatment plants were examined, and the untreated entry wastewater contained a large variety of helminth eggs [14]. In Egypt, examination of raw water samples from drains, canals and rivers revealed the presence of larvae and eggs of the nematode infecting humans and animals [15]. In a city in India, water samples were collected from open wells, overhead storage tanks, ground level reservoirs and other types of storage containers, and about one fourth of the samples showed the presence of pathogenic parasites [16].

In our study area, the sewage flows into roadside ditches and ends up in the village pond or ditches in cultivated fields. Human and animal excrement was used as a fertilizer in cultivated fields. The previous survey showed that one third and two thirds of households were using human and animal excrement as a fertilizer on their cultivated fields, respectively (unpublished data). In the present survey, a large number of helminth eggs were found in the water of ditches that run through rice and vegetable fields (D1-7) as well as in the water of roadside ditches in dwelling areas (D 8-23). Two water samples (D16 and 20) in the rainy season contained more than a thousand eggs of *Ascaris* sp. These unexpected results can be explained as follows. When inhabitants reconstruct their houses, they change the toilet system from a pit toilet to a flush toilet. However, the water-purifier tank is not adequately maintained, and human excrement is often discharged directly into ditches by the roadside. Small children and animals defecate on the road, and farmers spill human and animal excrement while carrying it to the cultivated fields. Our water sample collection in

the rainy season was interrupted in June by heavy rain and flooding. The floodwater, which took four days to recede in the study area, might have increased the egg contamination in ditches near dwelling areas.

There are three shallow ponds in our study area. All water samples from ponds contained helminth eggs during both rainy and dry seasons. The sewage from households flows into ditches and ends up in these ponds. The small and middle-size ponds were covered with water hyacinths, and few people seemed to come in contact with this pond water. On the other hand, the large pond was being used to produce spinach.A major vegetable in Vietnam, spinach is sold in the market of the village. There is a high possibility that the spinach is contaminated with helminth eggs. Indeed, helminth eggs were found in 26% of vegetables sold in the market [unpublished data].

In our study area, well water is pumped up to overhead concrete storage tanks, and rainwater collecting on the roof is often led to the tanks. The water in the storage tank is used in daily life after filtration with sand and gravel to remove the metallic taste. A low level of contamination with helminth eggs was noted in this house water during both rainy and dry seasons. This result indicated that the tip of the pipe in the well water and storage tank was contaminated with helminth eggs. In the present study, a small number of helminth eggs was found on the adhesive strips placed outside at four points during the rainy season. These helminth eggs seem to contaminate the water stored for daily life after being carried by the wind. Adhesive strips suspended below the ceilings of houses in communities of the Peruvian rainforest contained *Ascaris* eggs at a high rate [3]. In the systematic observation on the occurrence of *Ascaris* eggs in a Japanese village, *Ascaris* eggs were detected in vats filled with water placed under the eaves [17].

Results of previous and present surveys indicate the necessity for improvements in environmental conditions. When inhabitants used pit toilets, human excrement was carried out outside the dwelling area. However, inhabitants using flush toilets discharged human excrement into ditches in the dwelling area. The proper maintenance of water-purifier tanks is an important prerequisite for the prevention of contamination in dwelling areas.

ACKNOWLEDGEMENTS

We thank the staff members of the Helminth Unit of the National Institute of Malariology, Parasitology, and Entomology, Hanoi Vietnam, for their assistance during the survey. This study was conducted as part of a joint university program between Vietnam and Japan sponsored by the Japan Society for Promotion of Sciences (JSPS).

REFERENCES

1. Hoek WVD, De NV, Konradsen F, Cam PD, Hoa NTV, Toan ND, Cong LD (2003) Current status of soil-transmitted helminthes in Vietnam. Southeast Asian J Trop Med Public Health 34: 1-11 (suppl).
2. Uga S, Hoa NTV, Thuan LK, Noda S, Fujimaki Y (2005) Intestinal parasitic infections in schoolchildren in a suburban area of Hanoi, Vietnam. Southeast Asian J Trop Med Public Health 36: 1407-1411.
3. Kroeger A, Schulz S, Witte B, Skewes-Ramm R, Etzler A (1992) Helminthiasis and cultural change in the Peruvian rainforest. J Trop Med Hyg 95: 104-113.
4. Habbari K, Tifnouti A, Bitton G, Mandil A (2000) Geohelminthic infections associated with raw wastewater reuse for agricultural purposes in Beni-Mellal, Morocco. Parasitol Int 48: 249-254.
5. Amahmid O, Bouhoum K (2005) Assessment of the health hazards associated with wastewater resuse: Transmission of geohelminthic infections (Marrakech, Morocco). Int J. Environment Health Res 15: 127-133.
6. Ensink J H J, Blumenthal U J, Brooker S (2008) Wastewater Quality and the risk of intestinal nematode infection in sewage farming families in Hyderabab, India. Am J Trop Med Hyg 79: 561-567.
7. Blumenthal UJ, Cifuentes E, Bennett S, Quigley M, Ruiz-Palacios G (2001) The risk of enteric infections associated with wastewater reuse: the effect of season and degree of storage of wastewater. Trans Roy Soc Trop Med Hyg 95: 131-137.
8. Gunawardena G S A, Karunaweera N D, Ismail M M (2004) Socio-economic and behavioural factors affecting the prevalence of *Ascaris* infection in a low-country tea plantation in Sri lanka. Ann Trop Med Parasitol 98: 615-621.
9. Hughes R G, Sharp D S, Hughes M C, 'Acau'Ola S, Heinsbroek P, Velayudhan R, Schulz D, Palmer K, Cavalli-Sforza T, Galea G (2004) Environmental influences on helminthiasis and nutritional status among pacific schoolchildren. Int J Environment Health Res 14: 163-177.
10. Aompum P, Hshieh P (2004) Survey for intestinal parasites in Berieze, Central America. Southeast Asian J Trop Med Public Health 35: 506-511.
11. Carneiro F F, Cifuentes E, Tellez-Rojo M M, Romieu I (2002) The risk of *Ascaris lumbricoides* infection in children as an environmental health indicator to guide preventive activities in Caparao' and Alto Caparao', Brazil. Bull WHO 80: 40-46.
12. Gungoren B, Latipov R, Regallet G, Musabaev E (2007) Effect of hygiene promotion on the risk of reinfection rate of intestinal parasites in children in rural Uzbekistan. Trans R Soc Trop Med Hyg 101: 564-569.
13. Ulukanligil M, Seyrek A, Aslan G, Ozbilge H, Atay S (2001) Environmental pollution with soil-transmitted helminthes in Sanliurfa, Turkey. Mem Inst Oswaldo Cruz, Rio de Janeiro 96: 903-909.

14. Mahvi A H, Kia E B (2006) Helminth eggs in raw and treated wastewater in the Islamic Republic of Iran. Eastern Mediterranean Health Journal 12: 137-143.
15. El Shazly A M, Soliman M, El Nemr H I, Moafyo N, Abdel Gawad A B E, El Bendary M (2003) Nematodes and water pollution in eastern part of Nile Delta. J Egypt Soc Parasitol 33: 631-636.
16. Jonnalagadda P R, Bhat R V (1995) Parasitic contamination of stored water used for drinking/cooking in Hyderabad. Southeast Asian J Trop Med Public Health 26: 789-794.
17. Morishita K, Mishimura T, Imamura H (1959) Observation on the stereic distribution of *Ascaris* eggs in a rural village. Jpn J Parasitol 8: 532-541.