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Soil contamination by parasite eggs in rural village in the Philippines

Saori Horiuchi[1], Vachel Gay V. Paller[2] and Shoji Uga[*1]
[1]Department of Parasitology, Faculty of Health Sciences, Kobe University Graduate, School of Health Sciences, Kobe, Japan
[2]Animal Biology Division, Institute of Biological Sciences, University of the Philippines Los Baños, Laguna, Philippines
[*]Corresponding author email: ugas@kobe-u.ac.jp
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Abstract. Infectious diseases caused by soil-transmitted helminths (STHs) are important diseases of humans, which affect about one third of the world's population. Examination of soil can be used to estimate the risk of STH infection in humans. We carried out this survey to clarify the current status of soil contamination by parasite eggs and to assess the risk of STH infection. During survey periods, we examined soil, faeces, and the lifestyle of residents. Six genera and eight species of parasite eggs including *Ascaris lumbricoides*, *Toxocara cati*, *Toxocara canis*, and *Trichuris trichiura* were recovered from 85 out of 120 soil samples (71%). Contamination of soil by parasite eggs had spread widely throughout the village, and 50% of eggs recovered had already developed into fertilized eggs. It is remarkable that *Ascaris* eggs were recovered from inside the houses. Prevalence of STH in school children was 63%. This may indicate that school or preschool children cause soil contamination. Some of the eggs recovered were not only from humans but also from dogs and cats. From the results obtained, the need for health education with regards to zoonoses was revealed because 77% of fertilized *Toxocara* spp. eggs were detected. We conclude that the risk of STH infection in residents was extremely high, because the soil in this village was highly contaminated by infective parasite eggs.

INTRODUCTION

Infectious diseases caused by soil-transmitted helminth (STH) remain issues of major importance and are the most common diseases in humans, affecting up to an estimated 2 billion people (de Silva *et al.*, 2003). STH proliferation occurs in soil in unsanitary environments, in which STH eggs in human or animal faeces develop and cause faecal oral infection (Balen *et al.*, 2011). Surveys of soil contamination of STH eggs have been conducted in many countries, especially in developing countries in South America and Asia. Korkes *et al.* (2009) have reported a recovery rate of 7-20% for *Ascaris* spp. and hookworm eggs in urban slums in Brazil. In suburban areas in Indonesia (Uga *et al.*, 1995), a fishing village in Thailand (Chongsuvivatwong *et al.*, 1999), and surrounding fields and houses in Nepal (Rai *et al.*, 2000), 20-83% of *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm eggs have been recovered from soil.

STH infection is concern both in developing and developed countries. STH infection in developed countries is mainly caused by imported cases from tropical countries and larva migrans is caused by *Toxocara canis* or *Toxocara cati* (Holland & Smith, 2005). Larva migrans caused by *Toxocara* spp. has been given special attention owing to its high prevalence, pathogenicity, and wide distribution area. Soil examinations in the United States (Childs, 1985), Canada (Ghadirian *et al.*, 1976), and the United Kingdom (Gillespie & Ramsay,

1992) revealed that 6-78% of soil in sandpits in public parks, backyards, and gardens were contaminated by *Toxocara* spp. eggs.

Although studies in the Philippines have revealed that 67-88% of school children and adolescents were infected with intestinal parasites, mainly STH (Belizario *et al*., 2005), the current status of soil contamination by parasite eggs is not clear. Faecal examination is an effective and reliable method for evaluating the prevalence or distribution of parasites in certain areas (Uga *et al*., 1995) where STH eggs are present in human or animal faeces. However, the risk of STH infection of residents cannot be clarified using only faecal examination, because the survival rate of STH eggs depends largely on the environment. On the contrary, conducting soil surveys to determine parasite egg contamination allows direct observation of STH eggs, making it an effective method of assessing the risk of STH infection of residents.

This survey aimed to assess comprehensively the risk of STH infection of residents in a village in the Philippines, using soil and faecal examinations, as well as a questionnaire for residents and school children.

MATERIALS AND METHODS

Survey area and study period

The surveys were conducted in Barangay Bayog, Los Baños, Luzon Island, the Philippines, over two periods: September-October 2011 (rainy season) and February-March 2012 (dry season). Los Baños (14º10´N, 121º13´E) is located approximately 60 km southeast of Manila, and is bordered by Laguna de Bay to the north and Mt. Makiling to the south. The mean temperature in this area is 28.2ºC, and the mean rainfall volumes during the rainy and dry seasons are 317 and 34 mL, respectively (World Weather and Climate Information, 2012).

The survey area, is approximately 193 ha; half of which is agricultural and the other half a fishing village. Residents live in crowded quarters along the main street of the village, which has a public elementary school and a health center (Fig. 1).

Soil examination

A total of 120 soil samples were collected along the main street of the village at approximately the same sites during the rainy (60 samples) and dry seasons (60 samples). In each season, the soil samples were

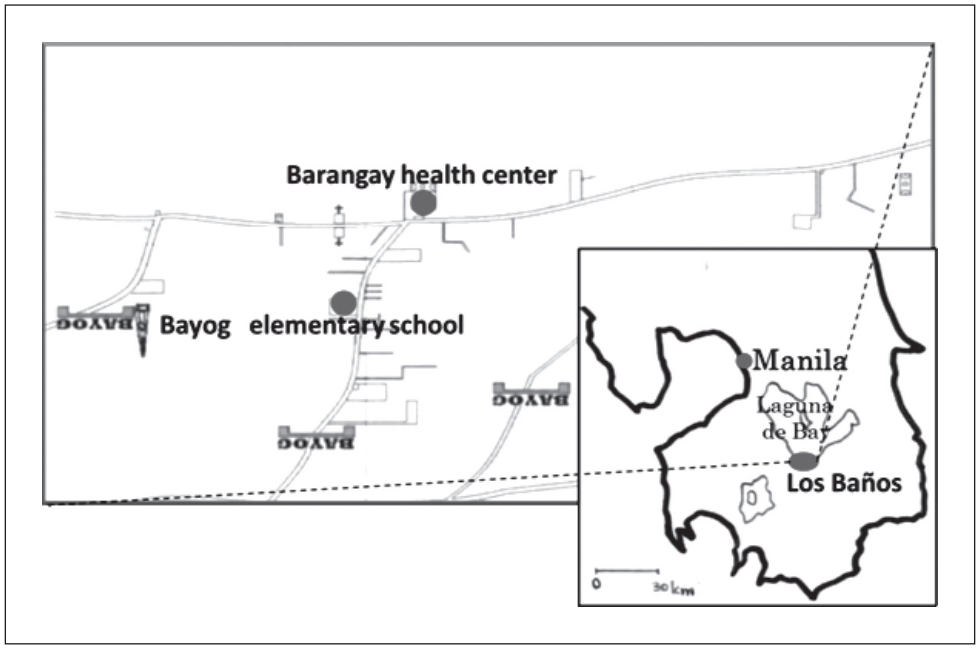

Figure 1. Map of survey area

collected from four different locations: backyards, open areas, paths, and inside living quarters.

The sucrose centrifugal flotation method, originally developed by Uga *et al.* (1993) was used for egg detection. Each specimen contained approximately 200 g of soil from a depth of 2-5 cm in an area that was not exposed to direct sunlight. The soil samples were dried overnight at room temperature. The samples were filtered through 150-µm-mesh sieves, yielding approximately 2 g of powdered sand. The powdered sand was moved to a 10-mL test tube (Eikenkizai, Japan), suspended in approximately 8 mL of Tween-80 solution whose concentration was 0.05 and centrifuged at 450 *g* for 10 minutes. After discarding the supernatant, the test tube containing the sediments was filled to approximately 1 cm from the top with sucrose solution (specific gravity of 1.200), vortexed, and centrifuged again. The test tube was then filled up to the brim with sucrose solution and centrifuged at 55 *g* for 5 minutes. The cover glass was removed and examined for parasite eggs, with an optical microscope. For each prepared specimen, the number of eggs was counted and the developmental stages of all the STH eggs were recorded. Some of the detected eggs were measured along major and minor axes by a micrometer.

Faecal examination

Faecal samples were collected from 70 school children aged 8-11 years who attended a public elementary school in Barangay Bayog. Faecal samples were taken to the laboratory, suspended in 10% formalin, and stored at room temperature. After centrifugation of the faecal suspension, 0.5 g of sediment was used for the faecal examination. The formalin-ether sedimentation technique was used to detect STH eggs, and 20 µL of a mean 40 µL plug was examined under a microscope.

Questionnaire survey

The questionnaires were answered by 44 residents and 70 school children. The residents were asked 25 questions related to daily living habits and lifestyle; the school children were asked 20 items related to daily living habits.

Data analysis

A chi-square test was used to examine the STH egg detection rate differences among the soil samples. The level of significance was set at 0.05.

Ethical considerations

The proposals for this study were approved by the municipal health office of Los Baños, University of the Philippines Los Baños, and the Ethical Committee of Kobe University Graduate School of Health Science.

RESULTS

Table 1 shows the parasite eggs isolated from 120 soil samples. In total, six genera of ecto- and endoparasites were recovered from 85 (71%) of 120 samples. The soil contamination rates for the rainy and dry seasons were 85% (51/60) and 57% (34/60), respectively, with the former being significantly higher than the latter ($p < 0.01$).

We measured major and minor axes of the eggs of *Toxocara* spp. and *Trichuris* spp. More than 70% of the *Toxocara* spp. eggs were the size of *T. cati*, 53-73 x 63-88 µm (Fig. 2a), or *T. canis* 72-91 x 63-79 µm (Fig. 2b) eggs. Thus, two species were differentiated. Similarly, *Trichuris* spp. eggs were the size of *T. trichiura* (20-26 x 40-60 µm (Fig. 2c)) or *T. vulpis* (32-40 x 72-89 µm (Fig. 2d)). Thus, these species were also differentiated. Although not all the detected eggs were measured, we recovered six genera and eight species of parasite eggs.

Figure 3 compares the STH egg soil contamination rates in the rainy and dry seasons at each of the four sites for *A. lumbricoides*, *Toxocara* spp., and *Trichuris* spp. The prevalence rates of eggs were extremely high. The soil contamination rates during the rainy season (77-100%; mean, 86%) were markedly higher than during the dry season (54-69%; mean, 63%) for all the four sites, including backyards (28 samples), open areas (13 samples), paths (15 samples), and

Table 1. Parasite eggs and ectoparasites isolated from soil

Species	No. of positive (%)	
	Rainy season (n = 60)	Dry season (n = 60)
Ascaris lumbricoides	37 (62)	19 (32)
Toxocara spp.	24 (40)	11 (18)
Trichuris spp.	32 (53)	17 (28)
Capillaria sp.	1 (2)	0 (0)
Hymenolepis diminuta	3 (5)	0 (0)
Tick	15 (25)	3 (5)
Sub total	51 (85)*	34 (57)
Total	85 (71)	

*p < 0.01

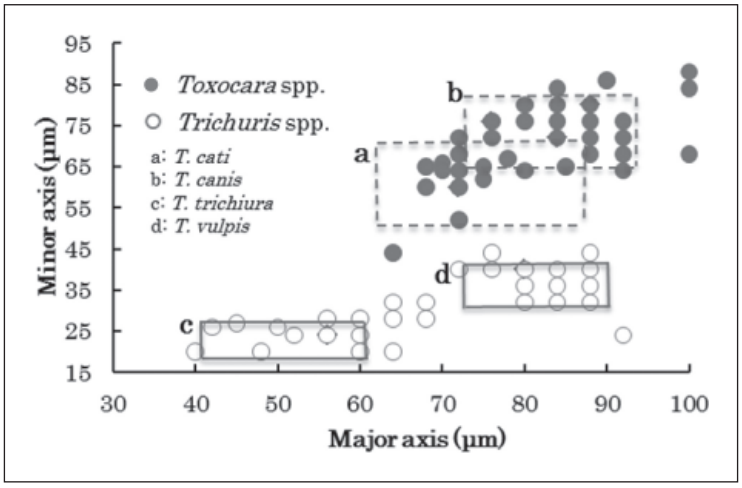

Figure 2. Measurement of *Toxocara* spp. and *Trichuris* spp. eggs

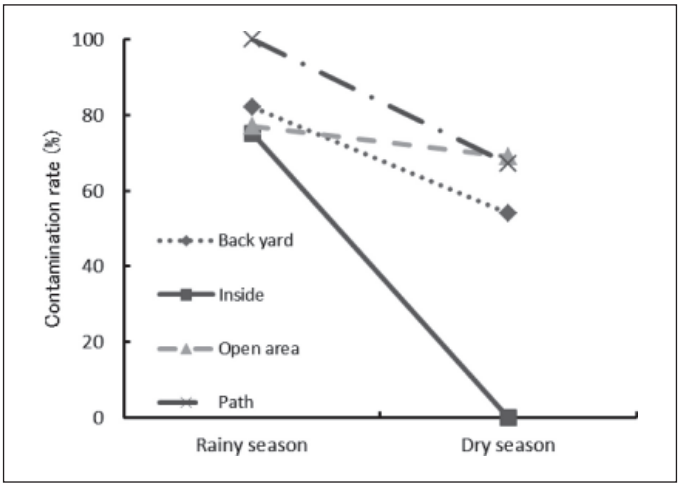

Figure 3. Comparison between rainy season and dry season of respective sites

inside living quarters (four samples; $p < 0.01$). Despite the small number of samples, the fact that STH eggs were detected in the samples taken from inside living quarters during the rainy season indicate widespread soil contamination in this village.

Furthermore, the number of detected STH eggs and the rates for fertilized eggs of each parasite from each of the four sites were compared to assess the risk of STH infection of residents (Table 2). An extremely large number of STH eggs were recovered from all the sites, except from inside living quarters, from which only a small number of samples were collected. Among a total of 1,450 STH eggs, 909 were *A. lumbricoides*, 301 were *Toxocara* spp., and 240 were *Trichuris* spp. The level of soil contamination by *A. lumbricoides* eggs was four times higher than that of *Trichuris* spp. Of all the recovered STH eggs, 777 were fertilized. Among those, 445 were *A. lumbricoides*, indicating that residents were at highest risk for *A. lumbricoides* infection. Moreover, based on the number of STH eggs and the rate of embryonation, the embryonated egg recovery rate for *Toxocara* spp. (77%) was significantly higher than that of the other two species ($p < 0.01$). The results reveal that the soil was highly contaminated by infective parasite eggs, and this contamination was widespread throughout the village.

Moreover, the responses of the residents and school children to the questionnaires clarified the sources of soil contamination (Table 3). Almost 100% of the adults answered that they had latrines in their homes and never defaecated in places other than latrines. In contrast, although almost 100% of the school children answered that they had never defaecated outside, >80% of them had seen other children defaecating outside. Approximately 50% of the residents kept dogs and cats, and almost none of them kept their pets on leashes. However, none of them kept swines (Table 3). This result confirmed the existence of *T. canis*, *T. cati* and *T. vulpis* eggs in the soil (Fig. 2).

Table 2. Recovery of eggs of soil-transmitted helminth at each site

Site	Species	No. of		%
		egg detected	emb. egg	
Back yard	*A. lumbricoides*	406	140	34
	Toxocara spp.	113	103	91
	Trichuris spp.	134	56	42
Open area	*A. lumbricoides*	91	64	70
	Toxocara spp.	134	85	63
	Trichuris spp.	36	13	36
Path	*A. lumbricoides*	410	239	58
	Toxocara spp.	48	40	83
	Trichuris spp.	70	30	43
Inside	*A. lumbricoides*	2	2	100
	Toxocara spp.	6	5	83
	Trichuris spp.	0	0	0
Four sites	*A. lumbricoides*	909	445	49
	Toxocara spp.	301	233	77*
	Trichuris spp.	240	99	41
Total		1,450	777	54

Embryonation rate of *Toxocara* spp. egg was significantly higher than that of *A. lumbricoides* and *Trichuris* spp. ($p < 0.01$).

Table 3. Result of questionnaire survey

Items	No. of answers	No. of "yes"	%
Do you have latrine in your house?	44	44	100
Do you defecate outside of latrine?	113	1	1
Have you witnessed someone's defecation?	66	54	82
Do you have a dog?	70	39	56
Do you have a cat?	70	29	41

Table 4. Soil-transmitted helminth eggs isolated from faeces of school children

Species	No. of samples		Total no. of eggs detected
	examined	positive (%)	
A. lumbricoides	70	25 (36)	8,366
T. trichiura	70	34 (49)	1,238
Total	70	44 (63)	9,604

The faecal examination was done for school children to specify the sources of soil contamination based on the soil contamination results and the questionnaire responses (Table 4). The prevalence of *A. lumbricoides* and/or *T. trichiura* was 63% (44/70). The number of *A. lumbricoides* eggs recovered through faecal examination was almost seven times larger than that of *T. trichiura*. Although the prevalence of *T. trichiura* (49%) was higher than that of *A. lumbricoides*, the total number of *A. lumbricoides* eggs (8,366) was larger than that of *T. trichiura* and accounted for >80% of all the eggs recovered from faeces (Table 4). The species and proportion of recovered STH eggs from faecal samples from school children were similar with those found in the soil samples.

DISCUSSION

This survey revealed that >70% of soil samples were contaminated by parasite eggs. Similar surveys have also been conducted in developing countries, including Brazil (Korkes *et al*., 2009), Czech Republic (Dubná *et al*., 2007), and Nepal (Rai *et al*., 2000), where 19-57% of soil samples contained parasite eggs. The contamination rates in our survey were markedly higher than those in previous surveys. As found in previous studies, humidity and appropriate temperature are important factors for the survival of STH eggs in the soil (Ghadirian *et al*., 1976; Bakta *et al*., 1993). Contamination rates have been found to be 2-4 times higher during the rainy season in Indonesia (Uga *et al*., 1995), Thailand (Chongsuvivatwong *et al*., 1999), and Nepal (Rai *et al*., 2000), and our survey yielded similar results. O'Lorcain (1994) and Ghadirian *et al*. (1976) have reported similar results for *Toxocara* spp. eggs.

The prevalence of STHs is closely associated with living environments and sanitation levels. In this study, we examined soil samples collected and found that all sites were highly contaminated by STH eggs. Although the number of samples was small, STH eggs were detected inside living quarters, which is associated with widespread soil contamination.

STH infection is inversely proportionate to the diffusion rate of latrines in an area (Ngui *et al*., 2011). However, Chongsuvivatwong *et al*. (1999) have reported an extremely high prevalence of STH infection despite an 88% latrine diffusion rate; thus, the two are not always correlated. Almost all of the adults who participated in the questionnaire survey indicated that they had latrines in their homes and had never defaecated in places other than latrines. Meanwhile, >80% of the school children indicated that they had seen others defaecate in places other than latrines. Moreover, the correspondence between eggs recovered from the soil samples and those found in the faecal samples from the school children strongly suggests that soil contamination had been caused by human faeces.

The percentage of *A. lumbricoides* embryonated eggs from soil samples was 49%, which was lower than the 77% of *Toxocara* spp. However, *A. lumbricoides* was considered to present the highest risk of infection to residents among all STH species because the number of eggs recovered from soil samples was particularly high and the number of embryonated eggs was approximately twice that of *Toxocara* spp. Faecal examination is generally used in epidemiological surveys for STHs with humans as their definitive host (Baldo *et al*., 2004; Belizario *et al*., 2011). Faecal examination can reveal the point prevalence or intensity of STH infection, but it is difficult to clarify the infection-risk for residents directly. Moreover, the collection of faeces can be troublesome from an ethical perspective. In contrast, soil examination has the advantage of easy sample collection and the possibility of assessing the infection-risk of eggs by observing their developmental stage. This means that soil examination is a highly effective method that can be used in epidemiological surveys intended to support the primary prevention of STH infection. Furthermore, in addition to easy treatment, soil examination reveals the actual state of soil contamination by STH eggs of humans and animal origin.

In this survey, *Toxocara* spp. eggs were detected. The detection rate of *Toxocara* spp. fertilized eggs (77%) was significantly higher than those of any other STH eggs ($p < 0.01$). This suggests that residents faced an extremely high risk of infection by *Toxocara* spp. Larva migrans is considered to be a significant disease based on its prevalence and difficulty in diagnosis, and on the occurrence of serious clinical manifestations (Sariego *et al*., 2012). Epidemiological surveys of soil contamination by *Toxocara* spp. have been conducted worldwide with a 10-75% recovery rate for *Toxocara* spp. eggs (Childs, 1985; Ruiz *et al*., 2001). In developed countries, prevention strategies have been created owing to concerns over toxocariasis among children, because dogs and cats often defaecate in sandboxes where children play (Uga *et al*., 1995). Investigations aimed at the prevention of zoonoses have rarely been conducted in developing countries. In the Philippines, STHs in humans is considered to be an important public health problem. Thus, the Department of Health has performed helminth control initiatives mainly in schools located in endemic areas, using anthelmintic drugs to reduce the prevalence of STH infection among children, adolescent women, pregnant women, and other special population groups (Republic of the Philippines Department of Health, 2011). However, zoonoses prevention strategies have not been very effective. Therefore, it is important to examine not only on STH species whose definitive hosts are human, but also zoonoses in developing countries. Pets are allowed to run loose by almost 50% of residents in this survey area, which has led to widespread soil contamination, and thus a high risk of toxocariasis infection among residents.

In our survey, the number of *A. lumbricoides* eggs tended to be larger than that of *Trichuris* spp. eggs recovered from both soil and faecal samples. However, the ratio of *A. lumbricoides* to *Trichuris* spp. eggs recovered from faecal samples was higher than that from soil samples. This means that the number of *Trichuris* spp. eggs recovered from soil was apparently larger

than that of *A. lumbricoides* eggs. One possible explanation is that *Trichuris* spp. eggs are highly resistant in the environmental conditions of the study site, and live longer than *A. lumbricoides* eggs in the soil (Mahmoud, 2002). *Trichuris trichiura* and *T. vulpis* eggs were detected in the soil because of contamination in this area caused by both human and animal faeces.

From a public health perspective, soil examination is an effective substitute for faecal examination in epidemiological surveys of STH infection. Soil in the village in Luzon was widely contaminated, not only by human faeces but also by animal faeces. Moreover, a large number of STH eggs had become infectious. These results suggest that the residents in this area were at extremely high risk for STH infection, resulting in a need for health education aimed at disease prevention.

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