

Comparative Study on Waterborne Parasites between Malaysia and Thailand: A New Insight

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Abstract. This study investigated the distribution of parasites as main contaminants in water environments of peninsular Malaysia (October 2011–December 2011) and the southeastern coast of Thailand (June 2012). Sixty-four water samples, 33 from Malaysia and 31 from Thailand, of various water types were examined according to U.S. Environmental Protection Agency guidelines. Drinking or household water types from both countries were free from parasitic contamination. The recreational/environmental (except a swimming pool in Malaysia) and effluent water types from these two countries were contaminated with waterborne parasites: *Giardia* (0.04–4 cysts/L), *Cryptosporidium* (0.06–2.33 oocysts/L), hookworm (6.67–350 ova/L), *Ascaris* (0.33–33.33 ova/L), and *Schistosoma* (9.25–13.33 ova/L). The most contaminated sites were recreational lake garden 3 in Malaysia and river 2 in Thailand. Higher concentrations of *Giardia*, *Cryptosporidium*, and hookworm were found in samples from Malaysia than in samples from Thailand. The presence of *Giardia* cysts showed a significant association with the presence of *Cryptosporidium* oocysts ($P < 0.005$).

INTRODUCTION

Water shortages and limited access to clean water sources caused by water contamination with pathogenic bacteria, viruses, protozoa,¹ and helminths² represent a major human health hazard. These contaminations occurred mainly because of improper management of water supplies, reuse of wastewaters, poor sanitation systems, and lack of awareness and poor hygienic behavior among human populations.^{1,3–5} In addition, parasite contamination of open water resources, such as reservoirs, lakes, and rivers, may also have been contributed by domestic and wild animals inhabiting these surroundings.^{3,5,6}

These parasites can be transmitted to humans through accidental consumption of contaminated waters (i.e., *Giardia*, *Cryptosporidium* and *Ascaris* ova) or via direct skin penetration (i.e., miracidia of *Schistosoma*).² The clinical symptoms for parasitic infections (i.e., giardiasis and cryptosporidiosis) may include loss of appetite, nausea, vomiting, diarrhea, loose or watery stool, malabsorption, dehydration, and stomach cramps.⁶ For helminthiasis, symptoms such as intestinal bleeding, abdominal pains, anemia, severe diarrhea and malnutrition are observed in humans.^{2,7}

To obtain and maintain parasite-free waters, various eradication methods, such as free chlorination have been applied to reduce parasite contamination in water resources.⁸ However, this chemical inactivation method, which is effective and safe at low dosages, could not completely eliminate waterborne protozoa, particularly *Giardia* and *Cryptosporidium* because of resistant characteristics of these contamination agents during their transmissive stages.⁸

Approximately 536 protozoa-related waterborne outbreaks have been reported worldwide since 1948–early 2012; 207 cases of *Giardia lamblia* infection and 293 cases of *Cryptosporidium parvum* infection.^{5,9–15} A recent outbreak of *Cryptosporidium* occurred in public swimming pools in Iowa, United States during June–August 2013; 528 cases were

reported.¹² However, during 1948–2012, only scanty data for outbreaks could be obtained from countries in Southeast Asia because of lack of documentation and recorded cases, especially in rural areas.⁹ There has been a slight increase in these infections during the past few years in Malaysia and Thailand.

In Malaysia, extensive studies on parasites detection in water environments have been conducted since 2004. *Giardia* was isolated from water bodies in zoos, recreational lakes, and rivers; *Cryptosporidium* was isolated from recreational lakes and rivers; *Blastocystis* was isolated from drinking water and sewage samples; free-living amoeba were isolated from swimming pools and recreational lakes; and helminths were isolated from recreational lakes.^{16–21} In Thailand, extensive water studies related to parasites have been conducted since 2006. *Giardia* and/or *Cryptosporidium* were isolated from wastewaters, rivers and coastal areas; *Blastocystis* was isolated from drinking water and freshwater; free-living amoeba were isolated from freshwater and hot springs; and liver flukes were isolated from the Mekong River basin.^{2,5,22,23} However, in other countries in Southeast Asia, less data are available for parasite detection directly from water compared with data for the prevalence of parasites in humans.^{20,21}

The present study was conducted to obtain current data on the distribution of waterborne protozoa and helminths in various water types in western peninsular Malaysia and along the southeastern coast of Thailand. This information would provide a better understanding of parasite contamination in water in the tropical regions of Southeast Asia, especially in Malaysia and Thailand.

MATERIALS AND METHODS

Sampling sites and sample collection. Water samples (10 liters from each sampling station) were collected in Petaling Jaya (101.61°E, 3.10°N) and Puchong (101.61°E, 3.00°N) areas in Selangor State, Malaysia during October–December 2011 and in Songkhla (100.61°E, 7.16°N) on the southeastern coast of Thailand in June 2012 (Figure 1). Malaysia and Thailand are neighboring countries and both have similar cultural and climate characteristics. The climate

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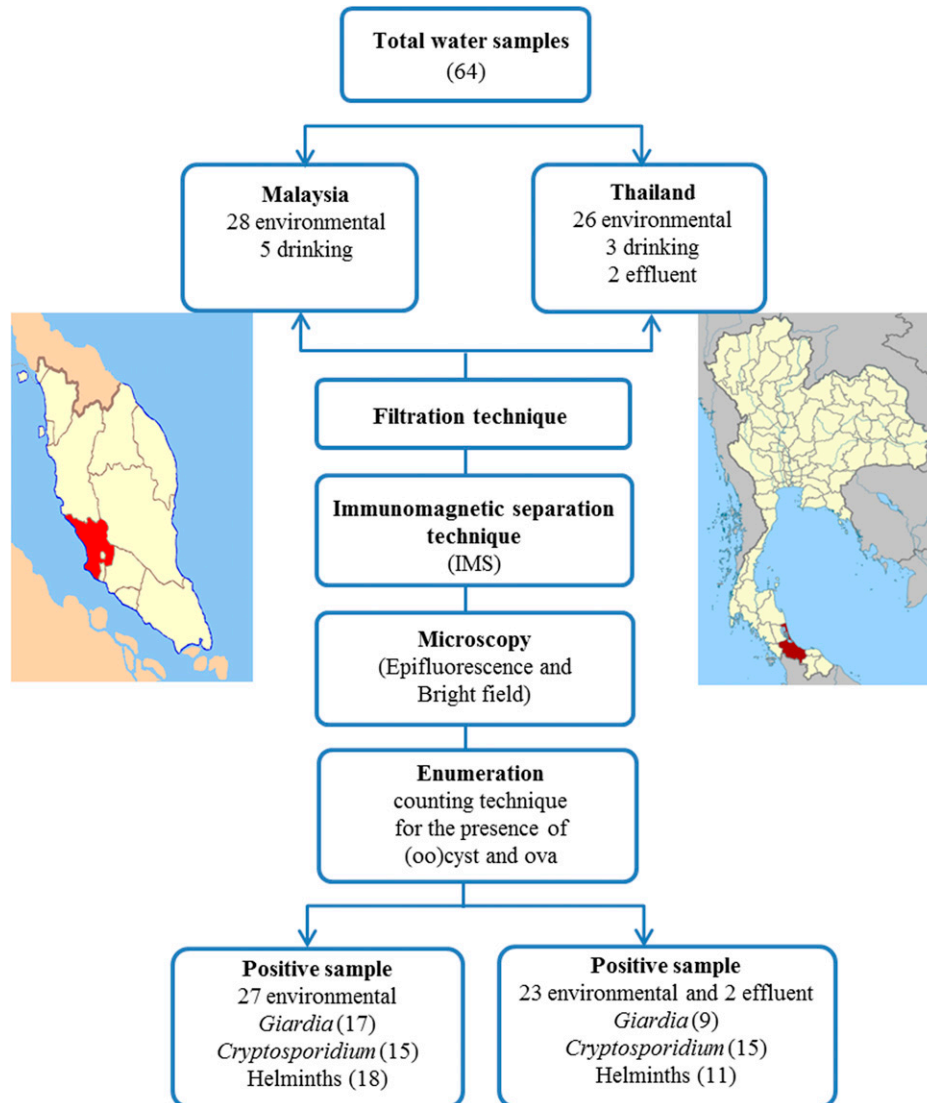


FIGURE 1. Study sites in Malaysia and Thailand. IMS = immunomagnetic separation.

in peninsular Malaysia and southern Thailand is generally hot and humid. Water sampling was conducted during the rainy season in both countries.

A total of 33 water samples were obtained in Malaysia and 31 water samples were obtained in Thailand. These water samples were grouped into three main categories: drinking or household water, recreational or environmental water, and waste or effluent water. For reservoirs, ponds, and lakes, water samples were obtained at a depth of 10 meter or from a mixture of surface and bottom water in shallow areas. For rivers and rainwater, water samples were obtained from surface water and open spaces, respectively.

Laboratory investigations. *Filtration of water samples and purification of parasites.* Collected water samples were filtered by using a flat-bed membrane filtration technique (EMD Millipore Corp., Billerica, MA). Oocysts and/or ova in soil sediments and debris from water were trapped on the surface of a nitrocellulose membrane (1.2 μm pore size, 142 mm diameter; EMD Millipore Corp.), scrapped off the membrane, and collected in 0.1% Tween 80. This eluate was concentrated to a volume of 10 mL by centrifugation (Kubota

Corporation, Osaka, Japan) at $1,050 \times g$ for 10 minutes at room temperature before being transferred to a 10-mL Leighton tube (L tube). Samples were analyzed by using an immunomagnetic separation (IMS) technique according to Method 1622/1623 (Environmental Protection Agency, Washington, DC [EPA], 2005) for *Cryptosporidium* (Chemical Abstracts Service Registry no. 137259-50-8) and *Giardia* (Chemical Abstracts Service Registry no. 137259-49-5) as per registered by the U.S. Government.

Subsequently, SL buffers and antibody-coated magnetic beads (Dynabeads[®] *Giardia*-Combo and Dynabeads[®] *Crypto*-Combo; Life Technologies Corp., Grand Island, NY) were added to L tubes and mixed by using a Dynal rotator (Life Technologies Corp.) for 1 hour. The Dynabeads–oocysts were purified from the supernatant (sediment and debris) by using a magnetic particle concentrator (MPC[®]-1; Life Technologies Corp.). The supernatant was decanted into a Falcon (Brookings, SD) tube, stored at 4°C, and used for detection of parasites other than *Giardia* and *Cryptosporidium*. The (oo)cysts were separated from the Dynabeads by the addition of 50 μL of 0.1N HCl.

Detection of *Giardia* cysts and *Cryptosporidium* oocysts. After the IMS technique, 50 μ L of sample (presumably containing oocysts) was applied to single-well microscope slides (Invitrogen Dynal AS, Oslo, Norway) and stained with fluorescein isothiocyanate-conjugated *Giardia/Cryptosporidium* monoclonal antibody reagent (Cellabs, Brookvale, New South Wales, Australia) and 4',6'-diamidino-2-phenylindole solution (Sigma-Aldrich, St. Louis, MO). Fixed slides were examined by using epifluorescence microscope (Model BX51 microscope; Olympus, Tokyo, Japan). Positive controls were included in a kit provided by Cellabs. Negative controls were prepared by using phosphate-buffered saline, pH 7.4. Putative *Giardia* cysts and *Cryptosporidium* oocysts showed a typical fluorescent appearance (based on size, shape, and internal structures (e.g., round to ovoid *Giardia* cysts 8–18 μ m \times 5–15 μ m and ovoid or spherical *Cryptosporidium* oocysts 4–6 μ m diameter; Method 1622/1623; EPA, 2005).

Detection of other parasites. Supernatants obtained during the IMS technique (stored at 4 °C) were re-suspended by using Pasteur pipette to ensure that all sediments were mixed well with eluate. Approximately 50 μ L of eluted sediments (from 10 mL) was applied onto a plain slide and mounted with Lugol's iodine or normal saline (1:2) and examined by using a bright-field light microscope (Model-CX40; Olympus). A negative control slide was prepared and examined with every 15–20 sample slides.

Enumeration of parasites. (Oo)cysts or ova were counted at least two times per slide and counts were adjusted to a volume of 1 liter water to minimize error. For example, if 10 cysts/L of *Giardia* were detected in a volume of 50 μ L, which is equivalent to 10 liters, this finding indicates that 1 cyst was detected in a 1-liter water sample. For other parasites, if 2 hookworm ova found in 50 μ L of slide (from 10ml supernatant equivalent to 10 L), it would be 400 ova in 10 L of water sample and subsequently 40 ova in 1 L of water sample.

Measurements of physical parameters and meteorologic information. Five physical parameters (temperature, pH, dissolved oxygen [DO], salinity, and turbidity) were simultaneously measured *in situ* at all sampling sites by using a handheld multi-parameter instrument (model 556; YSI Inc., Yellow Springs, OH) and turbidity parameter instrument (HACH-Model 2100P; ICM, Oregon). In addition, monthly rainfall and relative humidity for each sampling day was obtained from the nearest weather station (Malaysian Meteorological Department, Petaling Jaya Station; Thai Meteorological Department, Songkhla Station; and Norwegian Meteorological Institute and World Weather Online API, WeatherSpark.com).

Statistical analysis. Statistical analysis was performed by using Microsoft (Redmond, WA) Excel, Pearson's correlation (version 1.0.6) in Free Statistics Software,²⁴ and SPSS version 21.0 software (IBM, Armonk, NY) to analyze correlations between enumerated parasites and their physical parameters. Statistically significant results had an α value < 0.005.

RESULTS

Parasitic contamination in water. Of 33 samples from Malaysia, 5 were from drinking or household water (mineral, drinking, tap, and rain) and 28 samples were from recreational or environmental water (swimming pool, recreational

lakes, rivers, and a waterfall). Of 31 samples of Thailand, 3 were from drinking or household water (mineral, drinking and rain), 26 were from recreational or environmental water (ponds, lakes, reservoirs, rivers, and a waterfall), and 2 were from waste or effluent water from hospitals.

Numbers of parasites found in water samples are shown in Table 1. There was no parasite contamination of drinking or household water in Malaysia and Thailand. A selected swimming pool in Malaysia was also found to be free of parasites. However, other untreated environmental water in both countries and effluent water in Thailand were contaminated with waterborne parasites. The main waterborne parasites found in both countries were *Giardia*, *Cryptosporidium*, hookworm, *Ascaris*, and *Schistosoma*. All parasites were detected in recreational lake garden 3 (LG3) in Malaysia and river 2 in Thailand. River 1 in Thailand was the least contaminated site (only one parasite, *Cryptosporidium* oocysts). In the water sites in Malaysia, at least 2 types of waterborne parasites were detected (e.g., *Giardia* and *Cryptosporidium* in LG2 and a waterfall). Malaysia had a higher concentration of *Giardia* cysts (4 cysts/L) and *Cryptosporidium* oocysts (2.33 oocysts/L) in river water and a high hookworm ova concentration (70.33 ova/L) in LG3. However, a higher concentration of helminths was found in Thailand, particularly *Ascaris* ova (33.33 ova/L) in a waterfall and *Schistosoma* ova (13.33 ova/L) in pond 1.

Other waterborne parasites, such as *Blastocystis* cysts, *Entamoeba* cysts, *Enterobius* ova, *Hymenolepis* ova, tapeworm ova, *Toxocara* ova, *Toxoplasma* oocysts, were also detected in these water samples (1 or 2 per 25 microscopic slides), as shown in Figure 2. In addition, we also found ectoparasites, such as members of the orders Anastroca and Cladocera, copepods, lice, and a *Brachinecta* sp., as indicated in Table 1.

Physical parameters and meteorologic data. Monthly rainfall at Petaling Jaya station, Malaysia, was 250–300 mm in October 2011, 300–400 mm in November 2011, and 250–350 mm in December 2011. At Songkhla station in Thailand, low rainfall (50–100 mm) was reported in June 2012. In Malaysia, there was no rain on sampling days in all recreational lakes except for rivers. It rained on previous sampling days at LG1, LG3, and a waterfall. In Thailand, it rained on the sampling day at a natural lake but it did not rain at other sites during sampling. However, it rained heavily on the day before sampling at river 1.

Physical parameters and meteorologic data for each sampling location in peninsular Malaysia and along the southeastern coast of Thailand are shown in Table 2. For the pipeline distribution system of tap water and for rain water, high humidity was reported in both countries. However, lower humidity (60–72%) was noted at sampling sites in Malaysia than at sampling sites in Thailand (68.2–87.25%). Water temperature was high during sampling in Malaysia (\leq 32.11 °C) or Thailand (\leq 31.64 °C). Turbidity was low in drinking or household water from Malaysia (0.13–2.01 nephelometric turbidity unit [NTUs]) and Thailand (0.16–1.37 NTU). Higher turbidity was observed for recreational or environmental water from Thailand (1.04–88.71 NTU) compared with water from Malaysia (1.02–24.64 NTU). Lower salinity was found in environmental water samples from Malaysia (0.02–0.23 ppt) than in samples from Thailand (0.02–1.68 ppt). Low levels (0.06–0.12 g/L) of total dissolved solids (TDS) were found in drinking and environmental water in both countries. However,

TABLE 1
Parasites found in water samples, Malaysia and Thailand*

Water sample	No.	No. positive	Mean \pm SD				
			<i>Giardia</i>	<i>Cryptosporidium</i>	Hookworm	<i>Ascaris</i>	<i>Schistosoma</i>
Malaysia							
Drinking/household water							
Mineral water (MM)	1	0	ND	ND	ND	ND	ND
Drinking water (MD)	1	0	ND	ND	ND	ND	ND
Tap water (MT)	2	0	ND	ND	ND	ND	ND
Rain water (MR)	1	0	ND	ND	ND	ND	ND
Recreational/environmental water							
Treated							
Swimming pool (SP)	1	0	ND	ND	ND	ND	ND
Untreated							
Lake Garden 1 (LG1)	7	7	0.14 \pm 0.10	0.23 \pm 0.21	28.54 \pm 37.84	19.03 \pm 23.73	ND
Lake Garden 2 (LG2)	2	2	0.20 \pm 0.28	0.10 \pm 0.14	ND	ND	ND
Lake Garden 3 (LG3)	12	12	2.58 \pm 4.37	1.51 \pm 3.43	70.30 \pm 55.06	9.25 \pm 14.84	9.25 \pm 19.99
River (MR)	3	3	4 \pm 0.87	2.33 \pm 3.62	ND	0.33 \pm 0.58	ND
Waterfall (MW)	3	3	0.17 \pm 0.29	0.83 \pm 0.29	ND	ND	ND
Total	33	27					
Thailand							
Drinking/household water							
Mineral water (TM)	1	0	ND	ND	ND	ND	ND
Drinking water (TD)	1	0	ND	ND	ND	ND	ND
Rain water (TR)	1	0	ND	ND	ND	ND	ND
Recreational/environmental water							
Pond 1 (TP1)	2	2	ND	ND	ND	20 \pm 28.28	10 \pm 14.14
Pond 2 (TP2)	2	2	0.22 \pm 0.31	0.45 \pm 0.63	30 \pm 42.42	ND	ND
Natural lake (TNL)	7	7	0.21 \pm 0.33	0.73 \pm 1.33	ND	ND	ND
Reservoir lake (TRL)	6	5	0.04 \pm 0.10	0.26 \pm 0.49	ND	3.33 \pm 8.16	ND
River 1 (TR1)	3	2	ND	0.30 \pm 0.29	ND	ND	ND
River 2 (TR2)	3	3	0.52 \pm 0.50	1.29 \pm 1.54	6.67 \pm 11.55	20 \pm 34.64	13.33 \pm 11.55
Waterfall (TW)	3	2	0.11 \pm 0.19	0.06 \pm 0.10	ND	33.33 \pm 30.55	ND
Waste/effluent water							
Treatment plant (TTP)	2	2	0.22	0.89 \pm 0.31	350 \pm 494.97	ND	ND
Total	31	25					

*ND = not determined. Other detected parasites with negligible counts (1 or 2 per 25 microscopy slides examination) were *Blastocystis* cysts (LG1, TNL, TRL, TR2, TW, and TTP), *Entamoeba* cysts (TP1), *Enterobius* ova (TW), *Hymenolepis* ova (TP2 and TTP), tapeworm ova (TTP), *Toxocara* ova (LG3, TNL and TRL), *Toxoplasma* oocysts (LG1), ectoparasites of the orders Anastroca (LG2, LG3 and TTP) and Cladocera (LG1, LG2, TW, and TP2), copepods (LG3 and TR2), lice (LG1 and LG3), and *Brachinecta* organisms (LG3).

TDS level in a swimming pool was 0.23 g/L and in a treatment plant was 0.25g/L. Predictably, a high salinity (13.53 ppt) and TDS (14.48 g/L) was detected in a natural lake, which is also an estuary, in Thailand.

Correlation analysis of waterborne parasites. A significant positive correlation ($r = 0.583$) was observed between the presence of *Giardia* cysts and *Cryptosporidium* oocysts in recreational or environmental water (Figure 3). Otherwise, there was no significant correlation between each parasite or between parasites and physical parameters.

DISCUSSION

In our study, no parasites were detected in drinking or household water from Malaysia and Thailand. Thus, filtered or treated water was safe for drinking or domestic purposes. This study is the first to report on safe drinking water from the perspective of parasite contamination in Southeast Asia.

For recreational or environmental water, only a swimming pool was free from parasite contamination and this finding might have been the result of proper chlorination treatment at this site.¹⁷ For other sites of recreational or environmental water, five parasites were identified: *Giardia*, *Cryptosporidium*, hookworm, *Ascaris*, and *Schistosoma*. These parasites were detected on the basis of identification of (oo)cysts or ova. Because ova of *Ancylostoma* and *Necator* (and most other

hookworm species) are indistinguishable, additional laboratory tests (culture of fecal samples) are needed to distinguish these two genera. Malaysia had high contamination with *Giardia*, *Cryptosporidium*, and hookworm, and Thailand had high contamination with *Ascaris* and *Schistosoma*. These findings could be the result of the fact that samples from Malaysia were obtained from restricted-flow water, such as recreational lakes, whereas most samples from Thailand were obtained from free-flow water, such as rivers and a waterfall.

The most contaminated sites were LG3 in Malaysia and river 2 in Thailand; all five parasites were found at these sites. LG3 in Malaysia is situated in a city and it controls flash floods by functioning as a water storage area during heavy rains. Water from a row of shops (i.e., butchers, wet markets, and restaurants) drains directly into LG3, which probably plays an important role in parasite contamination found in our study.²¹ If lake water levels become too high, water would be channeled into a large drainage system. Furthermore, this recreational lake is mostly use for fishing activities. Thus, parasite contamination may contribute to human infections by accidental contact.

River 2 in Thailand is a confluence of two upstream rivers (from a mountainous area and a city area), flows through a small town, and ends in an area that contains remote villages. Samples from this river was obtained from a floating market (upstream), inside town temple (midstream), and village fishing docks (downstream). Contamination sources

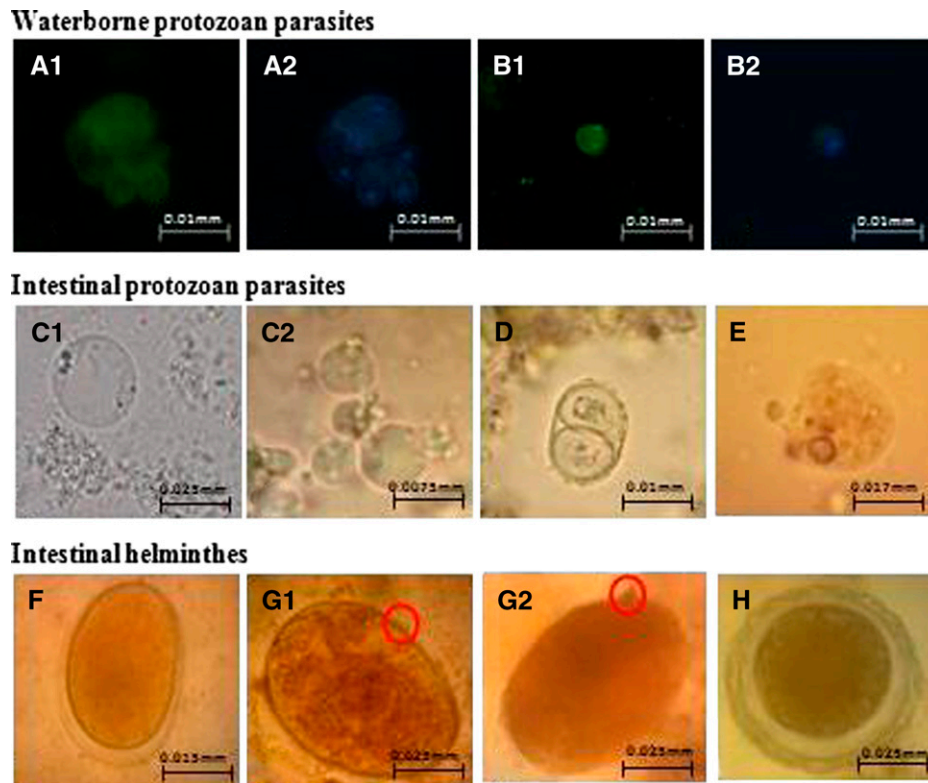


FIGURE 2. Photomicrograph of parasites detected in water samples from Malaysia and Thailand, 400 × magnification: *Giardia* (A), *Cryptosporidium* (B), *Blastocystis* (C), *Toxoplasma* (D), *Entamoeba* (E), *Hookworm* (F), *Schistosoma* (G), and *Ascaris* (H).

in this river might have been the floating market and the villages. In contrast, river 1 is located in a city area, and this river has a dense flow during rainy season.²² The day before sampling of this river, it rained heavily, which might explain why there was less contamination with parasites because of a dilution effect that might have washed away most contaminants.²⁵ Most environmental water in Thailand was used directly by villagers. This could lead to signs and symptoms, such as diarrhea, related to parasitic infections in affected persons.

Our study showed that the level of turbidity in drinking water was safe and conformed with standard regulations of the World Health Organization,²⁶ the Malaysia National Standard of Drinking Water Quality formulated by the Ministry of Health,²⁷ and the Thailand Ministry of Natural Resources and Environment.²⁸ River 1 and the natural lake had high turbidity and exceeded the U.S. EPA Ambient Water Quality Standard. This finding is also supported by fishing activity in surface water near banks and contaminated aquatic ecosystems at the shore and in shallow areas,²⁹ which results in parasite contamination at these water sites. Also, low-velocity settling characteristics of waterborne protozoan parasites (*Giardia* = 0.84 $\mu\text{m}/\text{second}$ and *Cryptosporidium* = 0.35 $\mu\text{m}/\text{second}$) can prolong their survival by attachment to suspended particles and decrease the mobilization rate of (oo)cysts in water.³⁰

The presence of waterborne protozoa in a natural lake (an estuary near a sea waterway) that has high salinity and distinct high TDS helps explain the tolerance adaptation of these parasites in adverse conditions, which has affected ecosystem diversity.^{31–33} There are many rivers, including river 1

and river 2, which join to become one river, and flow towards this natural lake. Although this lake contains brackish water, contaminants and pollutants from rivers indirectly affected the environment of this lake. No waterborne parasites, especially protozoan parasites, have been identified in the brackish water of this estuary, with the exception of related studies on sea-water fish helminths³¹ and *in situ* studies of *Cryptosporidium* in shellfish or salty water.^{33,34} To the best of our knowledge, this is the first study reporting on parasitic contamination with *Giardia* and *Cryptosporidium* in the brackish water of this estuary. No helminths were found in high-salinity natural lake water because of intolerance to hypertonic water, which would cause the helminths to burst.^{21,25}

A positive correlation between concentrations of *Giardia* cysts and *Cryptosporidium* oocysts in recreational or environmental water was found in our study. The average concentration of *Giardia* cysts was two times higher than that of *Cryptosporidium* oocysts in samples from recreational or environmental water from Malaysia and Thailand. This finding is consistent with data of the U.S. EPA, which reported that *Giardia* and *Cryptosporidium* are primary sources of water contamination.⁹ Our data was also supported by a study in Thailand, which reported a significant positive correlation between these two protozoan parasites in tropical water environments.⁵ In addition, the hot and humid climates with seasonal rainfall may sustain increasing occurrence of *Giardia* cysts and *Cryptosporidium* oocysts in these tropical water environments. However, studies reported that seasonal and climate changes (during the four seasons) in certain countries do not contribute to the correlation between these two waterborne parasites.^{35,36}

TABLE 2
Physical parameters and meteorologic data of each sampling location in peninsular Malaysia and southeastern coast of Thailand*

Location	Water sample (no.)	Baseline data of water samples on sampling day (mean ± SD)					Others
		Humidity (%)	Temperature (°C)	Turbidity (NTU)	Salinity (ppt)		
Drinking/household water							
Malaysia	Mineral water (1)	–	19.20	0.13	0.07	pH	6.93 ± 0.06
	Drinking water (1)	–	17.70	0.18	0.08	DO	5.7 ± 3.03
	Tap water (2)	84.67 ± 12.1	24.79 ± 0.69	2.01 ± 1.53	0.04 ± 0.01	TDS	0.06 ± 0.04
	Rain water (1)	88.67 ± 6.43	21.11	1.66	0		
Thailand	Mineral water (1)	–	25.47	0.16	0.24	pH	6.85 ± 0.36
	Drinking water (1)	–	28.28	0.27	0.06	DO	4.07 ± 2.74
	Rain water (1)	84	25.2	1.37	0	TDS	0.12 ± 0.14
Recreational/environmental water							
Malaysia	Treated: swimming pool (1)	72 ± 4.24	23.62	1.02	0.17	pH	6.98
						DO	16.1
	Untreated: Lake Garden 1 (7)	67.33 ± 4.51	31.55 ± 0.65	24.64 ± 2.62	0.05 ± 0.01	TDS	0.23
	Lake Garden 2 (2)	60	32.11 ± 1.48	9.99	0.03	pH	6.34 ± 1.16
	Lake Garden 3 (12)	69.8 ± 4.38	29.06 ± 0.48	15.45 ± 6.66	0.06 ± 0.01	DO	10.68 ± 3.77
	River (3)	68 ± 3.6	28.7	22.27	0.23 ± 0.003	TDS	0.07 ± 0.02
Thailand	Waterfall (3)	71 ± 5.44	27.8	1.07	0.02 ± 0.001		
	Natural lake (7)	87.25 ± 2.36	27.58 ± 0.6	88.71 ± 61.11	13.53 ± 6.55		
	Reservoir lake (6)	68.2 ± 12	30.85 ± 0.46	9.86 ± 1.89	0.02	pH	6.54 ± 0.92
	River 1(3)	79 ± 4.58	28.63 ± 0.57	61.57 ± 44.07	0.05	DO	13.68 ± 6.25
	River 2 (3)	71 ± 5.66	30.58 ± 0.48	15.3 ± 2.4	0.32 ± 0.36	TDS	0.1 ± 0.21
	Waterfall (3)	85 ± 1.41	24.86 ± 0.3	4.93 ± 0.06	1.68 ± 2.89	(except natural lake: 14.48 ± 6.57 g/L)	
	Pond 1 (2)	69 ± 2.83	29.80 ± 0.05	1.04 ± 0.03	0.05		
Pond 2 (2)	78.5 ± 0.71	30.34 ± 0.35	54.7 ± 11.03	0.05			
Waste or effluent water							
Thailand	Treatment plant (2)	73 ± 2.83	31.64 ± 1.32	38.1 ± 18.1	0.18	pH	7 ± 0.04
						DO	7.05 ± 0.92
						TDS	0.25 ± 0.01

*NTU = nephelometric turbidity units; DO = dissolved oxygen (mg/L); TDS = total dissolved oxygen (g/L) of total dissolved solids.

One limitation of this study was the use of the IMS technique. This technique was chosen because it has high sensitivity, is commercially available, and it effectively purifies waterborne protozoans from other debris. However, the IMS is not suitable for detecting other waterborne parasites, such as free-living amoeba cysts (i.e., *Acanthamoeba* and *Naegleria*).^{37,38} Our study focused on a simple method for identification of parasites contaminating water environments. Thus, species identification and obtaining of pathogenicity data by molecular analysis were limited. Based on these find-

ings, studies with larger sample sizes are needed, and global positioning systems and geographic information systems should be included to assess the distribution of waterborne parasites in this region.

In conclusion, waterborne protozoa parasites and helminths ova were found mostly in environmental water samples from Malaysia and Thailand, a finding that corresponds to human activities and local maintenance systems in the affected areas. Parasite contamination, especially with *Giardia* and *Cryptosporidium*, of various water types in these two neighboring

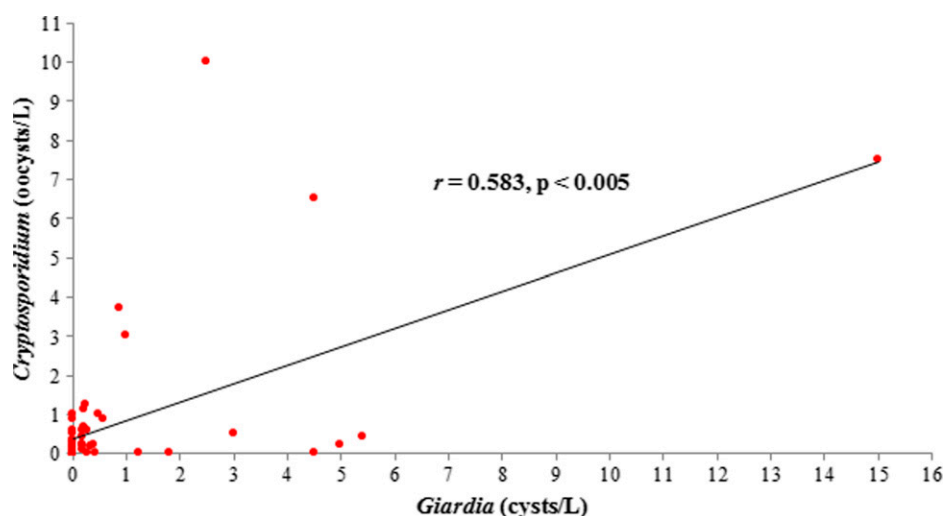


FIGURE 3. Positive correlation ($r = 0.583$) between presence of *Giardia* cysts and *Cryptosporidium* oocysts in recreational or environmental water type, Malaysia and Thailand.

countries is a serious health concern. On the basis of the data obtained, we make the following recommendations. First, recreational or environmental water should be treated by government and non-government sectors to eliminate parasites and effectively control these emerging pathogens to reduce waterborne parasite transmission. Second, procedures (storage, treatment, and distribution) are urgently needed to prevent release of waste into water environments and encourage use of biological filters in drainage systems before water is diverted into storages areas, such as recreational lakes. Third, rivers should be monitored regularly by conducting water analysis, including tests for the presence of waterborne parasites. In addition, persons involved in water activities should be provided with information about health education, waterborne health hazards, and primary behavior practices. Fourth, epidemiologic studies, microbiologic evaluations of water and food products, and quantitative microbial risk assessment should be conducted^{26,38} to provide better control strategies against these waterborne pathogenic parasites.

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